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Review

Biological and environmental monitoring of hospital personnel exposed to antineoplastic agents: a review of analytical methods

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Abstract

In order to assess occupational exposure of hospital personnel involved in the preparation and administration of antineoplastic drugs, biological and environmental monitoring are essential to identify the main exposure routes and to quantify potential health risks. If workplace contamination cannot be completely avoided, it is of utmost importance to reduce exposure to the lowest possible levels. To this aim, not only do education and training of the exposed subjects play an important role, but accurate standardized sampling techniques and analytical methods are also required. A critical overview of the most significant methods available in the literature is presented and their value is discussed, especially with respect to their sensitivity and specificity. In addition, attention is given to validation procedures and, consequently, to their reliability. The results from the most important surveys carried out at hospital departments are also discussed, with a view to improving both monitoring strategies and moreover working conditions.

Keywords: Reviews; Biological monitoring; Environmental monitoring; Antineoplastic drugs

Contents

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1. Introduction

The use of the so-called ''chemotherapy'' in the treatment of cancer has been constantly increasing over the last decades. Unfortunately, well-known are the side effects of antineoplastic drugs in cancer patients, due to the fact that these agents cannot distinguish between healthy cells and cancerous cells.

Anticancer agents include cytostatic drugs, hormones and antibiotics.

On the basis of their mechanism of action, cytostatic agents can be further divided into:

(1) *Alkylating drugs*, which form covalent bonds with DNA, RNA and proteins to form a DNA adduct. DNA adducts are thought to play a major role in mutagenesis and carcinogenesis. Nitrosoureas can also be included in this group, since they appear to function as alkylating agents, as well as through other mechanisms such as carbamoylation.

(2) *Antimetabolites*, which are structural analogues of nucleotides and are incorporated into cell components as if they were the essential pyrimidine or purine. As a consequence, they disrupt the synthesis of nucleic acids. Other antimetabolites disrupt essential enzymatic processes of metabolism.

(3) *Free radical generators* and *topoisomerase II inhibitors*, most of which intercalate between DNA base pairs and disturb the synthesis and/or function of nucleic acids. With regard to bleomycin, it appears to bind to DNA, resulting in single-strand breaks and double-strand scissions, thereby disrupting DNA synthesis. Doxorubicin not only intercalates between base pairs, but also alkylates macromolecules. Doxorubicin, daunorubicin, and their derivatives belong to a subclass called *anthracyclines*.

(4) *Mitotic spindle inhibitors*, which generally bind to microtubular proteins. This process halts cell replication at metaphase, and at high concentration, nucleic acid and protein synthesis is suppressed. Mitotic inhibitors include the vinca alkaloids vincristine and vinblastine and the epipodophyllotoxins teniposide and etoposide.

According to the International Agency for Research on Cancer (IARC), at least nine alkylating cytostatic drugs are classified as carcinogenic to

humans (Group 1) [1–8]. This classification is based on epidemiological studies showing secondary tumours in cancer patients treated with these drugs and primary tumours in subjects treated with the drugs for other purposes [9,10]. In addition, several cytostatic drugs are classified by the IARC in Groups 2A and 2B (probably and possibly carcinogenic to humans, respectively). Antimetabolites and mitotic inhibitors are not classifiable as to their carcinogenicity to humans (Group 3). Nevertheless, they are mutagenic and teratogenic (Table 1).

In most of the current chemotherapy protocols, cytostatic drugs are used as a combination of agents having different mechanisms of action. As a consequence, hospital personnel involved in the preparation, administration and disposal of these drugs may be exposed to a wide variety of cytotoxic substances.

In this review, cyclophosphamide, ifosfamide, methotrexate, 5-fluorouracil, taxol, platinum compounds, vinca alkaloids and anthracyclines are taken into consideration (Fig. 1). These substances are among the most frequently used and the most significant from a toxicological point of view. In addition, analytical standard products for the abovementioned drugs are commercially available. These are the main reasons why these substances have been analysed by a number of authors and therefore herein considered.

Occupational exposure to cytostatic drugs has been recognized as a potential health hazard since 1970s [11,12]. Falck first demonstrated the potential risk associated to nurses administering cyclophosphamide and vincristine by studying the mutagenicity of their urine [13].

Several subsequent investigations reported excretion of cytostatic drugs in the urine of exposed subjects [14–28] and also workplace contamination was documented [17,18,23–25,29–37].

Based on current scientific knowledge, it is impossible to set a level of exposure that can be considered to be safe. For this reason, exposure to cytostatic agents has to be kept at the lowest possible level. In order to reduce occupational risks associated with the handling of antineoplastic agents, a number of guidelines have been published in several countries [38–45]. Nevertheless, even when protective measures are taken and safety guidelines are adhered to, contamination occurs. Biological and environmental monitoring are therefore essential to identify the main exposure routes and to quantify potential health risks. However, risk assessment calls for accurate standardized sampling techniques and analytical methods. In this review, an overview of the exposure monitoring methods available in the literature is presented and their value is discussed, especially with respect to their reliability and validation.

2. Metabolism in humans and adverse effects

2.1. Cyclophosphamide

Cyclophosphamide (CP), 2-bis(2-chloroethyl) aminotetrahydro-2H-1,3,2-oxazophosphorine-2-oxide, is one of the most commonly used of the antineoplastic alkylating agents. It has also been used for its immunosuppressive properties in the treatment of autoimmune diseases as well as in renal and bone marrow transplants [46].

CP is a prodrug and thus requires activation in the liver. It is metabolized by cytochrome P-450 mixed function oxidase to 4-hydroxycyclophosphamide, which is in a steady state with the acyclic tautomer, aldophosphamide. These compounds may be oxidized further to carboxyphosphamide and 4-ketocyclophosphamide, which are inactive metabolites, whereas some aldophosphamide undergoes β-elimination to generate phosphoramide mustard, which alkylates DNA, and acrolein. This metabolite has been associated with the bladder toxicity observed after the administration of CP [47,48].

Cyclophosphamide is not specific for tumour cells but more toxic to rapidly proliferating cells. As a consequence, normal cells that are rapidly proliferating may also be affected.

CP is mainly administered orally and intravenously.

The unchanged drug found in urine ranged from 10 to 15%. About 85% of the metabolized drug is excreted renally [49,50].

Exposure to cyclophosphamide may occur from its manufacture, formulation, or distribution for use as an antineoplastic drug. Effects from exposure may

Table 1
Cytostatic drugs classified according to the IARC (last updated: 23 August 2002)

Group 1 Carcinogenic to humans	·Azathioprine [446-86-6] (Vol. 26, Suppl. 7; 1987) ·*N*,*N*-Bis(2-chloroethyl)-2-naphthylamine (Chlornaphazine) [494-03-1] (Vol. 4, Suppl. 7; 1987) · 1,4-Butanediol dimethanesulfonate (Busulphan; Myleran) [55-98-1] (Vol. 4, Suppl. 7; 1987) · Chlorambucil [305-03-3] (Vol. 26, Suppl. 7; 1987) · 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU; Semustine) [13909-09-6] (Suppl. 7; 1987) · Cyclophosphamide [50-18-0] [6055-19-2] (Vol. 26, Suppl. 7; 1987) · Etoposide [33419-42-0] in combination with cisplatin and bleomycin (Vol. 76; 2000) · Melphalan [148-82-3] (Vol. 9, Suppl. 7; 1987) · MOPP and other combined chemotherapy including alkylating agents (Suppl. 7; 1987) · Thiotepa [52-24-4] (Vol. 50; 1990) · Treosulfan [299-75-2] (Vol. 26, Suppl. 7; 1987)
Group 2A Probably carcinogenic to humans	· Adriamycin [23214-92-8] (Vol. 10, Suppl. 7; 1987) · Azacitidine [320-67-2] (Vol. 50; 1990) · Bischloroethyl nitrosourea (BCNU) [154-93-8] (Vol. 26, Suppl. 7; 1987) · 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) [13010-47-4] (Vol. 26, Suppl. 7; 1987) · Chlorozotocin [54749-90-5] (Vol. 50; 1990) · Cisplatin [15663-27-1] (Vol. 26, Suppl. 7; 1987) · *N*-Ethyl-*N*-nitrosourea [759-73-9] (Vol. 17, Suppl. 7; 1987) · Etoposide [33419-42-0] (Vol. 76; 2000) · *N*-Methyl-*N*-nitrosourea [684-93-5] (Vol. 17, Suppl. 7; 1987) · Nitrogen mustard [51-75-2] (Vol. 9, Suppl. 7; 1987) · Procarbazine hydrochloride [366-70-1] (Vol. 26, Suppl. 7; 1987) · Teniposide [29767-20-2] (Vol. 76; 2000)
Group 2B Possibly carcinogenic to humans	· Amsacrine [51264-14-3] (Vol. 76; 2000) · Aziridine [151-56-4] (Vol. 9, Suppl. 7, Vol. 71; 1999) · Bleomycins [11056-06-7] (Vol. 26, Suppl. 7; 1987) · Dacarbazine [4342-03-4] (Vol. 26, Suppl. 7; 1987) · Daunomycin [20830-81-3] (Vol. 10, Suppl. 7; 1987) · Merphalan [531-76-0] (Vol. 9, Suppl. 7; 1987) · Mitomycin C [50-07-7] (Vol. 10, Suppl. 7; 1987) · Mitoxantrone [65271-80-9] (Vol. 76; 2000) · Streptozotocin [18883-66-4] (Vol. 17, Suppl. 7; 1987)
Group 3 Not classifiable as to carcinogenicity in humans	· 5-Fluorouracil [51-21-8] (Vol. 26, Suppl. 7; 1987) · Isophosphamide [3778-73-2] (Vol. 26, Suppl. 7; 1987) · 6-Mercaptopurine [50-44-2] (Vol. 26, Suppl. 7; 1987) · Methotrexate [59-05-2] (Vol. 26, Suppl. 7; 1987) · Prednisone [53-03-2] (Vol. 26, Suppl. 7; 1987) · Vinblastine sulfate [143-67-9] (Vol. 26, Suppl. 7; 1987) · Vincristine sulfate [2068-78-2] (Vol. 26, Suppl. 7; 1987)

include fever, chills, shortness of breath, dizziness, headache, hemorrhagic colitis, leucopoenia, and pneumonitisor interstitial pulmonary fibrosis.

CP has been classified as a human carcinogen (Group I) by the International Agency for Research on Cancer (IARC) [5,51].

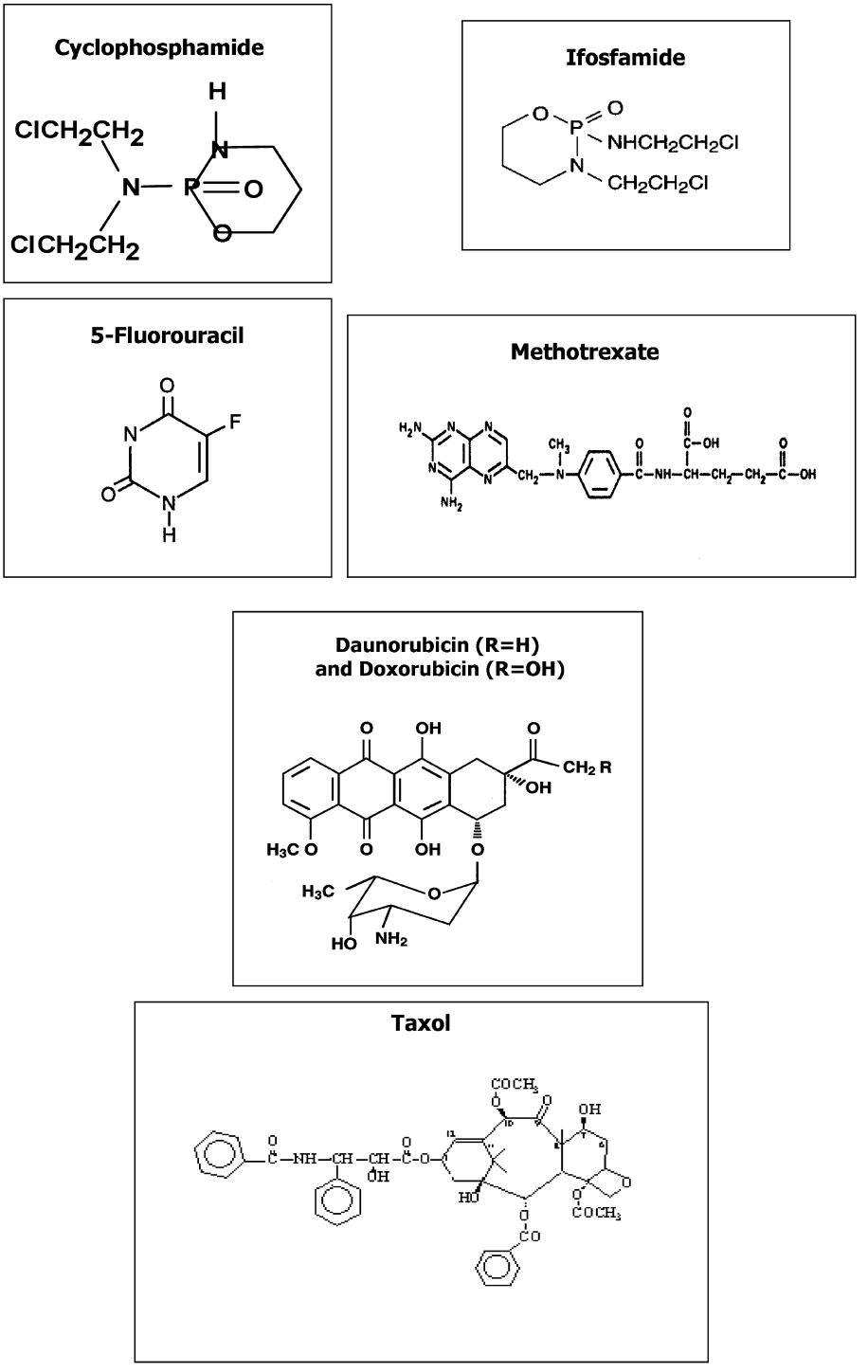
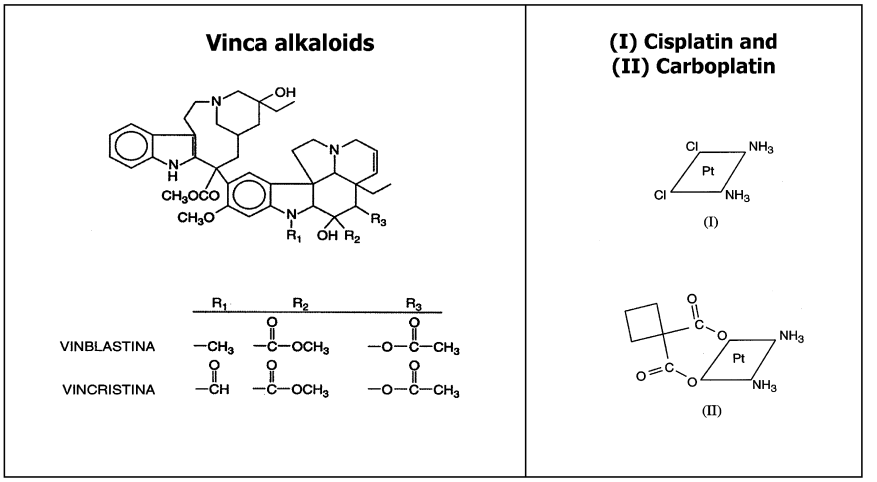


Fig. 1. Chemical structures.



(b)

Fig. 1. (*continued*)

2.2. Ifosfamide

Ifosfamide (IF), 3-(2-chloroethyl)-2-((2-chloroethyl)amino) tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide, is a structural analogue of cyclophosphamide (CP) having alkylating activity after enzymatic activation to cytotoxic compounds. IF differs from CP in the transfer of one 2-chloroethyl group from the extracyclic to endocyclic nitrogen. This results in differences in the physicochemical properties of the two drugs and different pharmacological and toxicological properties.

