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A LC-MS/MS based methodology for the environmental monitoring of healthcare settings contaminated with antineoplastic agents

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Abstract

Background: Adverse health events associated with the exposure of healthcare workers to antineoplastic drugs are well documented in literature and are often related to the chemical contamination of work surfaces. It is therefore crucial for healthcare professionals to validate the efficiency of safety procedures by periodic biological and environmental monitoring activities where the main methodological limitations are related to the complexity, in terms of chemical-physical features and chemical-biological stability, of the drugs analyzed.

Materials and methods: Here we describe the evaluation and application of a UHPLC-MS/MS based protocol for the environmental monitoring of hospital working areas potentially contaminated with methotrexate, iphosphamide, cyclophosphamide, doxorubicin, irinotecan, and paclitaxel. This methodology was used to evaluate working areas devoted to the preparation of chemotherapeutics and combination regimens at the University Hospital "San Giovanni di Dio e Ruggi d'Aragona" in Salerno (Italy).

Results: Our analyses allowed to uncover critical aspects in both working protocols and workspace organization, which highlighted, among others, cyclophosphamide and iphosphamide contamination. Suitable adjustments adopted after our environmental monitoring campaign significantly reduced the exposure risk for healthcare workers employed in the unit analyzed.

Conclusion: The use of sensitive analytical approaches such as LC-MS/MS coupled to an accurate wiping procedure in routine environmental monitoring allows to effectively improve chemical safety for exposed workers.

Keywords

Environmental monitoring, chemical risk, antineoplastic agents, LC-MS/MS, work surfaces

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Introduction

Occupational exposure to hazardous or potentially toxic drugs is a major problem for several healthcare workers. In particular, handling of antineoplastic agents (AAs) is associated to severe health risks, due to their carcinogenicity, mutagenicity, teratogenicity and to their lack of specificity toward cancer cells.[1–3] Moreover, skin rashes, allergic reactions, nausea, vomiting, an increased occurrence of adverse reproductive outcomes and infertility have been associated to chronic exposure to AAs.[4–6] Although all these effects might be unfortunately expected for patients undergoing chemotherapy, they should not be tolerated in healthy subjects, such as healthcare workers involved in AAs handling.[7] Exposure to AAs has been recognized since the early 1970s as a potential risk to health professionals. In 1979, Falck et al.[8] first showed a significant increase in mutagenicity risk in urine samples collected from a staff of nurses assigned to the preparation and administration of AAs. Ever since, several studies have confirmed the occurrence of adverse health outcomes for workers exposed to AAs, including impact on pregnancy rate,[9–12] chronic[13,14] and acute effects.[15–17]

The most frequent routes of involuntary or accidental absorption are transdermal penetration and inhalation.[18] Engineering supports, personal protective equipment (PPE) and handling protocols have been continuously improved to reduce contaminations,[19] but the potential exposure to antineoplastic drugs cannot be completely avoided.[20–22] Pharmacists and pharmacy technicians, nursing personnel, physicians and operating room personnel working in areas where AAs are prepared are among the most exposed personnel.[18] Indeed, several manual steps are required to prepare infusion bags specifically designed for each patient, thus many opportunities for accidental assumption effectively occur. A significant reduction of the risk might undoubtedly be obtained by limiting environmental contamination and drug dispersion during the different steps of preparation.

A key point to appraise the correct application of procedures aimed at reducing workplace contamination is the availability of robust and accurate methodologies to monitor the work environment,[18] particularly for the laboratories hosting the units for cytotoxic drug preparation (UCDP).[23–26] Indeed, such a methodology should allow carrying out a reliable quantitative assessment of contaminations, to rapidly evaluate the effectiveness of any environmental adjustment adopted.