The most important metabolic pathway is 4-hydroxylation generating 4-hydroxyifosfamide (4-OHIF), which exists in equilibrium with its tautomeric form aldoifosfamide. The latter compound can undergo oxidation by aldehyde dehydrogenase generating carboxyifosfamide, but can also be converted by β-elimination into acrolein and ifosfamide mustard, which is considered to be the major cytotoxic metabolite [47,52]. IF can also be deactivated in the liver by dealkylation of the 2-chloroethyl chains with the concomitant production of chloroacetaldehyde, which has been associated with the neurotoxicity observed in patients undergoing IF-therapies [53–55].

Usually IF is administered intravenously. It exhibits dose-dependent pharmacokinetics. The quote of unchanged drug in urine varied from 6 to 53%, greatly depending on the dose given. Approximately 73% of the metabolized drug is excreted renally [56–59].

Although IF has been shown to be carcinogenic in animals, there is no evidence for its carcinogenicity in humans and it is therefore included by the IARC in Group 3. Nevertheless, because of its structural resemblance to CP, IF may be considered a suspected carcinogen [3,5,60].

2.3. Methotrexate

Methotrexate (MTX), amethopterin or 4-amino-N^{10}-methylpteroylglutamic acid, is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis. MTX inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid and therefore exerts its chemotherapeutic effect by its ability to compete with folic acid in cancer cells resulting in folic acid deficiency within the cells. Normal cells are not immune from this effect of methotrexate either. As a result, it can cause significant side effects. The degree and severity of the side effects depend on the regimen of the administration of methotrexate. Most of these adverse effects can be either prevented or treated by using leucovorin

(folinic acid), which is normally administered 24 h after the methotrexate is given [61–63].

Renal excretion is the major route of MTX and metabolite elimination. Depending on the dosage and duration of intravenous infusion, from 60 to 95% of the administered MTX dose may be eliminated unchanged in the urine [64–67]. Its major metabolite is 7-hydroxymethotrexate (7-OHMTX), which is about 200-fold less cytotoxic than the parent compound [62,63].

MTX may be given by the intramuscular, intravenous, intra-arterial or intrathecal route. According to the IARC, MTX is not classifiable as to its carcinogenicity to humans (Group 3). However, it is mutagen and a human teratogen [3,5,68].

2.4. 5-Fluorouracil

5-Fluorouracil (5-FU), 5-fluoropyrimidine-2,4-dione, is an antimetabolite frequently used in the treatment of malignancies including cancers of the gastrointestinal tract, lung, and breast. It acts as a competitor of pyrimidine and needs to be converted to the nucleotide level so as to exert its effect. Several enzymes involved in pyrimidine metabolism are required for the conversion of 5-FU to nucleotides, which are incorporated into different RNA fractions [69–71].

The main portion of the drug is degraded in the liver. The metabolites are excreted as respiratory carbon dioxide and as urea, α-fluoro-β-alanine (FBAL), α-fluoro-β-guanidopropionic acid, and α-fluoro-β-ureidopropionic acid in urine. About 15% of the administered dose is excreted in urine as intact drug within 6 h. Approximately 80% is catabolized and excreted in urine mainly as FBAL [71–75].

5-FU is classified as Group 3 (not classifiable as to its carcinogenicity to humans) by the IARC based on inadequate evidence for carcinogenicity in humans and animals [3,5].

2.5. Taxol

Paclitaxel (TAX) or Taxol, tax-11-en-9-one, 4β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxy-4,10-diacetate-2-benzoate-13-(α-phenylhippurate), is a diterpene alkaloid isolated from the bark of the Pacific yew tree. Paclitaxel, an anti-microtubule agent, is the active component in Taxol, a clinically effective chemotherapeutic agent approved for the treatment of various cancers [76,77]. Microtubules control the migration of chromosomes during cell division (mitosis). Paclitaxel acts promoting the polymerization of microtubules, and consequently inhibits microtubule disassembly, arrests eukaryotic cell division, and causes DNA fragmentation which subsequently induces apoptosis, i.e., the destruction of proliferating tumour cells [78–81].

In addition to paclitaxel's recognized ability to inhibit cell division, it has been demonstrated that this drug inhibits several biological processes such as abnormal white blood cell activation, cell migration, angiogenesis and the production of enzymes responsible for tissue destruction [80,82].

Since it is highly lipophilic and insoluble in water, paclitaxel is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Each ml of sterile solution contains 6 mg paclitaxel, 527 mg of purified Cremophor EL-P (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol [83].

Less than 10% of the injected drug is excreted in urine within 24 h. In contrast, biliary excretion is thought to be the major route of elimination of taxol as is or taxol metabolites. Extensive tissue binding has also been suggested. From both in vitro and in vivo experiments it became clear that paclitaxel is extensively metabolized by cytochrome P450 enzymes. No metabolic products could be detected in plasma, while the three major metabolites (6α-hydroxypaclitaxel, 3′-*p*-hydroxypaclitaxel and 6α,3′-*p*-dihydroxypaclitaxel) have been detected in bile of rats and humans [82,84–86].

Due to its relatively recent use, so far it has not been possible to classify taxol as to its carcinogenicity, mutagenicity and teratogenicity. However, it has to be considered like the other potentially toxic compounds and therefore caution should be exercised in handling paclitaxel during its preparation and administration.

2.6. Platinum compounds

Cisplatin (*cis*-diaminedichloroplatinum[II]) and carboplatin (*cis*-diamine-1,1-cyclobutane-dicarboxylateplatinum[II]) are widely used in the treatment of

a variety of solid tumours [87]. The antineoplastic activity results from an interaction with the DNA. Carboplatin appears to be equivalent to cisplatin in efficacy but with less overall toxicity [88,89].

The IARC includes cisplatin in Group 2A (probably carcinogenic in humans). However, all platinum-containing cytostatic drugs are mutagenic in vitro, teratogenic and carcinogenic in animals [5].

To assess the extent of exposure of hospital personnel involved in the preparation and administration of antineoplastic drugs containing platinum, the excretion of platinum in 24-h urine was measured [90].

2.7. Vinca alkaloids

Vinca alkaloids, including vincristine, vinblastine, vindesine and vinorelbine, derive from the periwinkle plant and are widely used antineoplastic drugs, either as single agents or in combination with other drugs [91–94].

They all work by inhibiting mitosis in metaphase. These alkaloids bind to tubulin, thus preventing nuclear microtubule formation. They cause metaphase arrest and stop chromosome segregation.

These alkaloids also seem to interfere with the synthesis of DNA and RNA. They are all administered only intravenously in their sulphate form [95].

Both vincristine (VCR) and vinblastine (VBL) are vesicant and are widely distributed in the body, thus affecting both cancerous and noncancerous cells. They are metabolized in the liver and excreted mostly in the bile and to a lesser degree in the urine (about 10%) [96].

Vincristine and vinblastine are mutagen and teratogen, but there is no evidence of carcinogenicity in humans. For this reason, the IARC includes them in Group 3 (not classifiable as to their carcinogenicity to humans) [5].

2.8. Anthracyclines

Anthracyclines are glycosidic derivatives of tetrahydronaphthacene that belong to the tetracycline class of antibiotics. Because of their cytotoxicity, they are mostly used as cytostatic agents, particularly doxorubicin (DOXO) and daunorubicin (DAUNO) [97,98].

The antineoplastic activity of these drugs has been mainly attributed to their strong interactions with DNA in the target cells. These drugs can deform DNA structure and terminate its biological function. Furthermore, they can be reduced to their semiquinone form by biological reducing agents, such as NADH and NADPH. Free radicals can be generated, which can attack cell components, such as membrane and DNA, and stop cell growth [99–101].

After intravenous administration, the anthracyclines are rapidly transported into most of tissues, where they accumulate, and are mainly metabolized in the liver. The main reaction is the reduction of the keto group on the C_{13} atom to a hydroxyl function. The resulting anthracyclinols show cytotoxic properties as well [99–101].

The anthracyclines are primarily eliminated with bile and also via the kidneys. About 5–20% of the anthracyclines are excreted unchanged in urine [97].

According to the IARC, these agents are probably carcinogenic to humans (Group 2A). There is inadequate evidence of carcinogenicity in humans, but sufficient evidence of carcinogenicity in animals [5].

3. Environmental monitoring

Hospital personnel may be exposed to antineoplastic agents by inhalation of drug dusts or droplets, which has been considered the main exposure route for a long time. As it results from more recent investigations, the main exposure route appears to be direct skin contact, especially through hands and forearms, as nurses and technicians often wear short-sleeves uniforms. Exposure may also occur by inadvertent ingestion, due to hand-to-mouth contamination or accidental events.

Since workers are generally exposed to a wide number of drugs, the first step is the choice of the most significant analytes. Firstly, the most toxic (Group 1 according to the IARC) and the most frequently used drugs should be taken into account, not to mention the sensitivity of the available analytical methods, which must be necessarily considered.

Some authors preferred to monitor and analyze just one or more ‘‘markers’’, on the basis of their

frequency of use. However, this can be done only in the first phase of a survey, since it may result in an underestimate of the risk.

The results of all the determinations are strongly influenced by the detection limit of the analytical methods used and consequently depend on the length of the working shift. For example, as regards air monitoring, if the preparation of the therapies takes 4 h and the sampling flow is 2.5 l/min, a total sampling volume of 600 l is obtained. In the presence of an airborne 5-FU concentration of 10 ng/m^3, 6 ng of the analyte should be detected on the filter, calling for a very sensitive analytical method. Actually, the length of the working time is often even shorter, since in accordance with guidelines, exposure time should be reduced as much as possible.

Moreover, few methods available in the literature have been completely validated, which prevents the scientists from working according to standardized and thus comparable procedures.

In order to assure an accurate risk assessment, the monitoring strategy should meet the following:

(1) contamination routes in working areas have to be identified;

(2) exposure routes in hospital personnel have to be identified and particularly intake by inhalation and skin absorption should be quantified;

(3) the effectiveness of the personal protective equipment and of biological safety cabinets (BSCs) should be verified with the aim of improving working conditions, i.e., reducing contamination to the lowest practicable level;

(4) contamination of other areas located next to the preparation and administration rooms should be controlled and possibly avoided;

(5) information about working activities should be collected so as to have a sort of photography of workplaces and to compare situations relative to different periods.

With regard to the effectiveness of biological safety cabinets (BSCs), use of vertical laminar air flow safety hoods is strongly recommended by guidelines published in all countries. However, good practice and appropriate maintenance are crucial points.

Moreover, a number of surveys have demonstrated that in most workplaces a background level of contamination can be measurable, even if care is taken and safety guidelines are adhered to.

Furthermore, it has been recently observed that vapours smaller than the pore size of the BSCs HEPA (high efficiency particle arrestor) filter can be produced during the preparation of the therapies, suggesting the possibility that these small droplets pass through the filter, contaminating the work area of a Class II, type A BSC (partial-exhaust). This means that Class II, type B BSCs (total-exhaust) are the minimum choice [102–104].

All these considerations must be taken into account when interpreting the results from the environmental monitoring, bearing in mind that the main objective is the implementation of working conditions.

Obviously, the results from the environmental monitoring should be combined with those from biological monitoring so as to identify the exposure routes and establish the extent to which workers are really exposed, provided that reliable analytical methods are available.

3.1. Sampling and storage

3.1.1. Air samples

During the preparation and the administration of cytostatic drugs, overpressure may result in the release of aerosols. In addition, the front opening of the BSCs might be a source of environmental contamination since it may cause leakage toward the worker and into the preparation area, especially if strong air drafts or frequent personnel traffic occurs. Finally, the effectiveness of the safety hoods is compromised if there is interference with the inward airflow through the work area access opening and if appropriate engineering controls are not maintained according to regulations and standards. As a consequence, determination of airborne particulate matter and aerosols is needed for the quantification of external exposure to cytostatic drugs. To this aim, air is sucked through a proper filter and then the filters are extracted and analyzed.

deWerk Neal et al. first provided data on ambient concentrations of antineoplastic agents in preparation areas. The objectives of this study were to determine the air concentrations of CP, IF, DOXO and MTX and evaluate handling practices with the aim of

minimizing workers exposure to cytostatics. The sampler consisted of a portable pump connected by a tube to an open-faced 47-mm cassette which housed a 47-mm, 0.5-μm pore PTFE filter. In the preparation room, the cassette was mounted at breathing-zone height so as to provide a reasonable measure of worker exposure [29]. Nevertheless, this cannot be considered to be a real personal sampling.

In order to assess the degree of protection offered by a BSC vented into the work area (type A), McDiarmid et al. [30] measured levels of 5-FU outside the safety hood according to the same technique used by deWerk Neal et al. [29]. However, a 37-mm filter and a constant flow-rate of 2 l/min instead of flow-rates varying from 1 to 4 l/min were used. The pump with the attached filter was located at breathing-zone levels, 30 cm from the safety cabinet [30].

Hansen and Wadden [105] tested the reliability of a method for monitoring airborne antineoplastic drugs. They took into consideration the possibility that the drugs may evaporate, sublimate or degrade on the filters during the sampling. Thus, they investigated the extent of sample loss for 5-FU and MTX and also evaluated the stability of the drugs while stored. Thirty-seven-mm glass-fiber filters were placed in closed-faced cassettes, and air was drawn through the filters at a flow-rate of 2–2.3 l/min. Sets of spiked and blank filters were stored for 1 day, 1 week and 3 weeks either at room temperature (25 °C) or in freezer (−21 °C). A set of filters spiked with MTX and blanks were stored also at 2 °C. No mass loss of 5-FU was found on filters stored for different times or at different temperatures. A small mass loss (about 5%) of MTX was observed after storage at 25 °C, in accordance with the manufacturers' recommendations that MTX has to be stored below 0 °C and with the observation that it is sensitive to hydrolysis, oxidation and light. With regard to the airflow experiments, it resulted that the amount of drug removed from the spiked filters was directly correlated to the cumulative volume of air sampled. Consequently, the authors suggested that the best technique would be to sample with a new filter on each 8-h shift but to extract all filters together with the same volume of mobile phase (see Section 3.2) as if they were one. This procedure increases the sensitivity of the method, allowing at the same time long sampling periods [105].

Pyy et al. first used personal samplings to identify potential exposure situations [31]. Samples were collected on 37-mm glass-fiber filters, pre-washed with cyclohexane and dried for 2 h at 250 °C. For stationary samples the flow-rate was 20 l/min, and the air sample volumes varied from 0.5 to 30 m^3. For personal samples the sampling rate was 2 l/min and the air sample volumes were 100–1000 l. Stability of CP, chosen as model compound, was also measured, and a decrease of 15% of the CP concentration on filter and 25% in the water extracts was observed in 8 days.

Similar techniques were used by Sessink et al. [17,18,23], McDevitt et al. [32] and Minoia et al. [25,33] within surveys carried out at several hospital departments. It should be also noted that airborne Pt (as a measure of contamination with cisplatin and carboplatin) was determined only by two authors [24,33] and that in both cases sampling was performed by using cellulose esters filters.

In Table 2, sampling conditions reported in the most significant studies available in the literature are summarized.

3.1.2. Wipe samples

In several surveys, wipe samples were taken from different surfaces and objects and analyzed for the presence of cytostatic drugs (Table 3).

Wipe sampling is very useful to evaluate the presence of residual contaminants in the workrooms and moreover the effectiveness of personal protective equipment and decontamination techniques.

A variety of materials were used as well as surface area, type of wetting solution and volume of desorption solution may change.

In order to assess surface contamination at several hospital departments, Sessink et al. [17] swept clean spots and objects by using tissues (Kleenex professional wipes, 20×21 cm) wetted with a 0.03 *M* sodium hydroxide solution. They used two tissues and 5 ml of the wetting solution for the boxes, drug vials/ampoules, chamber pots and urinals and four tissues and 10 ml of the above-mentioned solution for the floors, the working trays, the tables and the sink unit. With regard to the floors, the tissues were not wetted and the solution was pipetted on the floor.

Within a survey carried out in a large university hospital oncology pharmacy and outpatient department, McDevitt et al. collected individual samples

Table 2
Sampling methods for the determination of cytostatics in air

Analytes	P–S[a]	Type of filter	Ø (mm)	Pore size (μm)	Flow rate (l/min)	Refs.
CP, 5-FU, MTX, DOXO	S	PTFE	47	0.5	1–4	deWerk Neal, 1983 [29]
5-FU	S	PTFE	37	0.5	2	McDiarmid, 1986 [30]
CP	P	Glass fiber	37	–	2	Pyy, 1988 [31]
	S	Sartorius SM 13400		–	20	
CP, IF, 5-FU, MTX	P–S	Cellulose	50	0.45	23.5	Sessink, 1992 [17]
CP	P–S	Glass fiber	25	–	13–15	McDevitt, 1993 [32]
CP, 5-FU, MTX	P	PTFE	37	1.2	2	Sessink, 1994 [18]
	S	PTFE	50	1	23.5	
CP, 5-FU, MTX	P	PTFE	37	1.2	2	Sessink, 1997 [23]
Pt	P	Mixed cellulose	n.s.	0.8	2	Nygren, 1997 [24]
	S	esters			10	
CP, IF	P–S	Glass fiber	20	–	2	Minoia, 1998 [25]
CP, IF, 5-FU, MTX	P–S	Glass fiber	20	–	2	Minoia, 1999 [33]
Pt	P–S	Cellulose nitrate	20	0.8	2	

n.s., not specified.
[a] P, personal sampling; S, stationary sampling.

from an area of about 900 cm^2 using 25-mm Type A/E glass fiber filters saturated with a solution of 40:60 (v/v) methanol and water. Each sampling area was wiped only once using one face of the filter [32].

In a first survey, Minoia et al. [25] measured contamination of working areas by using for each sample five cotton gauzes soaked in ethyl acetate and surfaces of 225 cm^2 were swept clean. At the end of the sampling, each sample was stored in a Petri dish at +4 °C until analysis. During a second survey in the same hospital [33], the authors searched for a higher number of analytes (not only CP and IF, but also 5-FU, MTX, TAX and Pt) and used non-woven swabs wetted with a pH 7 buffer solution to sweep surfaces of 100 cm^2. In this respect, cotton gauzes are not recommended as they may release fibers which interfere with the extraction procedure. In addition, it has been verified that the type of the wetting solution does not influence on the recovery, since the contaminants are swept away from the surfaces independent of the composition or the pH of the solution itself. For this purpose, even distilled water could be used without affecting the results. The crucial step is instead the choice of the desorption solution (see Section 3.2).

Connor et al. [34] measured all the surfaces to be sampled in cm^2 and then a solution of sodium hydroxide 0.03 *M* was spread over the surfaces with a pipette (typically 20 ml for a 4900-cm^2 area). The surfaces were then wiped with one or two absorbent tissues. Uneven objects were sampled by applying 5 ml of the wetting solution to the tissue and wiping the surface. Samples were stored at −40 °C.

For the determination of MTX, 5-FU, cytarabin (CYA) and gemcitabin (GCA), Floridia et al. [35,36] thoroughly rinsed accurately measured surfaces with cotton swabs dipped in measured volumes of 0.1 *M* ammonium acetate solution (typically 20 ml for a 0.5-m^2 area).

As it can be noted, some authors measured the concentration of the contaminants relative to the size of the gauzes, some others (the majority of them) refer to the surface of the sampled area.

Obviously, this is not possible when sampling objects of different shapes. In this case, the results can be provided as an absolute amount on each object or on the wipe, even if they should not be considered to be quantitative data.

Recently, the results from a study aiming at identifying an acceptable method for measuring surface contamination have been published [106]. The main objectives were to find an effective material for wiping surfaces of different types (stainless steel, resins and vinyl) and to develop a method having acceptable absorption and desorption capabilities and sufficient sensitivity to detect the analytes

Table 3
Wipe sampling for the assessment of surface contamination

Analytes	Sampling location	Refs.
CP, 5-FU, MTX	Packings, boxes, chamber pots, urinals, floors, working trays of the hoods, tables, sink unit	Sessink,1992 [17]
CP	Inside BSC, floor, countertop, video display terminal, sink	McDevitt, 1993 [32]
CP, IF	Door-handle, working tray inside BSC, protection glass, floor, top side of BSC next to the HEPA filter	Minoia, 1998 [25]
CP, IF, 5-FU	Work surface inside BSC, top side of BSC airfoil, bottom side of BSC grille, floor in front of BSC, floor in preparation room, top of pushcart, floor outside preparation room	Connor, 1999 [34]
5-FU, CYA, GCA	Board and inside surfaces of the preparation hood, nearby floor, window panes, cupboards, drawer handles, telephones	Floridia, 1999 [35]
MTX	Board and inside surfaces of the preparation hood, nearby floor, window panes, cupboards, drawer handles, telephones	Floridia, 1999 [36]
CP, IF, 5-FU, MTX, Tax, Pt	Work surface inside BSC, top side of BSC airfoil, bottom side of BSC grille, floor in front of BSC, floor in preparation room, countertop, door-handle, fridge handle, under the shoes of the operators, telephone	Minoia, 1999 [33]
CP, IF, 5-FU, Pt	Floor in front of the BSC, floor in the preparation room's central area, bench-top surfaces, storage shelves, transport boxes and waste bins	Schmaus, 2002 [37]

at low concentrations. Cyclophosphamide, ifosfamide, doxorubicin, fluorouracil and taxol were tested. Two types of wipes materials were considered, a 55-mm diameter ash less cellulose circle filter (Whatman) and an 11.4×21.6-cm analytical wipe of 100% virgin wood fiber (Kimwipes). These ones were evaluated on stainless steel for all five drugs, but an additive in the wipes interfered with ifosfamide and, consequently, Whatman filters appeared to be the best choice. Nevertheless, some disintegration of these filters was observed during use.

Within a survey carried out in 14 hospital pharmacies in Germany, Schmaus et al. [37] tested for contamination with CP, IF, 5-FU and Pt (as a measure for cisplatin and carboplatin) by using blue ribbon filters, 90-mm diameter, as wipes. The filters were purified in ethyl acetate in a Soxhlet apparatus for 24 h and dried at room temperature. Sampling was performed by wiping in three different directions (down, left, and right) per spot, using one filter for each direction, to cover an area of 20×20 cm. The three filters were then placed in a 60-ml flask and kept in a freezer until analysis. Stability was also tested for all the drugs. CP and IF were stable for 4 days at 4 °C and 1 day at room temperature. No decline of 5-FU was observed after 1 week, meaning that samples can be stored at room temperature for 7 days before analysis. The stability of platinum-contaminated wipe samples stored at room temperature and 4 °C was good over a period of 6 days.

3.1.3. Pads

In order to evaluate the extent of exposure, Minoia et al. introduced the use of pads [25]. Each operator

wore 13 pads (11 if the workers made use of short-sleeved gowns), on the right and left arm over and under the gown, on the right and left leg over and under the gown, on the chest and on the back over and under the gown, and on the mask. Three cotton gauzes were used for each pad and the gauze size varied from 25 to 81 cm^2, depending on the part of the body monitored.

Within a following survey [33], lint-free swabs were substitute for the cotton gauzes, since cotton fibers may interfere with the analytes, requiring filtration. In addition, the size of all the pads used was of 100 cm^2.

These data provide a sort of map of the body contamination, thus allowing to identify the most crucial handling techniques and to verify the effectiveness of the safety hoods and the protective equipment.

3.1.4. Other matrices

Within a number of surveys carried out in hospital departments, gloves were commonly collected and analyzed for the presence of cytostatic drugs [17,18,23,25,33]. The analysis of both the external and internal side of the gloves worn during the preparation and administration of anticancer therapies enables to evaluate the potential dermal uptake deriving from these activities.

Besides, many studies were aimed at establishing the permeability of different materials to antineoplastic drugs [107–114]. In particular, vinyl, nitrile rubber, latex, polyurethane, and neoprene were compared and latex chemotherapy gloves appear to be the best choice. Nevertheless, it should be noted that the permeability of the gloves depends on the kind of drug, the contact time, and the glove thickness. That is why the use of a double pair of gloves is strongly recommended. Again, good practice is a crucial point.

In Table 4, the most significant investigations dealing with the evaluation of dermal uptake are reported.

Sessink et al. [17] collected a total of 20 pairs of latex gloves used during preparation of CP, 5-FU and MTX and cleaning of the hoods. The left and the right glove were analyzed together. During a following survey, the permeation of these gloves was determined by wearing cotton gloves under the latex gloves [18]. The two types of gloves were collected separately and the analysis of the cotton gloves was performed in the same manner as for the latex gloves. In 1997, an other investigation was carried out for a re-evaluation after additional protective measure [23]. In contrast to the previous studies, all the technicians used a double pair of latex sterile surgical gloves.

Minoia et al. [25] analyzed the internal side of the gloves for contamination with CP and IF. After use, the left and the right gloves were collected together. In order to test the permeability of the gloves, one subject wore a double pair of vinyl gloves. During the following survey [33], the internal side of the gloves was analyzed also for MTX, 5-FU, Pt and TAX.

In order to avoid contamination with other materials (e.g., the gloves), masks were collected after drug

Table 4
Evaluation of dermal uptake of cytostatic drugs

Analytes	Activity	Type of sample	Refs.
CP, 5-FU, MTX	Preparation of cytostatics in hospital departments	Gloves (latex)	Sessink [17]
CP, 5-FU, MTX	Preparation of cytostatics in hospital departments	Gloves (latex and cotton)	Sessink [18]
CP, 5-FU, MTX	Preparation of cytostatics in hospital departments	Gloves (latex), masks	Sessink [23]
CP, IF	Preparation and administration of cytostatics in hospital departments	Gloves (latex, vinyl)	Minoia [25]
CP, IF, 5-FU, MTX, TAX	Preparation and administration of cytostatics in hospital departments	Gloves (latex, vinyl)	Minoia [33]

preparation during the investigation carried out in 1997 by Sessink et al. [23]. The masks were packed in aluminium foil until analysis.

3.2. Sample preparation

deWerk Neal et al. extracted the filters with 1 ml of filtered distilled water acidified to pH 2.5 with phosphoric acid, and shaken for 30 min. [29]. Practically 100% of the drug was recovered for 5-FU, DOXO, and MTX, and about 75% for CP. The same procedure was followed by McDiarmid et al. for the analysis of 5-FU [30]. Hansen and Wadden used the same extraction solution, which was in fact the mobile phase for the chromatographic analysis, but the amount added to the samples varied from 2.5 to 4 ml. MTX was extracted with a 10:90 (v/v) acetonitrile–0.02 *M* buffer solution [105].

In order to test the analytical methods for the determination of CP in air samples, Pyy et al. divided the filters into halves [31]. One half was extracted with 1 ml sterile water, filtered and analyzed by HPLC. In this case the recovery of CP from filter resulted to be 97%. The other half was extracted with 5 ml dichloromethane, filtered, and evaporated to dryness. The dried residue was then reconstituted in 50 μl dichloromethane, to which *N*-(2-chloro-6-methylphenyl)-3-chloropropionamide as internal standard was added. Five μl of this solution were evaporated to dryness and analyzed by direct probe MS. The advantages of this method were the high sensitivity and the stability of CP in dichloromethane (at least 2 weeks).

According to the procedure described by Sessink et al. [17,18,23], the filters, the tissues used as wipe samples, and the gloves were put in glass pots containing a 0.03 *M* sodium hydroxide solution. The amount varied from 45 to 90 ml, depending on the size of the sample. After sonication (90 min) and shaking (10 min), the extracts were centrifuged before analysis of the supernatant for the presence of CP, MTX and 5-FU. The gloves were only shaken. The masks were cut into pieces before sonication and then extracted with 250 ml of sodium hydroxide solution.

The same procedure was followed by Connor et al. for the determination of CP, IF and 5-FU on working surfaces in six cancer treatment centres [34].

McDevitt et al. used 25-mm glass fiber filters for both air samples and wipe samples. All the filters were extracted with 2 ml of a 40:60 (v/v) solution of methanol and water and then filtered to remove particulate material [32].

Within both the surveys carried out by Minoia et al. in two Italian hospitals [25,33,115], filters, wipe samples and pads were extracted with distilled water adjusted to pH 7 (10 ml for filters and 30 ml for wipe samples and pads). After shaking, three 10- and 5-ml aliquots of ethyl acetate were added, respectively. The samples were vortexed, centrifuged, and 15 ml were evaporated to dryness. The dried samples were dissolved in 200 μl of mobile phase (see Section 3.3), mixed, and filtered before injection onto the chromatographic system. For the analysis of the internal side, each glove was filled with 200 ml of water adjusted to pH 7 and 10 ml were extracted with 10 ml of ethyl acetate. Finally, 5 ml were evaporated to dryness and the dried samples were treated as reported for wipe samples and pads. With regard to the determination of MTX [116] and 5-FU [117] in filters, sample enrichment was required, since the expected airborne levels are generally very low. Thus, a solid-phase extraction (SPE) procedure was developed, and Isolute C_{18} and ENV+ (styrene divinylbenzene) cartridges were used for MTX and 5-FU, respectively. In both cases, SPE tubes were conditioned with 6 ml of methanol and 6 ml of 0.02 *M* ammonium acetate buffer, but the pH of the buffer was different for MTX (pH 4.0) and 5-FU (pH 5.0). Five ml (10 ml for 5-FU) of the pre-treatment solution were then loaded on the SPE tube and the analyte was eluted either with three 1-ml aliquots of methanol (MTX) or of methanol–ethyl acetate (1:1, v/v). As regards the determination of Pt, 1 ml of the pre-treatment solution was diluted with nitric acid 1% (v/v) and, after addition of ^{193}Ir as internal standard, was injected onto the instrumental system. The filters were treated with 2 ml with nitric acid 65% and after 4 h, the samples were treated in the same manner as the wipes [33,118].

Platinum in workroom air was also determined by Nygren [24]. Each filter was divided into equal pieces, which were placed in silica crucibles. After addition of 100 μl of nitric acid, the samples were

heated on a hotplate to dryness and then ashed in a muffle furnace according to the following temperature program: 200 °C for 60 min, 250 °C for 30 min, 350 °C for 75 min, 425 °C for 45 min and finally 800 °C for 150 min. After cooling, 1.5 ml aqua regia was added and the next morning, the remaining aqua regia was slowly heated to dryness. Finally, 600 μl HCl was added and the crucibles were transferred to the measuring cell.

For the assessment of occupational exposure to MTX, 5-FU, cytarabine and gemcitabine, Floridia et al. [35,36] measured the surface contamination in hospital departments by using cotton swabs dipped in known volumes of an 0.1 *M* ammonium acetate solution. The liquid was then collected from the swollen swabs by thoroughly pressing them in a plastic syringe and measuring the volume of the recovered solution. As regards determination of 5-FU and nucleoside analogues, all washings were analyzed as such, after adding an appropriate amount of internal standard to a 1 ml sample. With respect to MTX, some environmental extracts required enrichment before analysis. For this reason, a pre-concentration solid-phase extraction (SPE) procedure was developed. RP-18 cartridges were activated with 3 ml of methanol and 3 ml of water. After filtration through filter paper, a volume of 20–100 ml of sample was loaded onto the cartridge. The analyte was eluted with 2×1-ml portions of a freshly prepared methanol solution containing 1% (v/v) aqueous ammonia. The dried extracts were then reconstituted with 1 ml of aqueous solution of the internal standard (aminoptherin) before analysis.

Within the study carried out by Larson et al. for the identification of an acceptable method of evaluating surface contamination, tests of various blends showed that the most effective desorption solution was a mixture of 10% acetonitrile, 25% methanol, and 65% Milli-Q water buffered to pH 6.0. This mixture provided desorption rates of nearly 100% for cyclophosphamide, ifosfamide, fluorouracil, and taxol and of about 60% for doxorubicin. After sampling, 4 ml of desorbing solution was injected onto the surface of the filters, and the samples were shaken for 30 min. In order to remove fiber particulates, the desorbate was then removed with a syringe equipped with a 0.2-μm pore size filter and placed into a 15-ml test tube until analysis [106].