Here, we describe the development of a novel UHPLC-MS/MS based protocol for the environmental monitoring of AAs belonging to different categories, widely used in cancer therapy: methotrexate (MTX), cyclophosphamide (CFA), iphosphamide (IFA) doxorubicin (DXR), irinotecan (IRT), and paclitaxel (PTX). The analytical method was tested to verify selectivity, accuracy and sensitivity following current EMA guidelines.[27,28] Afterward, this methodology was used for the environmental monitoring of the UCDP at the University Hospital "San Giovanni di Dio e Ruggi d'Aragona" in Salerno (Italy), to evaluate handling procedures adopted to guarantee workers safety. Our environmental monitoring activities highlighted some criticisms in both working protocols and workspace organization; appropriate adjustments of these two aspects, which were carried out after our analysis, significantly reduced the exposure risk for healthcare workers employed in that unit.

Materials and methods

Materials and reagents

Standard drugs (MTX, CFA, IFA, IRT, DXR, and PTX) were European Pharmacopoeia (EP) reference standard, purchased from Sigma-Aldrich (St Louis, MO, USA). Solvents for pre-analytical sample treatments, water and ultra-pure solvents for LC-MS/MS analyses were from Romil (Cambridge, UK). Surface sampling kit consisted of a paper-wipe collection system and a wetting hydroalcoholic solution containing an internal standard (Italian patent no. 102019000007227). The chemical composition of the wetting solution was optimized to achieve a high recovery of the most widely used AAs and the presence of the standard allowed monitoring the correctness and efficacy of the surface sampling procedure (Italian patent no. 102019000007227).

UHPLC-MS/MS analysis

The LC-MS/MS apparatus was composed by a Ultimate3000 UHPLC system (Thermo-Fisher, Waltham, MA, USA) and a TSQ-Endura electrospray triple quadrupole mass spectrometer (Thermo-Fisher). A Phenomenex® Luna-Omega C18 column (50 mm × 1.0 mm; 1.6 μm) maintained at 40°C and a mobile phase composed of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) were used for chromatographic separation; total flow was set at 0.06 ml/min and a gradient from 20% to 40% of solvent B over 5 min was used. Mass spectra were acquired in positive selected reaction monitoring (SRM) mode using a ESI source, selecting two transitions for each compound (one, called quantifier, for quantitative analysis and the other, qualifier, to confirm the identification) to maximize selectivity and sensitivity. Ion transfer tube temperature and vaporizing temperature were set at 350°C and 100°C, respectively. Direct injections of pure compounds and standards were carried out to identify suitable transitions and optimal instrumental parameters for all the analytes considered in this study.

Standard solutions, surfaces preparation and sample collection

To obtain concentrated initial stock solutions, compounds (MTX, CFA, IFA, IRT, DXR, PTX) were initially dissolved in DMSO at a final concentration of 0.1 mg/ml;

working solutions were obtained by diluting stock solutions to the desired final concentration (typically 10 µg/ml) in DMSO. To optimize and validate the analytical method, 1 ml of each desired dilution, prepared in pure methanol, was poured over a 30 cm × 30 cm area (according to manufacturer) of a laminar flow hood using a glass pipette and air-dried for at least 2 h. Afterward, 2 ml of an appropriate solvent solution (from now on referred to as "recovery solution") were deposited on the surface analyzed, which was then rubbed using a paper-wipe. Wipes were transferred to a 50 ml conical centrifuge tube containing 7.5 ml of recovery solution; compounds extraction was obtained by sonicating the samples for 20 min at 25°C in an ultrasonic bath at 40 kHz. Samples were then centrifuged at maximum speed for 2 min at Room Temperature (RT); wipes were mechanically squeezed and the supernatants were transferred into a 2 ml autosampler vial.