Schmaus et al. analyzed simultaneously the content of CP and IF in wipe samples by GC–MS. A derivatization procedure was therefore necessary. Thirty ml ethyl acetate were first added to each flask containing three filters used to wipe a spot. The flasks were shaken for 15 min and 10 ng of cyclophosphamide-d_6 as internal standard was added to 10 ml of the resulting solution. The solutions were dried and 100 μl of ethyl acetate and 50 μl of trifluoroethyl acetate were then added to each tube. The derivatization process lasted 30 min at 70 °C. Finally, the solvent was evaporated at room temperature, and the residue was dissolved in 100 μl of toluene. A very similar procedure was applied to 5-FU. Methanol was used as the organic solvent, and 5-chlorouracil was used as internal standard. Derivatization was performed by adding 100 μl of acetonitrile and 50 μl of *N-tert*.-butyldimethylsilyl-*N*-methyltrifluoroacetamide to the samples, which were then heated for 15 min at 70 °C. For platinum analysis, 20 ml of 0.5 N hydrochloric acid was added to the wipe samples and flasks were shaken for 1 h. One ml was removed and digested by ultraviolet radiation before analysis by voltammetry [37].

3.3. Analytical methods

3.3.1. Instrumentation

Instrumental techniques used for the determination of cytostatic drugs in environmental matrices vary depending on the sampling matrices and on the analytes. For the analysis of cyclophosphamide (CP), high-performance liquid chromatography with ultraviolet detection (HPLC–UV) was used [29,31, 32,119]. CP was also determined by gas chromatography coupled with mass spectrometry (or tandem mass spectrometry) after derivatization [17,18,23, 34,37]. More recently, high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS–MS) was applied to the determination of a number of cytostatic drugs (CP, IF, TAX, MTX), and higher sensitivity and specificity were obtained [33].

5-FU and MTX were generally analyzed by using HPLC–UV [17,18,23,29,30,34–36,105,117]. With respect to MTX, Turci et al. recently developed and validated a method that allowed the comparison between ultraviolet and tandem mass spectrometry

detection [116]. Schmaus et al. used GC–MS for the analysis of 5-FU [37].

As a measure of contamination with platinum-containing cytostatic drugs, Pt can be determined either by voltammetry [24,37] or by inductively coupled plasma mass spectrometry with an ultrasonic nebulizer [33,118].

In Table 5, the instrumental techniques used within the most significant surveys and the corresponding detection limits, are reported. Note that some authors provided the detection limit (LOD) of the analytical method, some others gave the limit of detection for each kind of sample.

3.3.2. Instrumental conditions

As regards determination of cyclophosphamide by HPLC–UV, a μBondapak C_{18} reversed-phase column was used and the mobile phase was 25% acetonitrile and 75% 0.12 *M* $NH_4H_2PO_4$ acidified with 0.2% H_3PO_4. CP was monitored at 195 nm using a variable-wavelength ultraviolet detector [29]. Other authors [31,119] used a 150×4.6-mm I.D. Spherisorb 3 ODS, and the flow-rate of the mobile phase (35% acetonitrile in water) was 1.5 ml/min. The wavelength was set at 193 nm. The instrumental parameters of the method used by McDevitt were not provided [32].

Sessink [17] analyzed CP by GC–MS on a 30-m DB-5 0.25-mm×0.25-μm column connected with a deactivated fused-silica retention gap (5 m×0.53-mm I.D.). The on-column injection mode was used with an initial injector temperature of 110 °C. After 1 min, the temperature was increased by 180 °C/min to 280 °C and after 8 min, it was decreased to the initial temperature by cooling with liquid carbon dioxide. With regard to the oven, the program temperature was 110 °C (1 min), 15 °C/min to 280 °C(5 min). The interface temperature was 280 °C and electron impact was used as ionization mode. IF was used as internal standard. Identification was carried out by the combination of full scan spectra and retention times. Quantification was performed on the selected ion fragment *m*/*z* 307.

Connor et al. [34] analyzed CP and IF by gas chromatography with tandem mass spectrometer detection on a GC–MS–MS system comparable to the above-mentioned GC–MS system used by Sessink [17].

Separation of CP and IF was performed by Schmaus et al. [37] on a GC–MS system with a DB-5 column after derivatization. The injector and interface temperatures were 200 and 300 °C, respectively. The oven program temperature was: 100 °C (1 min), 20 °C/min to 160 °C, 4 °C/min to 200 °C, 30 °C/min to 280 °C (3 min). For selected ion monitoring, the masses were set at *m*/*z* 307 (target ion) and 309 (qualifier ion) for CP and IF and at *m*/*z* 313 and 315 for the internal standard, which was cyclophosphamide-d_6.

CP and IF in environmental matrices were determined by Minoia et al. [25] by HPLC with tandem mass detection (HPLC–MS–MS). Chromatographic separation was performed on a 5-μm Hypersil C_8 BDS (15 cm×4.6 mm) column equipped with a 5-μm H5ODS pre-column. The mobile phase was a mixture of methanol–0.02 *M* acetate buffer, pH 4.0 (1:1, v/v), and the flow-rate was 1.0 ml/min under isocratic conditions. A volume of 10 μl was injected for all the samples. The triple quadrupole mass spectrometer operated in the positive-ion mode and was equipped with an atmospheric pressure ionization (API) source and an ion spray interface. The target ions were *m*/*z* 261.2 for both CP and IF, and the fragment ions were *m*/*z* 140.2 and 92, for CP and IF, respectively.

5-FU was determined by HPLC with UV detection. Samples extracts were injected onto a μBondapak C_{18} reversed-phase column with a mobile phase of 0.05% H_3PO_4 in water (pH 2.5) and a flow of 2.0 ml/min. Monitoring was carried out at 254 nm [29,30,105]. Sessink used a Nucleosil RP 18 (150×4.6 mm, 5 μm) and a 0.05 *M* sodium acetate buffer (pH 4.0) as mobile phase with a flow of 1.0 ml/min. The selected wavelength was 260 nm [17,34].

Within occupational hygiene studies carried out at Italian hospital departments [35], all separations were accomplished on a reversed-phase column system composed of a 10×4.6-mm I.D. guard column placed before a 33-mm Supelcosil LC_{18} analytical column, both packed with spherical 3-μm RP-18 bonded silica particles. The mobile phase was a 10 m*M* ammonium acetate aqueous buffer at pH 6.9. Fluorouracil and the other nucleoside analogues (cytarabin and gemcytabin) were detected by absorbance monitoring at 272 nm. The same columns

Table 5
Instrumental techniques used for the determination of cytostatic drugs in environmental matrices and limits of detection (LOD)

Refs.	Analyte	Instrumentation	LOD
deWerk Neal [29]	CP	HPLC–UV	120 ng/m^3 (air sample volume=3.68–18.32 m^3)
	5-FU	HPLC–UV	0.065 ng/m^3 (air sample volume=3.68–18.32 m^3)
	DOXO	HPLC–FLUOR	0.55 ng/m^3 (air sample volume=3.68–18.32 m^3)
	MTX	HPLC–UV	1.6 ng/m^3 (air sample volume=3.68–18.32 m^3)
McDiarmid [30]	5-FU	HPLC–UV	0.2 ng/m^3 (air sample volume=6.28–11.51 m^3)
Hansen and Wadden [105]	5-FU	HPLC–UV	25 ng/m^3 (after an 8-h sampling time)
	MTX	HPLC–UV	
Pyy [31]	CP	HPLC–UV	0.05 μg/m^3 (air sample volume=1 m^3)
Sorsa [119]	CP	HPLC–UV	0.05 μg/m^3 (air sample volume=1 m^3)
Sessink [17]	CP	GC–MS	5 ng/m^3 (air sample volume=5.55–6.73 m^3)
			0.02 ng/cm^2 (wipe on floors)
			0.01 ng/cm^2 (wipe on working trays)
			0.06 μg (wipe on drugs boxes and packings)
			0.1 μg/pair of gloves
Sessink [18]	CP		0.003–0.5 μg/m^3 (sampling time=6–80 min)
			0.08 μg/pair of gloves
Sessink [23]		GC–MS	0.02–0.15 μg/m^3 (sampling time=6–80 min)
			0.13 μg/pair of gloves
			0.2 μg/mask
Sessink [17]	5-FU	HPLC–UV	30 ng/m^3 (air sample volume=5.55–6.73 m^3)
			0.1 ng/cm^2 (wipe on floors)
			0.04 ng/cm^2 (wipe on working trays)
			0.3 μg (wipe on drugs boxes and packings)
			0.7 μg/pair of gloves
Sessink [18]	5-FU		0.2–2.2 μg/m^3 (sampling time=6–80 min)
			4.0 μg/pair of gloves
Sessink [23]		HPLC–UV	1.6–9.9 μg/m^3 (sampling time=6–80 min)
			2.0 μg/pair of gloves
			5 μg/mask
Sessink [17]	MTX	HPLC–UV	300 ng/m^3 (air sample volume=5.55–6.73 m^3)
			1.0 ng/cm^2 (wipe on floors)
			4.0 ng/cm^2 (wipe on working trays)
			3.0 μg (wipe on drugs boxes and packings)
			6.0 μg/pair of gloves
Sessink [18]	MTX		0.5–5.4 μg/m^3 (sampling time=6–80 min)
			20 μg/pair of gloves
Sessink [23]		HPLC–UV	3.8–24 μg/m^3 (sampling time=6–80 min)
			10 μg/pair of gloves
			13 μg/mask
McDevitt [32]	CP	HPLC–UV	0.06–0.30 μg/m^3 (air sample volume=19–45 m^3)
			0.003–0.025 μg/cm^2 (surface area sampled=100–900 cm^2)
Minoia [25]	CP	HPLC–MS/MS	2 ng/m^3 (air sample volume=0.5 m^3)
	IF	HPLC–MS/MS	1 ng/dm^2 (referred to wipe and pads size)
			0.02 μg/pair of gloves
Nygren [24]	Pt	Voltammetry	40 pg/filter
Floridia [35]	5-FU	HPLC–UV	4–30 μg/m^2 (depending on the surface area sampled)
	CYA	HPLC–UV	
	GCA	HPLC–UV	
Floridia [36]	MTX	HPLC–UV	About 1 μg/m^2

Table 5. Continued

Refs.	Analyte	Instrumentation	LOD
Connor [34]	CP	GC–MS/MS	0.1 ng/ml of extract
	IF	GC–MS/MS	
	5-FU	HPLC–UV	20 ng/ml of extract
Turci [116]	MTX	HPLC–MS/MS	1 ng/m^3 (air sample volume=0.5 m^3)
			2 ng/wipe
			10 ng/glove
Sottani [115]	TAX	HPLC–MS/MS	4 ng/m^3 (air sample volume=0.5 m^3)
			10 ng/dm^2 for wipe and pads
			0.2 μg/pair of gloves
Micoli [117]	5-FU	HPLC–UV	15 ng/m^3 (air sample volume=0.5 m^3)
			150 ng/wipe
			1.5 μg/glove
Minoia [33]	Pt	ICP-MS	4 pg/filter
			4 pg/dm^2 for wipe and pads
			0.4 ng/pair of gloves
Schmaus [37]	CP	GC–MS	1 ng/wipe (2.5 pg/cm^2 for a 20×20 cm^2 area)
	IF	GC–MS	
	5-FU	GC–MS	0.1 ng/wipe (0.25 pg/cm^2 for a 20×20 cm^2 area)
	Pt	Voltammetry	0.005 ng/wipe (0.01 pg/cm^2 for a 20×20 cm^2 area)

were used for the analysis of MTX, whereas the mobile phase was prepared by adding 4 ml of acetonitrile to 96 ml of 10 m*M* ammonium acetate aqueous buffer, acidified to pH 6. The selected wavelength was 310 nm [36].

As regards the method developed and validated by Micoli et al. [117] for the determination of 5-FU in environmental samples, chromatographic separation was accomplished on a LiChrospher 100 RP18, 250×4 mm, 5 μm and a guard column C_{18} (4×4 mm). The selected wavelength was 265 nm and the mobile phase consisted of a methanol–0.02 *M* ammonium acetate buffer, pH 4.7 (2:98, v/v).

Schmaus et al. [37] analyzed 5-FU by GC–MS after derivatization. A DB-5 column was used and the injector and interface temperatures were 200 and 300 °C, respectively. The oven temperature was the following: 100 °C (1 min), 10 °C/min to 200 °C, 50 °C/min to 280 °C (3 min). 5-Chlorouracil was used as internal standard. The monitored masses were *m*/*z* 301 for 5-FU and *m*/*z* 317 for 5-CU.

With regard to the analysis of MTX, a μBondapak C_{18} was used and the mobile phase was 10 parts of acetonitrile mixed with 90 parts of 0.02 *M* monobasic ammonium phosphate acidified with 0.2% phosphoric acid. Monitoring was carried out at 313 nm [29,105]. HPLC–UV was also used by Sessink et al. [17]. The column was a 150×4.6-mm Nucleosil RP-18 and MTX was eluted with a mixture of 72.5% sodium acetate buffer (0.05 M; pH 4.0) and 27.5% methanol and analyzed at 300 nm.

In a more recent paper, a method using high-performance liquid chromatography coupled with tandem mass spectrometry, incorporating solid-phase extraction (SPE), was validated for the determination of MTX in air and wipe samples. Each step of the method was first developed using UV detection, and afterwards tandem mass spectrometry was used to obtain a lower limit of detection. As regards HPLC–UV analysis, a diode array detector was interfaced to an HPLC system. The chromatographic analysis was performed using a Lichrospher 100 C_{18} cartridge equipped with a C_{18} precolumn. The selected wavelength was 313 nm. The mobile phase consisted of methanol and 0.02 *M* ammonium acetate buffer, pH 4.0 (30:70, v/v). The HPLC–MS/MS system consisted of a triple quadrupole mass spectrometer interfaced to an HPLC system and separation was carried out on a Discovery C_{18} equipped with a guard column. The isocratic mobile phase was methanol–ammonium acetate, 0.02 *M*, pH 4.0 (30:70, v/v), delivered at a flow-rate of 1 ml/min. The target ions and the product ions were *m*/*z* 454.9 and 308.4 for MTX and *m*/*z* 471.0 and 324.1 for 7-OHMTX, used as internal standard [116].

The same HPLC–MS/MS system was used to

validate a method for the determination of taxol in environmental samples [115]. The analyses were achieved on a 5-μm Hypersil ODS 30×4.6-mm column. The mobile phase used for the chromatographic separation was acetonitrile with 0.1% formic acid at a flow-rate of 1 ml/min under isocratic conditions. 2-Methyl taxol was used as internal standard. The target ions for taxol and the internal standard were 854.4 m/z and 868.2 m/z, while the product ions were 286.2 m/z and 300.2 m/z, respectively.

As for Pt, the airborne concentration was determined with the use of a polarograph and a three-electrode measuring cell, equipped with a hanging mercury drop electrode as working electrode, an Ag/AgCl electrode as reference electrode and a glassy carbon rod as auxiliary electrode. The Pt concentration was determined at about −0.95 V [24]. The same technique was used by Schmaus et al. [37], although they determined Pt concentration at −0.85 V.

A novel method using ICP–MS was developed and validated by Ronchi et al. [118] for the determination of platinum as a measure of contamination with cisplatin and carboplatin in environmental samples. ^{195}Pt was identified as the isotope with the lowest number of interferences. Iridium was chosen as internal standard because it is very close to platinum as far as chemical and physical properties are concerned. The main instrumental parameters are reported in Table 6.

3.3.3. Recovery

Recovery was calculated by comparison of the instrumental responses of the aqueous solutions and those of the spiked matrices. In Table 7, recoveries of cytostatic drugs from environmental matrices are reported. Note that the ranges of concentration were often not specified by the authors.

3.3.4. Reliability of the methods

Since protective measures and safety precautions have been introduced, expected exposure levels became lower and lower. Determination of the analytes in different samples at trace and ultra trace levels is therefore necessary, as even very low concentrations may result in health hazard. For this reason, sensitivity is of utmost importance as well as specificity of the analytical methods is essential. Use of HPLC–UV assures good specificity as selected wavelengths are characteristic for each drug, while acquisition of specific ions is possible by using GC–MS. HPLC–MS–MS or ICP–MS further enhance both sensitivity and specificity for the analysis of a number of drugs in environmental matrices.

With regard to accuracy, it must be noted that no certified materials are available for antineoplastic drugs, so that standard addition method should be used for evaluating the reliability of the methods. Nevertheless, as it results from Table 8, few authors properly validated the analytical procedures. The majority of them did not measure either precision or accuracy, or specified the range of linearity. Only in more recent papers validation procedures using quality controls were reported. In most cases, precision (expressed as C.V.%) was calculated at one or two levels of the calibration curves. Some authors just carried out experiments in duplicate at one concentration level.

Table 6
Determination of platinum by ICP–MS: instrumental conditions (Ronchi [118])

ICP parameters		MS conditions		Acquisition	
Plasma RF power	1100 W	Bessel Box Barrel	52^+	Resolution	Normal
		Bessel Box Plate	44^+	Signal	Peak hopping
Plasma flow	12 l/min	Bessel Box Stop	33^+	Point across peak	1
		Einzel lens 1 and 3	26^+	Number of replicates	10
Auxiliary flow	0.8 l/min	Einzel Lens 2	130 V		
		Interface pressure	1 Torr	Sweep/replicate	3
Nebulizer gas flow	0.99 l/min	High vacuum pressure	1.4×10^{-5} Torr	Dwell time	300 ms

Table 7
Recoveries of the analytes from environmental matrices

Refs.	Analyte	Matrices	Range	Recovery
deWerk Neal et al. [29]	CP	Filters	Not specified	75%
	MTX			100%
	5-FU			100%
Pyy et al. [31]	CP	Filters	Not specified	97%
Hansen and Wadden [105]	MTX, 5-FU	Filters	Not specified	100%
McDiarmid [30]	5-FU	Filters	Not specified	95%
Sessink et al. [17]	CP, MTX, 5-FU	Filters, wipe samples, gloves	Not specified	Not specified
McDevitt et al. [32]	CP	Wipe samples	Not specified	85%
Minoia et al. [25]	CP, IF	Filters, wipe samples, gloves	1–5 ng/filter or wipe 0.5–50 μg/pair of gloves	>85%
Turci et al. [116]	MTX	Filters	3–24 ng/filter	80%
		Wipe samples	5–160 ng/wipe	88%
Micoli et al. [117]	5-FU	Filters	15–480 ng/filter	>94%
		Wipe samples	1–32 μg/wipe	>94%
Ronchi et al. [118]	Pt	Filters, wipe samples Gloves	Not specified	>90% >80%
Floridia et al. [36]	MTX	Wipe samples	99 μg/l	93%
Floridia et al. [35]	5-FU, CYA, GCA		Not specified	Not specified
Connor et al. [34]	CP, IF	Wipe samples	Not specified	Not specified
Sottani et al. [115]	TAX	Filters	3 and 30 ng/filter	>90%
		Wipe samples	10 and 40 ng/wipe	>90%
		Gloves	Not specified	>80%
Nygren et al. [24]	Pt	Filters	Not specified	>90%
Schmaus et al. [37]	CP, IF, 5-FU, Pt	Wipe samples	0.5–50 ng/glass plate	60–100%

4. Biological monitoring

In 1979, Falck et al. [13] first demonstrated that a potential health hazard may be associated with the handling of antineoplastic agents. Since then, several studies have been carried out concerning biological monitoring of subjects occupationally exposed to these compounds (Fig. 2). In order to evaluate the adverse effects of the simultaneous exposure to a wide number of drugs, biological endpoints were studied, such as urinary mutagenicity (UM), sister chromatid exchange (SCE), chromosomal aberrations (CA), thioethers excretion (TE), micronuclei (MN), hypoxanthine guanine phosphoribosyltransferase (HPRT), DNA damage (DNA), and *d*-glucaric acid (*d*-GA). Some of these methods (UM, SCE, CA) are well documented and have been used in several settings, some others (HPRT, DNA damage, *d*-GA) have been recently applied to the monitoring of professional exposure and thus their reliability is still to be demonstrated. Nevertheless, even the relatively well-established methods have drawbacks. For example, UM and TE are useful only during the excretion period (generally 1 or 2 days). In addition, many confounders are known, such as smoking, diet, use of other drugs or other chemical exposures, which means that these tests lack specificity and an overestimate of the risk is possible.

Table 8
Precision and accuracy of the methods for the determination of cytostatic drugs in environmental samples

Analyte	Range		Precision (C.V.%)	Accuracy (%)	Refs.
CP	Intra-day	Not specified	2–4	–	deWerk Neal [29]
CP	–	–	–	–	Pyy [31]
CP, IF	–	–	–	–	Sessink [17]
CP	–	0.25–2 μg/injection	–	–	McDevitt [32]
CP	Intra-day	Not specified	2.9	–	Connor [34]
	Inter-day		0.9	–	
IF	Intra-day	Not specified	14.6	–	
	Inter-day		6.9	–	
DOX	Intra-day	Not specified	2–4	–	deWerk Neal [29]
CYA	Intra-day	31.25–1000 μg/l	60.6 (*)	2.4% (*)	Floridia [35]
	Inter-day		94.5 (*)	−16.9% (*)	
GCA	Intra-day	31.25–1000 μg/l	94.6 (*)	86.4% (*)	Floridia [35]
	Inter-day		−0.5 (*)	−0.2% (*)	
5-FU	Intra-day	Not specified	2–4	–	deWerk Neal [29]
5-FU	–	Not specified	–	–	McDiarmid [30]
5-FU	Intra-day	0.025–250 μg/ml	0–1.5	–	Hansen and Wadden [105]
5-FU	–	–	–	–	Sessink [17,18,23]
5-FU	Intra-day	31.25–1000 μg/l	78.6 (*)	3.8% (*)	Floridia [35]
	Inter-day		97.1 (*)	−6.3% (*)	
5-FU	Intra-day	15–480 ng/filter	0–4.5	99–108.8	Micoli [117]
	Inter-day		3.3–10	96.9–110	
5-FU	Intra-day	Not specified	1.5	–	Connor [34]
	Inter-day		2.7	–	
MTX	Intra-day	Not specified	2–4	–	deWerk Neal [29]
MTX	Intra-day	0.025–250 μg/ml	0–1.5	–	Hansen and Wadden [105]
MTX	–	–	–	–	Sessink [17,18,23]
MTX	Intra-day	62.5–1000 μg/l	17.4 (*)	−8.1% (*)	Floridia [36]
	Inter-day		32.3 (*)	7.3% (*)	
MTX	Intra-day	1–32 ng/filter	8.3–14.3	95.6–123.9	Turci [116]
	Inter-day		21–22.9	90.9–124	
TAX	Intra-day	2–64 ng/filter	10–13	107–114	Sottani [115]
	Inter-day		7–19	97–124	

In contrast to biological endpoints, direct chemical analysis of the parent drugs or their metabolites assures higher sensitivity and specificity.

4.1. From biological endpoints to compound-selective analytical methods

Evaluation of urinary mutagenicity has been considered very useful in risk assessment studies for a long time. These tests rely on the ability of a compound to induce specific mutations in bacteria, such as *Salmonella typhimurium* or *Escherichia coli*, having deficiency in amino acid biosynthesis. After these bacteria are exposed to body fluids containing mutagenic agents, mutagenesis is measured by counting the number of mutations produced. A total of 29 studies using this method to evaluate possible adverse effects after exposure to cytostatic drugs are reported in the literature [13,15,119–145]. Of these, 14 showed positive tests associated with cytostatic drugs handling, and 15 had negative findings. However, a number of confounders are known, not to mention intraindividual and interindividual physiological variability and the importance of the time and the duration of urine collection in relation to drug exposure. Some studies [13,120,137] showed that if samples were collected at the beginning of the week, very low levels of drug were detected in urine. In

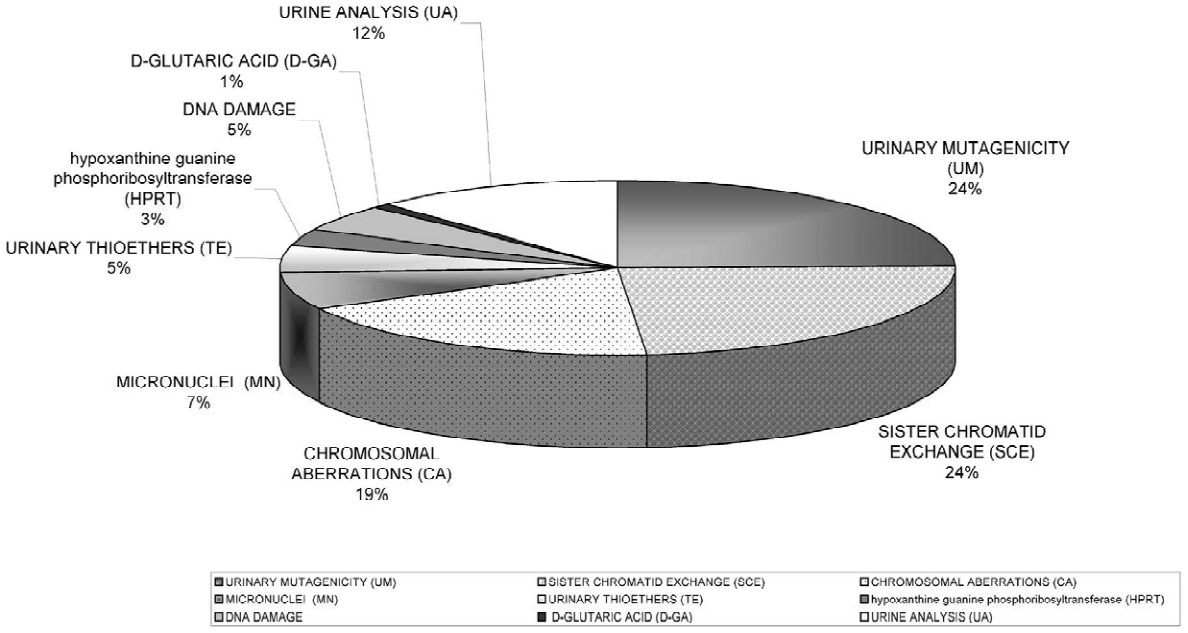


Fig. 2. Biological monitoring of the occupational exposure to antineoplastic agents (1979–2002).

most cases, concentration levels resulted to be even below detection limit. In essence, both sensitivity and specificity appear to be inadequate.

Another method used in the past to assess exposure to cytostatic drugs was urinary thioethers measurement [136,145–149]. The formation of thioethers derives from the main detoxification pathway of the alkylating agents, i.e., conjugation with glutathione. Two out of six studies had positive findings, and three had negative results. In one study, no significant differences between the exposed subjects and the controls were found. In addition, for exposed workers who smoked greater excretion was observed than for non-exposed workers who smoked [146].

Also the determination of *d*-glucaric acid, which is an index of mixed-function oxidase and β-glucuronidase activity, may provide information about cytostatic drugs exposure. Nevertheless, no data relating *d*-glucaric acid levels and exposure to anticancer agents are available. Only one study was carried out using this method, and no significant differences between oncology nurses and the control group were observed [136].

In order to assess mutation frequencies at the hypoxanthine-guanosine phosphoribosyltransferase (HPRT) locus in lymphocytes, Albertini et al. [150] developed a clonal assay. This may be an indicator of exposure to genotoxic compounds. In four studies, use of this technique for the biomonitoring of subjects exposed to antineoplastic drugs was reported [151–154]. Two had positive results, one had negative findings and one was equivocal. Since a limited number of studies are available, it is still difficult to establish the utility of this test.

A number of methods may be used to measure DNA damage directly, e.g., alkaline elution or the Comet assay. However, these techniques were applied to the biomonitoring of hospital personnel exposed to cytostatic drugs in a limited number of studies [155–160]. An excess of DNA strands was found in nurses handling antineoplastic agents without adequate safety precautions. After the introduction of proper safety equipment, no significant differences were observed between exposed personnel and controls [155].

With regard to cytogenetic methods, sister-chromatid exchanges (SCE), chromosomal aberrations (CA), and micronuclei (MN) are the most frequently used. Of the 28 studies assessing exposure to cytostatic drugs using SCE [119,121,132,133,

138,143–145,149,153,156,157,159,161–176], 12 provided positive results, 13 had negative findings and three were equivocal.

Several studies showed an increase in chromosomal damage in the lymphocytes of hospital personnel handling cytostatic drugs. Fourteen out of 22 studies [133,138,142–144,149,153,156,162–164,170, 172,174,175,177–183] had positive findings, seven showed no association between exposure and chromosomal aberrations, one provided inconclusive results. Within the survey carried out by Sessink et al. in 1994 [183], no correlation between CA and urinary excretion of cyclophosphamide was found.

Increased numbers of micronuclei were observed in lymphocytes of groups of workers potentially exposed to antineoplastic drugs [119,142,145,147, 163,166,173,174,177,184–187]. In general, the results indicated the presence of genotoxic damage in hospital personnel working with no or inadequate safety equipment. In contrast, from the findings of other four surveys, it appeared that micronuclei rates were not significantly different at various sampling time points and no correlation between urinary excretion and MN frequency was observed. One study resulted to be equivocal, since increased number of micronuclei was observed in binucleated lymphocytes of the worker groups as compared with controls, but the trend appeared to be statistically nonsignificant [119].

The effects registered by using SCE and CA are cumulative and age is a confounder when using MN [188]. In addition, these methods are non-selective and time consuming. It can be therefore concluded that these endpoints should be used at most in combination of other monitoring strategies.

4.2. Determination of the unchanged compounds or of their metabolites

The determination of parent drugs or their metabolites in biological fluids (blood, urine, etc.) is the recommended approach for assessing occupational exposure to antineoplastic agents, provided that highly sensitive analytical methods are used. Moreover, precision and accuracy of the methods should be measured. Since no reference certified materials are available, accuracy can be measured by using the standard addition method. A calibration curve should be prepared by spiking blank blood or urine with the analyte of interest and recovery should be calculated in the linearity range of this curve.

Analyses were often carried out just for one or two drugs, considered as model compounds. Nevertheless, it must be taken into account that physical and chemical properties, and metabolic pathways are different for each drug and thus determination of a wider number of compounds would be more representative for risk assessment. At least the most cytotoxic drugs according to the IARC (Groups 1, 2A, and 2B) should be considered.

In addition, no Official Methods are available to date, except for anthracyclines [189], and only a few analytical procedures were properly validated.

From Table 9, it results that the first study concerning the determination of a cytostatic drug (CP) in urine was published in 1984 [14] and analysis was performed by gas-chromatography equipped with a nitrogen-phosphorus detector (NPD). The detection limit was 250–300 ng CP in 24-h urine samples. The analyte was extracted in ethyl acetate and derivatized with trifluoracetic anhydride.

Since then, a lot of methods were developed for the determination of urinary CP and the most of them made use of gas chromatography with mass spectrometric detection [16,17,26,28,190]. Both Evelo et al. [16] and Ensslin et al. [19] purified the samples by using XAD-2, but the former used GC–MS while the latter used GC–ECD. Tandem mass detection coupled with either GC [191] or HPLC [192] further increased sensitivity and specificity. Moreover, if using HPLC–MS–MS, a simple and fast procedure for the preparation of samples allows to avoid derivatization. In addition, some authors determined CP and IF simultaneously [17,19, 28,191,192]. HPLC–UV was used also for the determination of CP and IF in human plasma [193].

With respect to the determination of urinary MTX, the minimum detection limit (0.2 μg/l) was obtained by using HPLC–MS–MS after solid-phase extraction [194]. Previously developed methods provided detection limits comprised between 4 and 4.54 μg/l [195].