Preparation of standards calibration curve and method validation

For the calibration curve, surfaces were spiked with 0.06, 0.11, 0.28, 0.56, 2.78, and 11.11 ng/cm^2 of MTX, CFA, IFA, IRT, DXR, PTX; the protocol previously described for wipe-sampling and compounds extraction was carried out. Cleaning procedure was also applied to a clean surface for comparison and to exclude any interference or false positive response derived from extractive procedure, reagents, or disposable material used. The carry-over was evaluated by analyzing a solvent aliquot immediately after a sample obtained from a surface spiked with a solution containing 11.11 ng/cm^2 of each of the six compounds. The lower limit of detection (LLOD) was defined as the lowest concentration at which the analytical assay can reliably differentiate the signal of the analyte peak (S) from the background noise (N) ($S/N \geq 3$). The lower limit of quantification (LLOQ) was considered as the lowest concentration that was characterized by a peak intensity at least five times higher compared to the baseline noise and providing precision and trueness within 20%, based on triplicate analyses. Intra- and inter-day precision and trueness for each analyte were evaluated at three concentrations (i.e. Low Level = LL: 0.39 ng/cm^2, Medium Level = ML: 0.78 ng/cm^2, and High Level = HL: 3.89 ng/cm^2), which were different from those used to build the calibration curves. Five replicates for each concentration were analyzed in the same day and aliquots of the same samples were analyzed again (three times for each day) after 1, 2 and 4 days. To evaluate method precision, percentage coefficient of variance (% CV) over the different measurements was calculated; trueness was defined by a percentage relative standard error (% RSE) between the nominal and the measured concentration. Linearity of the analytical response was determined by plotting the ratio analyte/internal standard peak areas as a function of the analyte concentration used; the resulting curves were plotted according to a linear regression. Experimental concentrations were back-calculated using the calibration curve to determine their deviation from the nominal ones. All procedures involving the use of hazardous substances were carried out under a chemical hood, using cytostatic gloves and disposable gowns.

Collection and analysis of samples from UCDP working areas

The wipe-sampling procedure described above was used to evaluate the contamination of different surfaces in the UCDP laboratory. Different areas were monitored to verify potential criticisms generated by inappropriate procedures, inefficient cleaning and/or individual mishandling. Sampling procedure was carried out on squared (30 cm × 30 cm) surface sections; in the case of the refrigerator handler where AAs are commonly stored, PPE wardrobe and door handle, the monitored area was of (1 cm × 30 cm). Samples were analyzed in triplicate by UHPLC-MS/MS along with blank samples, which were randomly injected several times during the analysis to continuously check the instrument performance and potential carry-over of the chromatographic run.

Preliminary assessment of cleaning operations for AAs contaminated surfaces

To evaluate the efficiency of the cleaning procedures for AAs contamination on working surfaces, a preliminary test was carried out using different washing solutions and supports used for the removal of contaminants. A surface contamination condition was simulated by spotting a solution with a final concentration of 11.11 ng/cm^2 for each tested AA molecule. Specifically, three supports were used to clean the contaminated surfaces: nonwoven cleaning cloth (NW), compressed gauze (CG), and absorbent paper (AP). Two washing strategies have been examined: a solution composed of 70% ethanol (Et70), and a washing procedure using water first, followed by 100% EtOH (W/E). Cleaning procedure was carried out on squared (30 cm × 30 cm) surface sections preventively spotted with contaminants. Following cleaning operations, for each support/procedure combination, the wiping protocol was carried out according to the procedure described above. The residual permanence on the surface of each AAs was evaluated and expressed by the percentage ratio between the analytical response derived from the contaminated area and the analytical response of the same area wiped after the cleaning operations. All samples were analyzed in triplicate.

Results and discussion

Environmental samples collected from work areas potentially contaminated with AAs using paper-wipes were analyzed

Table 1. UHPLC-MS/MS parameters used to identify and quantify the compounds described in this study.

Compound	Precursor (m/z)	Product (m/z)	CE (V)	Retention time (min)	Qualifier/quantifier area ratio range (%)
MTX	455	308*	20	1.58	35–41
		175§	30		
IFA	261	154*	21	3.06	47–53
		182§	16		
CFA	261	140*	21	3.19	30–36
		233§	16		
IRT	587	458*	35	3.39	70–80
		502§	30		
DXR/EPI	544	397*	11	3.49	52–58
		361§	25		
PTX	854	569*	11	5.82	55–61
		286§	15		

CE: collision energy.
*Quantifier ion.
§Qualifier ion.

through a multi-residual UHPLC-MS/MS-based analytical method. Taking into account frequency of use, theoretical harmfulness (in terms of biological effects and bioavailability), and ease of analysis in a single run of several species at the same time, we selected six different chemotherapeutics as common markers of contamination, namely MTX, IFA, CFA, DXR, IRT, and PTX. For method performance evaluation pure DXR was used, but, when our method is applied on UCDP working surfaces, it is worth noting that it is potentially not possible to discriminate DXR from its isomer Epirubicin (EPI). For this reason, the DXR/EPI notation is reported in the tables shown, even though only one of the two drugs was used during the environmental monitoring campaign. Selected reaction monitoring mode was used and two transitions were selected for each analyte (Table 1).