The main metabolite of 5-FU, α-fluoro-β-alanine (FBAL) was determined by Sessink et al. [196], but the limit of detection was inadequate (60 μg/l). A

Table 9
Methods for the determination of cytostatic drugs in biological fluids

Analyte/Matrix	Extraction	Derivatization	Purification	Instrumentation	Refs.
CP/urine	Ethyl acetate	TFAA	–	GC–NPD	Hirst, 1984 [14]
Pt/urine	–	–	–	GF-AAS	Venitt, 1984 [15]
CP/urine	Ethyl acetate	TFAA	XAD-2	GC–MS	Evelo, 1986 [16]
CP, IF/plasma	Elution solvent AcCN–pH 4 buffer (40:60, v/v)	–	SPE (cyclohexyl-silica)	HPLC–UV	Burton and James, 1988 [193]
CP, IF/urine	1. Ethyl acetate 2. *n*-Hexane	TFAA	–	GC–MS	Sessink, 1992 [17]
CP/urine	1. Ethyl acetate 2. *n*-Hexane	TFAA		GC–MS	Sessink, 1994 [18,23]
CP/urine	Et_2O	TFAA	–	GC–MS (ion trap)	Sessink, 1993 [190]
MTX/urine	Elution solvent: MeOH	–	SPE (phenyl-silica)	HPLC–UV	Mader, 1993 [193]
CP, IF/urine	Ethyl acetate	TFAA	XAD-2	GC–ECD	Ensslin, 1994 [19]
Pt/urine		(UV photolysis)		Voltammetry	Ensslin, 1994 [20]
Pt/urine and blood		(UV photolysis)		Voltammetry	Nygren, 1997 [24]
FBAL/urine	Ethyl acetate	*S*-ETFA+ *n*-ButOH	–	GC–MS/MS	Bos, 1998 [197]
TAX/plasma	Elution solvent: AcCN–TEA (1000:1, v/v)	–	SPE (CN)	HPLC–MS/MS	Sottani, 1998 [198]
CP, IF/urine	Ethyl acetate	–	–	HPLC–MS/MS	Sottani, 1998 [192]
Pt/urine	(dilution with HNO_3 1%, v/v)			ICP-MS	Ronchi, 1998 [118]
Pt/plasma	(dilution with HNO_3 1%, v/v)				
CP, IF/urine	Et_2O	HFBA	–	GC–MS/MS	Sannolo, 1999 [191]
CP/urine	Et_2O	TFAA	–	GC–MS/MS	Burgaz, 1999 [26]
Anthracyclines/ urine	(acidification and direct injection with column switching)			HPLC–Fluorescence	DFG, 1999 [189]
MTX/urine	Elution solvent: MeOH	–	SPE (C_{18})	HPLC–MS/MS	Turci, 2000 [194]
CP, IF/urine	TBME	TFAA	–	GC–MS	Pethran, 2002 [28]
Pt/urine		(UV photolysis)		Voltammetry	

TFAA, trifluoracetic anhydride; *S*-ETFA, *S*-ethyltrifluoroacetate; *n*-ButOH, *n*-buthanol; AcCN, acetonitrile; Et_2O, diethyl ether; MeOH, methanol; TEA, triethylamine; HFBA, heptafluorobutyric anhydride; TBME, *tert*.-butylmethylether; GF-AAS, flameless atomic-absorption spectrophotometry.

10-fold increase in sensitivity was later obtained by Bos et al. [197] by using tandem mass detection. Note that these methods were applied to the monitoring of subjects working in producing factories. However, this limit should be further improved.

Inductively coupled plasma mass spectrometry was used for the analysis of urinary platinum as a measure of contamination with platinum-containing drugs [118] and a LOD of 2 ng/l was obtained. Voltammetry was used by other authors [20,22,24,28]. In a recent study published by Pethran et al. [28], a limit of detection of 1 ng/l for a 0.5-ml urine sample was reported.

Taxol [198] and anthracyclines [28,189] were also analyzed in biological matrices. The procedures and validation parameters are reported in Tables 9 and 10, respectively.

4.2.1. Sample collection and storage

Hirst et al. [14] collected urine samples from two nurses each morning at the beginning of their shift and after the administration to the patients. The

Table 10
Methods for the determination of cytostatic drugs in biological fluids: validation parameters

Analyte	Range	Precision (C.V.%)		Accuracy	Detection limit	Refs.
CP	–	–	–	–	250–300 ng/24-h urine	Hirst, 1984 [14]
Pt	–	–	–	–	0.6 μg/l urine	Venitt, 1984 [15]
CP	–	–	–	–	0.5 μg/24-h urine	Evelo, 1986 [16]
CP	5–50 μg/ml	Intra-day[a]	3.3%	104.3%	1 μg/ml (using	Burton and James,
		Inter-day[a]	6.1%	–	250 μl of plasma)	1988 [193]
IF	5–100 μg/ml	Intra-day[a]	2.0%	89.5%		
		Inter-day[a]	8.6%	–		
CP, IF	–	–	–	–	0.1 μg/l urine	Sessink, 1992 [17]
CP	0–100 μg/l	Intra-day	5.1%	–	0.25 μg/l urine	Sessink, 1993 [190]
		Inter-day	18%	–		
MTX	4–40 μg/l	Intra-day	4.1%	97.3%	4 μg/l urine	Mader, 1993 [193]
		Inter-day	4.8%	101.5%		
CP, IF	–	–	–	–	2.5 μg/24-h urine	Ensslin, 1994 [19,20]
Pt	–	–	–	–	4 ng/l urine	
Pt	–	<6%	–	–	5 ng/l urine	Nygren, 1997 [24]
					20 ng/l blood	
FBAL	12.7–381 μg/l	6.9%	–	–	6 μg/l urine	Bos, 1998 [197]
TAX	5–500 μg/l	Intra-day	<12%	97.9–110.3	1.7 μg/l plasma	Sottani, 1998 [198]
		Inter-day	<10.2%	102.8–110.6		
CP, IF	0–3.2 μg/l	Intra-day	<10.9%	96.3–106.3%	0.05 μg/l urine	Sottani, 1998 [192]
		Inter-day	<9.3%	98.8–102.6%		
CP	0.5–20 μg/l	–	–	–	0.1 ng/l	Burgaz, 1999 [26]
CP	0–20 μg/l	Intra-day[b]	<13.3%	90–105.5%	0.1 μg/l urine	Sannolo, 1999 [191]
		Inter-day[b]	<10.1%	92.2–98.2%		
IF	0–40 μg/l	Intra-day[b]	<13.4%	93–107.1%	0.5 μg/l urine	
		Inter-day[b]	<11.5%	93.8–102.5%		
Doxo	–	Intra-day	2.1	95–99%	4.5 ng/l urine	DFG, 1999
	–	Inter-day	2.3			(Grimm and Boos)
Epi	–	Intra-day	2.7	94–100%	6.1 ng/l urine	[189]
	–	Inter-day	2.5			
Dauno	–	Intra-day	8.1	91–97%	35 ng/l urine	
	–	Inter-day	9.3			
Ida	–	Intra-day	2.3	94–97%	11.5 ng/l urine	
	–	Inter-day	3.4			
MTX	0.5–16 μg/l	Intra-day	<16.2%	<111.9%	0.2 μg/l urine	Turci, 2000 [194]
		Inter-day	<14.7%	<109.9%		
Pt	0–3 μg/l	<10%		<101%	0.002 μg/l urine	Ronchi, 2000 [118]
CP	0–5 μg/l	4–9%		93–102%	0.04 μg/l urine	Pethran, 2002 [28]
IF					0.05 μg/l urine	
Pt	–	5–10%		93–104% (10 pg)	0.001 μg/l	

Doxo, doxorubicin; Epi, epirubicin; Dauno, daunorubicin; Ida, idarubicin.
[a] These values are calculated at the lower level of the calibration curve.
[b] These values refer to the relative standard deviation % (RSD%).

nurses took note of the times and quantities of cyclophosphamide (CP) handled. Urine samples were collected into bottles containing hydrochloric acid (3 ml, 0.1 N) and then kept below 5 °C for less than 24 h before analysis.

Within the studies carried out by other authors [16–20,23,25,26], 24-h urine samples were collected from the start of the working day. Excretion periods and excreted amounts were registered. Preparation and administration were noted as separate handlings by Evelo et al. [16]. The urine samples were stored at −20 °C until analysis [17–19,22,23,26].

Minoia et al. collected urine samples from exposed subjects in two Italian hospitals at the beginning and at the end of their work shifts. The samples were first collected in polypropylene bottles, then transferred to 25-ml containers and stored at −22 °C [25].

According to the procedure reported by Sannolo et al., the collection of 24-h urine samples from hospital personnel started soon after the handling of the drug. Then, urine samples were acidified with 5% HCl so as to minimize the proliferation of bacteria and a 50-ml aliquot was stored at −20 °C [191].

Within a survey carried out in German hospital pharmacies, 24-h urine sampling started at the beginning of the work shift after 3 working days. Samples were collected in polypropylene bottles and stored at −20 °C until analysis [28].

With regard to the DFG (Deutsche Forschungsgemeinschaft) method for the determination of anthracyclines in urine, samples were collected in plastic bottles and stored in the deep-freezer at −20 °C until processing. Acidification of the samples with hydrochloric acid (37%) to a pH value of 2.0–4.0 soon after collection, was suggested [189].

In order to evaluate contamination of urine samples obtained from nurses involved in the care of patients undergoing high-dose methotrexate (MTX) therapy, Mader et al. [21] collected the samples during the 36 h after the first possible contact with the drug. A 4-ml aliquot of each urine sample was stored at −20 °C until analysis. MTX was found to be stable in these conditions for at least 1 month.

In a study aiming at the evaluation of exposure to platinum-containing drugs, single urine samples were collected and stored at −86 °C in polyethylene bottles [15]. Nygren et al. collected urine samples in acid-washed plastic bottles and froze them prior to analysis [24].

4.2.2. Extraction and clean-up

For the determination of cyclophosphamide, urine was eluted through an amberlite XAD-2 column, after addition of ifosfamide (IF) as internal standard. Then, the column was washed three times with 5 ml of distilled water to eliminate the more hydrophilic components. Elution was performed with 10 ml of ethyl acetate. A further purification with ethyl acetate after addition of a saturated $NaHCO_3$ solution, and subsequently of 0.05 N HCl was carried out. The combined organic layers were evaporated under nitrogen and the residue was dissolved in methanol–water (90:10, v/v). This phase was washed with hexane and, after evaporation, trifluoracetic anhydride (TFAA) was added to the residue [14,16,19].

Sessink et al. extracted 5 ml of urine twice with 10 ml ethyl acetate. The organic layers were combined and washed first with a 10% sodium hydrogen carbonate solution and then with a 0.05 *M* hydrochloric acid solution. The derivatization was performed with trifluoracetic anhydride. A further extraction with hexane was carried out before evaporation and reconstitution in toluene. IF was used as internal standard for CP and vice versa [17,18,23]. The same authors also published a method for the determination of urinary CP that can be considered as more appropriate for routine analysis [190], which was also used by Burgaz et al. [26]. After liquid–liquid extraction with diethyl ether, derivatization was performed with trifluoracetic anhydride. This procedure allowed to reduce amounts of solvents during clean-up.

Cyclophosphamide (CP) and ifosfamide (IF) were extracted from urine with ethyl acetate after adjusting pH to 7 so as to maximize extraction efficiency. The extraction procedure was repeated twice and the organic layers were combined and evaporated. The dried residue was reconstituted with the mobile phase used for the HPLC analysis and injected [25,33,192].

According to the procedure reported by Sannolo et al., CP and IF extraction was performed with diethyl ether (2×20 ml) after adjusting pH to 8. The ether layers were then combined, evaporated, and the dried residue was dissolved in ethyl acetate before derivatization with heptafluorobutyric anhydride. Trophosphamide was used as internal standard [191].

Pethran et al. used *tert*.-butylmethylether (2×20 ml) for the liquid–liquid extraction of CP and IF. Before extraction, cyclophosphamide-d_6 was added as internal standard and pH was adjusted to 9. After evaporation, the organic layers were re-dissolved in ethyl acetate and derivatized with tri-fluoroacetic anhydride [28].

Solid phase extraction was used for clean-up of urine samples for the determination of methotrexate (MTX) [194,195]. A 1-ml aliquot of urine was

adjusted to pH 5.0 and then applied to a silica-bonded phenyl column (100 mg of sorbent) conditioned with 1 ml of methanol and 1 ml of water. The column was then washed with water and ethyl acetate (2 and 1 ml, respectively). The analyte was finally eluted with 2 ml of methanol, evaporated to dryness, and reconstituted with distilled water before injection onto the HPLC system [195].

Octadecyl-bonded, endcapped silica columns (500 mg of sorbent) conditioned with 6 ml of methanol and 6 ml of 0.02 *M* ammonium acetate buffer (pH 4.0) were used by other authors [194]. A 5-ml urine sample was diluted 1:1 (v/v) with acetate buffer and the entire volume was applied to the C_{18} tubes. Then, the cartridges were washed with 6 ml of ethyl acetate. MTX was eluted with three 1-ml aliquots of methanol. The eluate was redissolved in 200 μl of the mobile phase used for the HPLC analysis.

Untreated urine was analyzed for platinum by Venitt et al. [15].

According to the procedure described by Ensslin et al. [20] and Pethran [28], 1 ml urine, 5 ml ultrapure water, 100 μl sulphuric acid, and 200 μl hydrogen peroxide were irradiated in a 705 UV Digestor. After photolysis, the total amount was poured into a voltammetric vessel where 1 ml of supported electrolyte was added.

A method for the determination of platinum in blood and urine was proposed by Nygren et al. [24]. Samples were pipetted into silica crucibles and, after addition of nitric acid, were heated to dryness. Thereafter, the crucibles were placed in a muffle furnace and the samples were ashed. After cooling, aqua regia was added and the samples were left to stand overnight. The following morning, after heating to dryness, hydrochloric acid was added and the samples were transferred to the measuring cell.

A 1-ml aliquot of urine was diluted 1:4 with distilled water and acidified with nitric acid to 1% (v/v) before analysis by ICP–MS. ^{193}Ir was added as internal standard [118].

A solid-phase extraction procedure was described by Sottani et al. for the determination of taxol in plasma. Sep-Pak-CN cartridges (100 mg, 1 ml) were conditioned with methanol, water, and finally with a pH 5 buffer. A 0.5-ml aliquot of plasma was mixed with the same volume of acetate buffer and, after addition of 2′-methylpaclitaxel as internal standard, was loaded onto the cartridge. The washing step was performed with acetate buffer and methanol, and the analyte was finally eluted with acetonitrile–triethylamine (1000:1, v/v) into borosilicate tubes. The eluate was dried and reconstituted with 200 μl of acetonitrile [198].

In order to determine anthracycline cytostatic agents, the pH of the urine samples was adjusted to 2.0–4.0 using hydrochloric acid and, after centrifugation, an aliquot of the supernatant was transferred to a sample vial for subsequent analysis [28,189].

4.2.3. Instrumentation

Most of authors analyzed cyclophosphamide (CP) and ifosfamide (IF) in the urine of potentially exposed subjects by using gas chromatography coupled with nitrogen-phosphorus (NPD), electron capture (ECD) or mass spectrometry (MS) detection [14,16–19,22,23,28,190]. Recently, tandem mass spectrometry was also used [26,191]. The instrumental conditions are summarized in Table 11.

High performance liquid chromatography with UV detector (HPLC–UV) was employed for the determination of CP and IF in plasma. The column used was a 5-μm Merck LiChrosorb C_8 RP-Select B (25 cm×4.0 mm). The mobile phase consisted of acetonitrile–0.025 *M* phosphate buffer, pH 4.0 (25:75, v/v), and the flow-rate was 1.0 ml/min. The detector wavelength was set at 203 nm [193].

In order to enhance both sensitivity and specificity, a triple quadrupole mass spectrometer interfaced to an HPLC system was used for the determination of CP and IF in human urine. Analyses were achieved on a 5-μm Hypersil BDS C_8 column (150×4.6 mm). The mobile phase was a mixture methanol–0.02 *M* ammonium acetate, pH 4.5 (1:1, v/v), and the flow-rate was 1 ml/min. A volume of 10 μl was injected onto the chromatographic system. Peak-area ratios obtained from multiple ion-reacting monitoring (MRM) of CP (*m*/*z* 261.2 and 104.2) and IF (*m*/*z* 261.2 and 92.0) were used for quantification [192].