Using the UHPLC-MS/MS based methodology, the analytical cycle was completed in 9 min (including column equilibration and washing steps), and a good separation among all the compounds investigated was obtained (Figure 1).

One of the main critical points to consider in order to guarantee the high accuracy of an environmental monitoring protocol concerns the method for collecting samples from contaminated surfaces. We developed a patented methodological procedure that allows to evaluate the efficacy of wiping-based samples collection, thanks to the presence of a specific internal standard (see Material and Methods section for details).

A partial validation to verify the performance of the methodology was carried out in accordance to EMA guidelines for bioanalytical methods.[27,28] No interfering signals were detected when blank samples underwent analytical processing, and carry-over was substantially absent for all the compounds investigated (data not shown). Lower limits of detection (LLOD) and quantification (LLOQ) on surfaces were evaluated (Table 2) and values ≤0.10 ng/cm^2 were obtained for all six compounds.

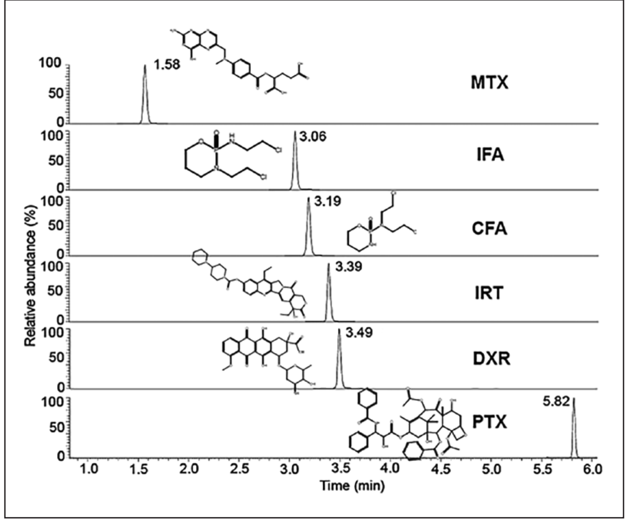

Figure 1. Reconstructed UHPLC–MS/MS chromatogram of a mixture of the six drugs analyzed (1 ng/ml) with their corresponding chemical structures. For each compound, the channel corresponding to the quantifier transition is shown. MTX: methotrexate; IFA: iphosphamide; CFA: cyclophosphamide; IRT: irinotecan; DXR: doxorubicin; PTX: paclitaxel.

For each molecule, linearity of the response was investigated over a concentration range from LLOQ to 11.11 ng/cm^2, and good correlation coefficients were retrieved (Figure 2).

Intra- and inter-day precision and trueness of the method were evaluated for all compounds at three different concentrations and the observed coefficient variants (CV) and relative standard errors (RSE) were lower than 18% for all drugs tested (Table 3). Considering the

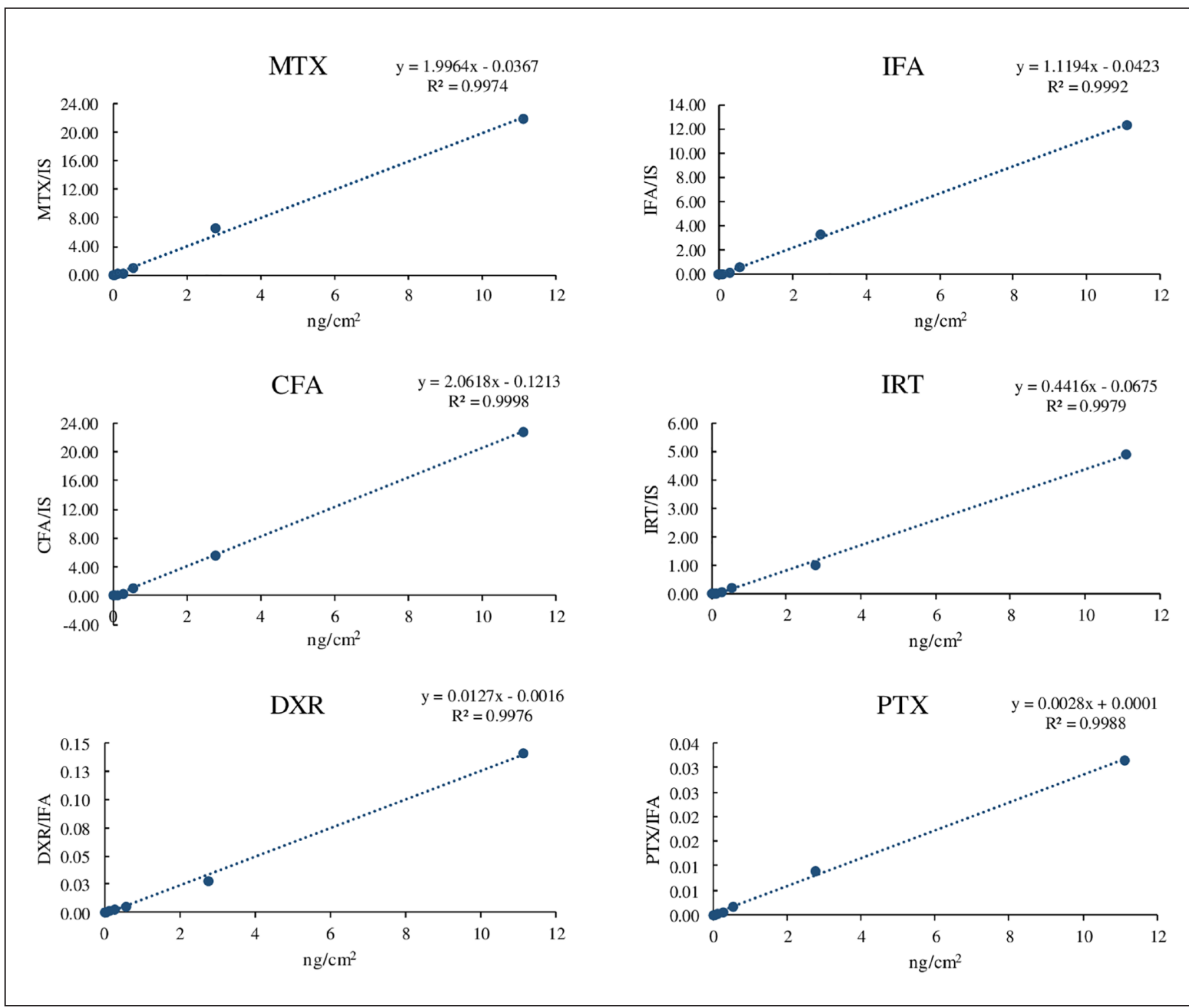

Figure 2. Linearity of the analytical method, evaluated ($n=6$) for each of the six compounds, recovered from spiked surfaces.

Table 2. Lower limits of detection (LLOD) and lower limits of quantification (LLOQ) evaluated for each compound.

Compound	LLOD (ng/cm^2)	LLOQ (ng/cm^2)
MTX	0.01	0.02
PTX	0.06	0.08
IRT	0.01	0.02
CFA	0.01	0.02
IFA	0.02	0.02
DXR	0.04	0.06

intrinsic variability of the wipe-sampling procedure, these values may be considered satisfactory for the analysis of surface contaminations. Finally, the stability of the samples at 5°C (autosampler temperature) was monitored for 24h and the response obtained for all analytes was substantially unmodified (CV ≤ 7%).