An HPLC method with column switching was developed by Mader et al. for the detection of traces of methotrexate (MTX) in urine. MTX was eluted isocratically with 1% acetonitrile from an anion-exchange column (Nucleosil 100 SB, 10-μm particles, 250×4 mm) and switched onto the analytical

Table 11
Gas chromatographic methods for the determination of cyclophosphamide in urine: instrumental conditions

Refs.	Hirst, 1984 [14]	Evelo, 1986 [16]	Sessink, 1992 [17]	Ensslin, 1994 [19]	Sannolo, 1999 [191]	Burgaz, 1998 [26]	Pethran, 2002 [28]
Apparatus	GC–NPD	GC–MS (EI mode)	GC–MS (EI mode)	GC–ECD	GC–MS–MS	GC–MS–MS	GC–MS (EI mode)
Injection volume	–	1 μl	–	1 μl	–	1 ml	2 μl
Flow/pressure	48 ml/min (nitrogen)	0.75 ml/min	14 p.s.i.	27 ml/min (nitrogen)	40 cm/s	14 p.s.i.	100 kPa
Injection mode	–	Splitless	On column	–	On column	On column	Splitless
Column	OV-17 on Chromosorb W-HP	Cross-linked dimethylsilicone (16 m, 0.2 mm, 0.3 μm)	DB-5 (30 m, 0.25 mm, 0.25 μm)	OV-1	DB-5MS (30 m, 0.25 mm, 0.25 μm)	DB-5MS (30 m, 0.25 mm, 0.25 μm)	DB-XLB (15 m, 0.25 mm, 0.25 μm)
Injector temperature	240 °C	250 °C	110–280 °C	300 °C	Cold	110–280 °C	250 °C
Column temperature	210 °C	80–185 °C	110–280 °C	190–280 °C	70–300 °C	110–290 °C	100–280 °C
Detector temperature	250 °C	270 °C	280 °C	–	275 °C	290 °C	280 °C
Specific ions	–	(SIM) *m/z* 307 *m/z* 309	*m/z* 307 abstracted from full scan spectra	–	Product ions: *m/z* 150, 212, 214	Product ion: *m/z* 212	(SIM) *m/z* 307 *m/z* 309

column (LiChrospher 100 RP-18e, 10-μm particles, 250 mm×4 mm). Acetonitrile was raised to 25% by a linear gradient to elute the analyte. The absorbance was measured at 310 nm [195].

A triple quadrupole mass spectrometry equipped with an atmospheric pressure ionisation source and interfaced to an HPLC system, was used to analyze urine samples for the presence of MTX [194]. The instrumental conditions were exactly the same reported for the determination of MTX in environmental samples (see Section 3.3.2).

The same HPLC–MS–MS system was used for the analysis of paclitaxel (TAX) in human plasma. The column was a 30-m×4.6-mm, 3-μm Hypersil ODS and the mobile phase was acetonitrile–0.1% formic acid 50:50 (v/v) delivered at 1 ml/min under isocratic conditions. Parent ions and product ions were *m/z* 854.4 and 286.2 for TAX, and *m/z* 868.2 and 300.2 for 2′-methylpaclitaxel, used as internal standard [198].

Voltammetry and inductively coupled mass spectrometry (ICPMS) were mainly employed for the determination of urinary platinum as a marker of exposure to platinum-containing antineoplastic drugs [20,24,28,118]. The main instrumental parameters for voltammetry and ICP-MS methods are reported in Tables 12 and 6, respectively.

An HPLC system with column switching and a fluorescence detector was used by Grimm and Boos [189] for the determination of the anthracycline cytostatic agents in urine. Enrichment of the sample (4 ml of urine) and separation from the matrix were carried out on a restricted access material phase. Then the analytes were transferred onto a reversed-phase column by means of an automatic switch valve, separated and quantified with fluorescence detection. The precolumn and the analytical column were, respectively, a LiChrospher RP-4 ADS (25×4 mm, 25 μm) and a LiChrospher RP Select B (250×4 mm, 5 μm). Different percentages of methanol, 0.015 *M* dipotassium hydrogen phosphate buffer (pH 7.5) and water, delivered at flow-rates varying from 0.5 to 1.5 ml/min, were used during the HPLC-integrated sample preparation. The mobile phase for the transfer to the analytical column was a mixture of acetonitrile and a pH 2.2 aqueous buffer (20:80, v/v). The analytical gradient separation was achieved by using different percentages of acetoni-

Table 12
Determination of urinary platinum by voltammetry: instrumental conditions

Refs.	Ensslin, 1994, 1997 [20,22] Pethran, 2002 [28]	Nygren, 1997 [24]
Working electrode	Hanging mercury drop	Hanging mercury drop
Auxiliary electrode	Glassy carbon	Glassy carbon
Reference electrode	Ag/3 *M* AgCl	Ag/3 *M* AgCl
Deaeration	300 s nitrogen bubbling and stirring	300 s nitrogen bubbling and stirring
Sweep	Differential pulse mode (−50 mV), 10 mV/s	Differential pulse mode (25 mV), 20 mV/s
Recording	−0.85 V	−0.95 V

trile and the aqueous buffer. The flow-rate during both transfer and analytical separation was 1.0 ml/min and the column was heated at 50 °C. With regard to fluorescence detection, excitation and emission wavelengths were set at 445 and 560 nm, respectively.

4.2.4. Recovery

Recovery was generally calculated by comparison of the instrumental responses of the aqueous/solvent solutions and those of the spiked matrices. In Table 13, recoveries of cytostatic drugs from urine and plasma are reported. Note that the ranges of concentration were often not specified by the authors.

4.2.5. Reliability of the methods

Rarely were the methods for the determination of antineoplastic agents in biological fluids as well as in environmental samples, properly validated (Table

Table 13
Recoveries of the analytes from urine and plasma

Refs.	Analyte/ Matrix	Range of concentration	Recovery (%)
Hirst et al. [14]	CP/urine	Not reported	Not reported
Evelo et al. [16]	CP/urine	2 μg/24-h urine sample	75±5%
Sessink et al. [17]	CP/urine	Not reported	Not reported
Ensslin et al. [19]	CP/urine	200 μg/200 ml	40.4%
Sottani et al. [192]	CP/urine	0.5–3.2 μg/l	85%
Sannolo et al. [191]	CP/urine	1–20 μg/l	80±5%
Sessink et al. [17]	IF/urine	Not reported	Not reported
Ensslin et al. [19]	IF/urine	200 μg/200 ml	80.6%
Sottani et al. [192]	IF/urine	0.5–3.2 μg/l	85%
Sannolo et al. [191]	IF/urine	1–20 μg/l	67±8%
Mader et al. [193]	MTX/urine	4–40 μg/l	98–102%
Turci et al. [194]	MTX/urine	0.5–16 μg/l	88%
Sottani et al. [198]	TAX/plasma	15–500 μg/l	85%
Venitt et al. [15]	Pt/urine	Not reported	Not reported
Ensslin et al. [20]	Pt/urine	Not reported	93–104%
Nygren et al. [24]	Pt/urine	Not reported	>90%
	Pt/plasma	Not reported	>90%
Ronchi et al. [118]	Pt/urine	0.25–0.75 μg/l	85–95%
	Pt/plasma	0.25–0.75 μg/l	85–95%
DFG	Doxorubicin/urine	10–500 ng/l	95–99%
(Grimm and Boos)	Idarubicin/urine	10–500 ng/l	94–97%
[189]	Daunorubicin/urine	10–500 ng/l	91–97%
	Epirubicin/urine	10–500 ng/l	94–100%

10). In particular, quality controls were introduced only recently.

Not only are sensitivity and specificity essential for risk assessment, but precision and accuracy should also be measured. Accordingly, standardization of the procedures is the main point for an accurate evaluation of potential health hazards.

5. Interpretation of the results

5.1. Environmental monitoring

With regard to environmental monitoring, Tables 14–18 depict the results from the most significant surveys carried out at several hospital departments from 1983 to 2002.

The number of positive samples relative to the total amount of analyzed samples is strongly influenced by the detection limit of the analytical method used and the working conditions. It is clear that introduction of safety equipment and precautions have gradually reduced contamination levels in work areas.

Moreover, from these results it can be concluded that inhalation is not the main exposure route, since the concentration of airborne cytostatic drugs resulted to be rather low (0.005–0.10 $\mu g/m^3$ is the range, but the majority of the samples were below the analytical detection limit). It should also be noted that the highest values generally related to accidental events or inappropriate engineering controls (BSCs should be periodically tested for the integrity of the HEPA filter and velocity of the airflows). Furthermore, use of vertical laminar-flow hoods and proper protective measures has been introduced recently.

In contrast, the analysis of the inner gloves or of the internal side of the gloves showed that dermal uptake was relevant. Permeation varied depending on the physicochemical properties of each drug, the contact time, and the glove thickness. Even though there is no ideal material for gloves yet, latex gloves specific for cytostatic drugs appear to provide good protection. Moreover, gloves should be changed every 30 min or immediately after a spillage or a puncture.

Also removal of contaminated gloves is to be considered as a critical step.

Surface contamination in working environments was observed by all authors. Wipe samples taken from different locations were found to be positive for a number of antineoplastic drugs. The presence of significant amounts of drugs was registered on working trays inside and outside the safety cabinets, floors, door handles, refrigerators, and even on the outside of vials received from manufacturers. Contaminants were also identified on shoe soles, which is a likely source of widespread contamination outside the preparation and administration areas. And what is more, this may result in an increase of the number of the exposed individuals.

As regards pads, arms, legs and chest were the most contaminated parts, which means that leakage from the inside of the hood to the environment occurred.

5.2. Biological monitoring

In Table 19, the results from biological monitoring of hospital personnel exposed to cytostatic drugs are reported.

Biological endpoints were widely used for evaluating cytogenetic effects in exposed subjects, but a number of confounders may interfere with the results. For this reason, the direct chemical analysis of urine (which can be obtained more easily than plasma) can be considered a more reliable tool for risk assessment.

Even when technical and personal protective equipment was used, a remarkable uptake of antineoplastic drugs was observed. However, the detection limit of the analytical method, the amount of drug handled and the interindividual differences in toxicokinetic parameters influenced the percentage of positive samples.

As far as urinary platinum levels are concerned, it must be noted that Pt can be found in the general population, so that it is difficult to establish the significance of the collected data. Moreover, sensitivity of the available analytical methods is rather high, and consequently the mean Pt level was often not different from the Pt level in the pooled reference urine. This could mean that a high number of positive samples is not necessarily an index of occupational exposure.

Table 14
Results from environmental monitoring of exposure to cyclophosphamide

Hospital Department	Matrix		No. of positive samples (%)	Range of concentration	Refs.
Oncology Out-patient Dept.	Air samples		1/14 (7.1%)	0.37 μg/m^3	deWerk et al., 1983 [29]
Hospital Pharmacy	Air samples		0/19 (0%)	nd	Pyy et al., 1988 [31]
Clinical Pharmacy Dept.	Air samples		0/2 (0%)	nd	Sessink et al., 1992 [17]
Clinical Pharmacy Dept.	Gloves		2/20 (10%)	0.1–21 μg/glove	
Outpatient Dept. (preparation)			0/11 (0%)	nd	
Clinical Pharmacy Dept.	Wipe samples (working trays of the hoods)		1/24 (4.2%)	0.1 ng/cm^2	
	Wipe samples (floor)		0/15 (0%)	nd	
Outpatient Dept. (preparation)	Wipe samples (working trays of the hoods)		1/12 (58.3%)	12 ng/cm^2	Sessink et al., 1992 [17]
	Wipe samples (floor)		0/9 (0%)	nd	
Outpatient Dept. (administration)	Wipe samples (floor)		5/120 (4.2%)	<0.02–4.5 μg/cm^2	
	Wipe samples (tables)		4/40 (10%)	0.2–4.5 μg	
Oncology Dept.	Wipe samples (dirty room)		1/12 (8.3%)	0.9 ng/cm^2	
	Wipe samples (patient rooms)		0/56 (0%)	nd	
	Wipe samples (urinals/chamber pots)		1/20 (5%)	8.3 μg	
Pharmacy	Air samples		3/34 (8.8%)	0.218–0.407 μg/m^3	McDevitt et al., 1993 [32]
Outpatient Dept.			0/39 (0%)	nd	
Pharmacy	Wipe samples		6/34 (18%)	0.005–0.035 μg/cm^2	McDevitt et al., 1993 [32]
Outpatient Dept.			6/42 (14%)	0.005–0.027 μg/cm^2	
Pharmacy	Air samples	P[a]	4/17 (23.5%)	1.0–10.1 μg/m^3	Sessink et al., 1994 [18]
		S[a]	1/17 (5.9%)	0.2 μg/m^3	
	Outer latex gloves		8/17 (47.1%)	1.5–9.6 μg/pair	
	Inner cotton gloves		5/8 (62.5%)	0.5–7.3 μg/pair	
Pharmacy	Air samples (P)[a]		2/45 (4.4%)	0.06–2.0 μg/m^3	Sessink et al., 1997 [23]
	Masks		10/45 (22.2%)	0.2–8.8 μg	
	Outer latex gloves		26/45 (57.8%)	0.13 –140 μg/pair	
	Inner latex gloves		7/45 (15.6%)	0.13–9.1 μg/pair	
Hospital A (five departments)	Air samples	P[a]	1/7 (14.3%)	0.24 μg/m^3	Minoia et al., 1998 [25]
		S[a]	0/13 (0%)	nd	
	Wipe samples		31/32 (96.9%)	0.002–82.4 μg/dm^2	
	Gloves	Prep.	5/6 (83.3%)	<0.02-63.4 μg/pair	
		Admin.	7/7 (100%)	0.633-11.8 μg/pair	
	Pads	Prep.	29/91 (31.9%)	0.02–113.98 μg/dm^2	
		Admin.	4/99 (4.0%)	0.02–0.5 μg/dm^2	
Hospital B (three departments)	Air samples		0/12 (0%)	nd	Minoia et al., 1998 [25]
	Wipe samples		17/17 (100%)	0.25–383.4 μg/dm^2	
	Gloves (preparation)		2/4 (50%)	17.5–47.9 μg/pair	
	Pads	Prep.	21/39 (53.8%)	0.004–23.61 μg/dm^2	
		Admin.	35/65 (53.8%)	0.001–0.53 μg/dm^2	

Table 14. Continued

Hospital Department	Matrix		No. of positive samples (%)	Range of concentration	Refs.
Pharmacy	Wipe samples (six cancer treatment centres)		46/46 (100%)	0.01–65.66 ng/cm^2	Connor et al., 1999 [34]
Administration area			33/36 (91.7%)	0.01–0.64 ng/cm^2	
Four departments	Air samples	P[a]	3/27 (11.1%)	5–20 ng/m^3	Minoia et al., 1999 [33]
		S[a]	3/12 (25%)	5–14 ng/m^3	
	Wipe samples		101/126 (80.2%)	0.001–966.1 μg/dm^2	
	Gloves (inner side)	Prep.	21/25 (84%)	0.03–13.61 μg/pair	
		Admin.	12/16 (88,9%)	0.04–1.37 μg/pair	
14 Pharmacies	Wipe samples		42/185 (22.7%)	max value 5.1 ng/cm^2	Schmaus et al., 2002 [37]

nd, less than detectable amount; Prep., worn during preparation; Admin., worn during administration.
[a] P, personal sampling; S, stationary sampling.

6. Conclusions

With regard to the occupational exposure to antineoplastic agents, environmental and biological monitoring are essential tools for risk assessment.

With the aim of identifying the main exposure routes (inhalation, dermal uptake or ingestion), a comprehensive sampling strategy can be summarized as follows:

(1) The first step is the choice of the analytes. The

Table 15
Results from environmental monitoring of exposure to ifosfamide

Hospital department	Matrix		Number of positive samples (%)	Range of concentration	Refs.
Hospital A	Air samples		0/20 (0%)	nd	Minoia et al.,
(five departments)	Wipe samples		22/32 (68.8%)	0.002–91.0 μg/dm^2	1998 [25]
	Gloves	Prep.	2/6 (33.3%)	6.5–60.1 μg/pair	
		Admin.	1/7 (14.3%)	3.9 μg/pair	
	Pads	Prep.	13/91 (14.3%)	0.11–298.7 μg/dm^2	
		Admin.	1/99 (1%)	0.056 μg/dm^2	
Hospital B	Air samples		2(P[a])/12 (16.7%)	20–47 ng/m^3	Minoia et al.,
(three departments)	Wipe samples		17/17 (100%)	0.01–141.6 μg/dm^2	1998 [25]
	Gloves (preparation)		1/3 (33.3%)	0.62 μg/dm^2	
	Pads	Prep.	7/39 (17.9%)	0.04–0.78 μg/dm^2	
		Admin.	4/65 (6.2%)	0.02–0.12 μg/dm^2	
Pharmacy	Wipe samples		24/32 (75%)	0.01–459.0 ng/cm^2	Connor et al.,
Administration area	(six cancer treatment centres)		10/20 (50%)	0.01–1.12 ng/cm^2	1999 [34]
Four departments	Air samples		0/39 (0%)	nd	Minoia et al.,
	Wipe samples		92/126	0.001–60.61 μg/dm^2	1999 [33]
	Gloves (inner side)	Prep.	5/25 (20%)	0.02–0.067 μg/pair	
		Admin.	2/16 (12.5%)	0.02 μg/pair	
14 Pharmacies	Wipe samples		28/185 (15.1%)	1.86 ng/cm^2	Schmaus et al., 2002 [37]

nd, less than detectable amount; Prep., worn during preparation; Admin., worn during administration.
[a] P, personal sampling; S, stationary sampling.