The performance of the analytical procedure developed was satisfactory, both for the ability to detect and quantify even very low levels of contamination, and for the reproducibility, which was high despite the complexity of the procedures required for the environmental monitoring.

The method was used to evaluate potential contamination affecting different work areas routinely used by the UCDP at the University Hospital "San Giovanni di Dio e Ruggi d'Aragona" in Salerno (Italy). The workspace examined consisted of two rooms: the laboratory and an anteroom (Figure 3).

To assess the correctness and the efficacy of the safety procedures adopted in drugs preparation, several samples were withdrawn from different potentially contaminated surfaces in both rooms (spots 1–7 in Figure 3). This procedure was carried out twice: a first sampling was performed during the working time and a second one at the end of the working shift, following the cleaning operations, which included a 5% hypochlorite-based washing solution and a sterile gauze for rubbing. On each surface, two intra-day samplings were carried out by the same operator on two identical and adjacent portions (Figure 4).

Table 3. Intra- and inter-day precision and trueness of the method evaluated at three different concentrations. LL (Low Level) = 0.39 ng/cm²; ML (Medium Level) = 0.78 ng/cm²; HL (High Level) = 3.89 ng/cm².

	Intra-day % CV			Inter-day % CV			Intra-day % RSE			Inter-day % RSE		
Compound	LL	ML	HL	LL	ML	HL	LL	ML	HL	LL	ML	HL
MTX	6	5	5	4	6	6	10	9	8	9	5	6
IFA	7	6	6	7	4	5	10	10	7	9	5	5
CFA	9	5	6	9	7	6	11	8	6	10	7	6
IRT	5	3	3	5	5	3	8	6	5	13	6	5
DXR	5	5	6	10	8	8	10	11	8	15	8	9
PTX	7	6	6	11	10	11	17	17	11	17	9	10

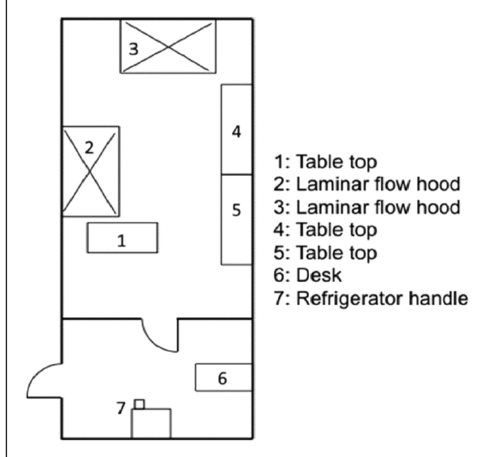

Figure 3. Schematic map of the monitored working areas in the UCDP.

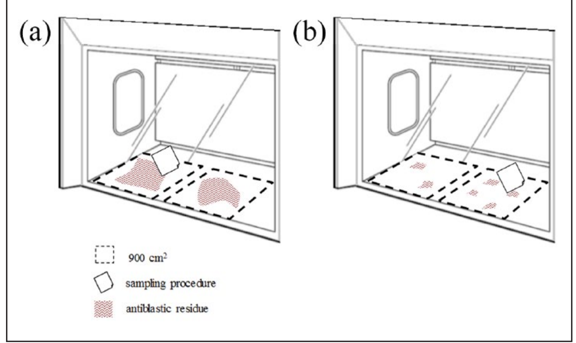

Figure 4. Simplified representation of the procedure used to evaluate hood surface contamination: (a) sampling during working time performed on a 30 cm × 30 cm surface and (b) sampling after cleaning procedure, performed on the adjoining area (30 cm × 30 cm).

The first cycle of analyses performed showed that significant amounts of CFA and IFA were present on the two hood workstations (areas 2 and 3), even after the surface had undergone the standard cleaning procedure (Table 4).

In addition, some contamination was found over an area that was expected to be clean, such as the handle of the refrigerator where drugs are commonly stored (area 7). The amount of IFA and CFA found on several surfaces might be related to the specific preparation procedure these drugs were subjected to; they were in fact provided as powder and needed to be solubilized before their dilution in the final infusion bag. Our data showed that the cleaning procedure was largely ineffective, as significant amounts of drugs were detected immediately after the different working surfaces were routinely cleaned by UCDP workers.