Table 16
Results from environmental monitoring of exposure to methotrexate

Hospital department	Matrix		No. of positive samples (%)	Range of concentration	Refs.
Outpatient Dept.	Air samples		0/14 (0%)	nd	deWerk., 1983 [29]
Clinical Pharmacy Dept.	Air samples		0/2 (0%)	nd	Sessink et al., 1992 [17]
Clinical Pharmacy Dept.	Gloves		5/20 (25%)	<6–49 μg/pair	
Outpatient Dept. (preparation)			2/11 (18%)	<6–40 μg/pair	
Clinical Pharmacy Dept.	Wipe samples (working trays of the hoods)		0/24 (0%)	nd	
	Wipe samples (floor)		0/15 (0%)	nd	
Outpatient dept. (preparation)	Wipe samples (working trays of the hoods)		0/12 (0%)	nd	
	Wipe samples (floor)		0/9 (0%)	nd	
Outpatient dept. (administration)	Wipe samples (floor)		0/120 (0%)	nd	
	Wipe samples (tables)		0/40 (0%)	nd	
Oncology Dept.	Wipe samples (dirty room)		0/12 (0%)	nd	
	Wipe samples (patient rooms)		2/56 (3.6%)	5.5–5.9 ng/cm^2	
	Wipe samples (urinals/chamber pots)		0/20 (0%)	nd	
Pharmacy	Air samples (P, S)[a]		0/34 (0%)	nd	Sessink et al., 1994 [18]
	Outer latex gloves		2/17 (11.8%)	220–1900 μg/pair	
	Inner cotton gloves		1/8 (12.5%)	63 μg/pair	
Pharmacy	Air samples		0/45 (0%)	nd	Sessink et al., 1997 [23]
	Masks		0/45 (0%)	nd	
	Outer gloves		1/45 (2.22%)	94 μg/pair	
Four preparation rooms	Wipe samples		20/34 (60%)	0.5–60 μg/m^2	Floridia et al., 1999 [36]
Four departments	Air samples	P[a]	1/27 (3.7%)	7 ng/m^3	Minoia et al., 1999 [33]
		S[a]	1/12 (8.3%)	10 ng/m^3	
	Wipe samples		30/125 (24%)	0.14–6.64 μg/dm^2	
	Gloves	Prep.	2/25 (8%)	0.023–0.033 μg/pair	
		Admin.	2/16 (12.5%)	0.024–0.135 μg/pair	

nd, less than detectable amount; Prep., worn during preparation; Admin., worn during administration.
[a] P, personal sampling; S, stationary sampling.

most significant drugs from a toxicological point of view and the most frequently used should be considered. Use of just one or two substances as ''markers'' should be avoided, since it can lead to an underestimate of the potential risk.

(2) Secondly, wipe samples should be used to measure contamination on working surfaces and objects (e.g., door-handles; working trays and protection glasses inside the hoods; the top of the safety hoods; top-counters in handling areas; adjacent floors, and in the administration areas around the patients' beds). On the basis of the results from this

Table 17
Results from environmental monitoring of exposure to 5-FU

Hospital department	Matrix		No. of positive samples (%)	Range of concentration	Refs.
Outpatient Dept.	Air samples		9/14 (64.2%)	0.12–0.082 μg/m^3	deWerk et al., 1983 [29]
Preparation area	Air samples		0/2 (0%)	nd	McDiarmid et al., 1986 [30]
Clinical Pharmacy Dept.	Air samples		0/2 (0%)	nd	Sessink et al., 1992 [17]
Clinical Pharmacy Dept.	Gloves		20/20 (100%)	19–87 μg/pair	
Outpatient Dept. (preparation)			8/11 (72.7%)	<0.7–140 μg/pair	
Clinical Pharmacy Dept.	Wipe samples (working trays of the hoods)		2/24 (8.3%)	0.2–0.5 ng/cm^2	
	Wipe samples (floor)		9/15 (60%)	0.2–10.7 ng/cm^2	
Outpatient Dept. (preparation)	Wipe samples (working trays of the hoods)		7/12 (58.3%)	0.2–1.8 ng/cm^2	
	Wipe samples (floor)		8/9 (88.9%)	0.5–3.1 ng/cm^2	
Outpatient Dept. (administration)	Wipe samples (floor)		High positivity (data not shown)		
	Wipe samples (tables)		3/40 (7.5%)	4.9–22 μg	
Pharmacy	Air samples (P, S)[a]		0/34 (0%)	nd	Sessink et al., 1994 [18]
	Outer latex gloves		11/17 (64.7%)	21–620 μg/pair	
	Inner cotton gloves		5/8 (62.5%)	130–760 μg/pair	
Pharmacy	Air samples		0/45 (0%)	nd	Sessink et al., 1997 [23]
	Masks		1/45 (2.2%)	15 μg	
	Outer gloves		9/45 (20%)	12–450 μg/pair	
Pharmacy	Wipe samples (six cancer treatment centres)		23/46 (50%)	0.72–208.6 ng/cm^2	Connor et al., 1999 [34]
Administration area			17/36 (47.2%)	0.7–15.1 ng/cm^2	
Preparation area	Wipe samples	Hoods	7/7 (100%)	89–111000 μg/m^2	Floridia et al, 1999 [35]
		Other surfaces	22/30 (73.3%)	20–11000 μg/m^2	
Four departments	Air samples	P[a]	3/27 (11.1%)	50-230 ng/m^3	Minoia et al., 1999 [33]
		S[a]	1/12 (8.3%)	43 ng/m^3	
	Wipe samples		78/125 (62.4%)	0.2–470.1 μg/dm^2	
	Gloves (inner side)	Prep.	17/25 (68%)	0.07–3.77 μg/pair	
		Admin.	10/16 (62.5%)	0.12–3.29 μg/pair	
14 Pharmacies	Wipe samples		25/37 (67.6%)	max value 1.4 ng/cm^2	Schmaus et al., 2002 [37]

nd, less than detectable amount; Prep., worn during preparation; Admin., worn during administration.
[a] P, personal sampling; S, stationary sampling.

cost-saving step of the survey, it is possible to identify the weak points of the whole system.

(3) Then, the effectiveness of the BSCs and the HEPA filters should be verified, also by means of area samplings. They are needed to measure airborne particulate matter inside the safety hoods; at the top

Table 18
Results from environmental monitoring of exposure to other cytostatic drugs

Analyte	Hospital department	Matrix		No. of positive samples (%)	Range of concentration	Refs.
Taxol	Four departments	Air samples		0/39 (0%)	nd	Minoia et al., 1999 [33]
		Wipe samples		65/126 (51.5%)	0.016–11.15 μg/dm^2	
		Gloves	Prep.	1/27 (3.7%)	0.29 μg/pair	
			Admin.	0/16 (0%)	nd	
Pt	Pharmacy Administration room	Air samples (three hospitals)		0/more than 300 samples	nd	Nygren et al., 1997 [24]
Pt	Four departments	Air samples	P[a]	3/20 (15%)	0.0069–0.0167 μg/m^3	Minoia et al., 1999 [33]
			S[a]	2/9 (22.2%)	0.0013–0.0044 μg/m^3	
		Wipe samples		126/127 (99.2%)	0.055–9.23 μg/dm^2	
		Gloves	Prep.	5/27 (18.5%)	0.02–0.193 μg/pair	
			Admin.	3/16 (18.8%)	0.016–0.018 μg/pair	
Cytarabin	Seven preparation areas	Wipe samples	Hoods	2/4 (50%)	30–14000 μg/m^2	Floridia et al., 1999 [35]
			Other surfaces	10/21 (47.6%)	17–2000 μg/m^2	
Gemcytabin	Seven preparation areas	Wipe samples	Hoods	2/2 (100%)	250–11200 μg/m^2	
			Other surfaces	1/4 (25%)	972 μg/m^2	
Pt	Four pharmacies	Wipe samples		147/147 (100%)	max value 2.7 ng/cm^2	Schmaus et al., 2002 [37]

nd, less than detectable amount; Prep., worn during preparation; Admin., worn during administration.
[a] P, personal sampling; S, stationary sampling.

of the hoods next to the HEPA (high efficiency particle arrestor) filters, and in the centre of the handling rooms.

(4) In addition, personal samplers can be worn by workers at breathing-zone level so that the intake of the drugs can be quantified. This step can be avoided if the percentage of positive wipe samples is very low and the amount of the drugs detected on the gauzes, is very close to the limit of quantification of the analytical methods.

(5) The analysis of the internal side of the gloves is necessary to verify if skin contact has occurred. Besides, pads may be worn by workers over and under the gown to see which part of their body is more contaminated and masks and clothes may be sometimes analyzed as well. This step is very important because dermal uptake is thought to be a major route of exposure.

(6) Urine samples from all exposed subjects must be collected at the beginning, at the end and possibly at the midpoint of the work-shifts. If even just a limited number of biological samples is found to be positive, handling practices should be controlled and revised as well as working conditions should be improved, making the required changes for the better.

(7) A database of the results from both environmental and biological monitoring should be built for each single unit, so that a comparison of situations associated with different working procedures can be made.

As regards biological monitoring, it must be emphasized that the analysis of parent drugs is usually preferred to that of their metabolites. This is mainly due to the fact that most of the metabolites of interest are not chemically stable (i.e., nitrogen mustard) and/or standard products are not commercially available. Besides, suitable compounds to be used as internal standards for validation procedures, are not easily available as well.

From an overview of the most significant surveys carried out from 1983 to 2002, it results that although safety precautions are taken, contamination still occurs.

On this respect, the effectiveness of the vertical, laminar airflow safety hoods has been discussed by several authors. Any interference with the inward airflow through the work area access opening may

Table 19
Results from biological monitoring of hospital personnel exposed to cytostatic drugs

Personnel	Analyte/ matrix	No. of positive samples (%)	Range of concentration	Refs.
Nurses	CP/urine	8/87 (9.2%)	0.35–9.08 μg/l	Hirst et al., 1984 [14]
Pharmacy technicians	CP/urine	5/20 (25%)	0.7 –2.5 μg/24 h	Evelo et al., 1986 [16]
Pharmacy technicians	CP/urine	4/18 (22.2%)	0.07 –0.5 μg/ excretion period[a]	Sessink et al., 1992 [17]
Nurses	CP/urine	3/5 (60%)	0.04–0.1 μg/ excretion period[a]	
Pharmacy technicians	CP/urine	8/9 (88.9%)	0.2–19.4 μg/24 h	Sessink et al., 1994 [18]
Pharmacy technicians	CP/urine	6/9 (66.7%)	0.2–2.6 μg/5 days	Sessink et al., 1997 [23]
Nurses/ Pharmacy technicians	CP/urine	12/13 (92.3%)	3.48–38.23 μg/24 h	Ensslin et al., 1994 [19]
Pharmacy technicians	CP/urine	2/13 (15.3%)	5–9 μg/l	Ensslin et al., 1997 [22]
Pharmacy technicians (Hosp. A)	CP/urine	4/12 (33.3%)	0.11–0.73 μg/l	Minoia et al., 1998 [25]
Nurses (Hosp. A)	CP/urine	3/18 (16.7%)	0.11-2.0 μg/l	
Pharmacy technicians (Hosp. B)	CP/urine	5/6 (83.3%)	0.66-2.1 μg/l	
Nurses (Hosp. B)	CP/urine	8/10 (80%)	0.11–1.7 μg/l	
Pharmacy technicians (Hosp. A)	IF/urine	2/12 (16.7%)	0.81–1.0 μg/l	Minoia et al., 1998 [25]
Nurses (Hosp.A)	IF/urine	1/18 (5.6%)	0.16 μg/l	
Pharmacy technicians (Hosp. B)	IF/urine	0/6 (0%)	–	
Nurses (Hosp. B)	IF/urine	0/10 (0%)	–	
Nurses	CP/urine	20/25 (80%)	0.02–9.14 μg/24 h	Burgaz et al., 1999 [26]
Pharmacy technicians Nurses	CP/urine	106/1415 (7.5%)	0.05–0.76 μg/l	Pethran et al., 2002 [28]
Pharmacy technicians Nurses	CP/urine	18/62 (29%)	0.05–10.03 μg/l	Turci et al., 2002 [27]
Nurses	IF/urine	1/8 (12.5%)	0.4 μg/excretion period[a]	Sessink et al., 1992 [17]
Pharmacy technicians Nurses	IF/urine	4/10 (40%)	5.05–12.74 μg/24 h	Ensslin et al., 1994 [19]

Table 19. Continued

Personnel	Analyte/ matrix	No. of positive samples (%)	Range of concentration	Refs.
Pharmacy technicians	IF/urine	0/5 (0%)	–	Ensslin et al., 1997 [22]
Pharmacy technicians Nurses	IF/urine	37/1415 (2.6%)	0.05–1.90 μg/l	Pethran et al., 2002 [28]
Pharmacy technicians Nurses	IF/urine	1/62 (1.6%)	0.153 μg/l	Turci et al., 2002 [27]
Pharmacy technicians	MTX/urine	3/5 (60%)	26–2348 μg/l	Mader et al., 1996 [21]
Nurses	MTX/urine	3/5 (60%)	12.8–27.3 μg/l[b]	
Pharmacy technicians Nurses	MTX/urine	0/62 (0%)	–	Turci et al., 2002 [27]
Pharmacy technicians Nurses	Pt/urine	10/10 (100%)	0. 6 –23.1 μg/l	Venitt et al., 1984 [15]
Pharmacy technicians Nurses	Pt/urine	14/52 (26.9%)	3.5–34.4 μg/l	Ensslin et al., 1994 [20]
Pharmacy technicians Nurses	Pt/urine	3/62 (4.8%)	0.92–1.30 μg/l	Turci et al., 2002 [27]
Pharmacy technicians Nurses	Doxorubicin/ urine	34/1752 (1.9%)	5–127 ng/l	Pethran et al., 2002 [28]
Pharmacy technicians Nurses	Epirubicin/ urine	45/1752 (2.6%)	10–182 ng/l	

[a] Excretion time (about 6 h) is intended to be the period from the start of the previous urine production to the end of this particular urine production. Time zero is the beginning of the working day.

[b] Maximal urinary concentration in urine.

result in contamination of the personnel and the workplace [38,39]. In addition, according to recent publications, vaporization of some antineoplastic agents at room temperature is possible and moreover it has been hypothesized that small droplets pass through the HEPA filter [102–104].

A dramatic reduction in the contamination inside the safety hoods can be provided by using closed, disposable syringe systems during reconstitution of the drugs [199]. An alternative may be the use of barrier isolators, especially when installing new equipment [200–202].

If sensitivity and specificity of the analytical methods, as well as the reliability of both the sampling and analytical procedures, are the prerequisites for an accurate risk assessment, adherence to safety standards and guidelines and use of proper protective equipment cannot be neglected. By comparing the results from different settings all over the world, it results that education and training of the exposed personnel must be implemented, since most of the contamination is due to poor technique. And furthermore, last but not least, common sense and the sense of responsibility of each single worker play a major role.

However, much can still be done. For example, cost- and time-saving procedures for the determination of a wider number of drugs should be developed and validated. In addition, since hospital personnel are usually exposed to mixtures of drugs,

simultaneous analysis of different compounds would be a significant step forward.

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