Based on these observations, the laboratory and the anteroom were deep cleaned with 70% ethanolic solution and the working procedures at the UCDP were completely reorganized. Moreover, the anteroom was converted in a dressing room where all personal protective equipment (PPE) were worn before starting routine drug manipulations. A wardrobe replaced the desk, and people were admitted in that room only immediately before entering the laboratory or to undress PPE coming out of it. About 1 month after this reorganization, the environmental monitoring of the working areas was repeated. The results of the analyses performed on these samples (Table 4) showed that AAs contamination had significantly decreased; drug concentration, in fact, was lower than 0.44 ng/cm² even over the hood workstation. At the end of this first round of sampling, an overview of the total AAs contamination pattern confirmed the efficacy of the modification performed on the working environment (Figure 5).

The data presented in Table 4 did not follow a normal distribution (confirmed by the Shapiro-Wilk test), therefore they were compared using the Wilcoxon paired test. The *p*-values obtained from the comparison between the contamination of the areas during working time and after routine cleaning (p-value = 0.01864) and those after deep cleaning (p-value = 0.0004545) confirmed their statistical significance and highlighted the need to improve the cleaning procedure. However, CFA was still found to be the most persistent compound and traces of IFA and CFA were still detected on the refrigerator handle, thus suggesting that working procedures required further adjustments. In

Table 4. Contamination levels (expressed as ng/cm^2 of each drug) detected in the monitored areas during working time, after routine cleaning operations and after deep cleaning operations and reorganization of working procedures. 1 (table top); 2 (laminar flow hood); 3 (laminar flow hood); 4 (table top); 5 (table top); 6 (desk/door handling); 7 (refrigerator handle).

Detection phase	Drug	Area 1	2	3	4	5	6§	7
Working time	MTX	0	0	0	0	0	0	0
	IFA	0.10 ± 0.03	2.21 ± 0.35	0.73 ± 0.03	0.34 ± 0.04	0	0	0.15 ± 0.01
	CFA	0.44 ± 0.09	4.36 ± 0.33	*13.26 ± 0.05	0.71 ± 0.04	0	0	0.18 ± 0.01
	IRT	0	1.64 ± 0.2	0.07 ± 0.03	0.02 ± 0.01	0	0	0
	DXR	0	0	0	0	0	0	0
	PTX	0.36 ± 0.13	0.23 ± 0.14	0.26 ± 0.12	0	0	0	0
After routine cleaning operations	MTX	0	0	0	0	0	0	0
	IFA	0.41 ± 0.04	0.17 ± 0.04	0.29 ± 0.04	0.12 ± 0.02	0	0	0.12 ± 0.04
	CFA	1.3 ± 0.68	1.47 ± 0.07	4.92 ± 0.46	0.55 ± 0.02	0	0	0.12 ± 0.06
	IRT	0	0.83 ± 0.08	0.06 ± 0.01	0	0	0	0
	DXR	0	0	0	0	0	0	0
	PTX	0.04 ± 0.06	0.21 ± 0.04	0.19 ± 0.06	0	0	0	0
After deep cleaning operations and working procedures reorganization	MTX	0.03 ± 0.03	0.02 ± 0.01	0	0	0	0	0
	IFA	0.02 ± 0.01	0	0.05 ± 0.02	0.02 ± 0.01	0	0	0.05 ± 0.01
	CFA	0.26 ± 0.15	0.43 ± 0.11	0.3 ± 0.1	0.04 ± 0.02	0	0	0.02 ± 0.01
	IRT	0	0.02 ± 0.01	0.02 ± 0.01	0	0	0	0
	DXR	0	0	0	0	0	0	0
	PTX	0	0.09 ± 0.03	0	0	0	0	0

*This value might be inaccurate since it is not included in the calibration range.
§After reorganization, door handle replaced the desk as analyzed area.

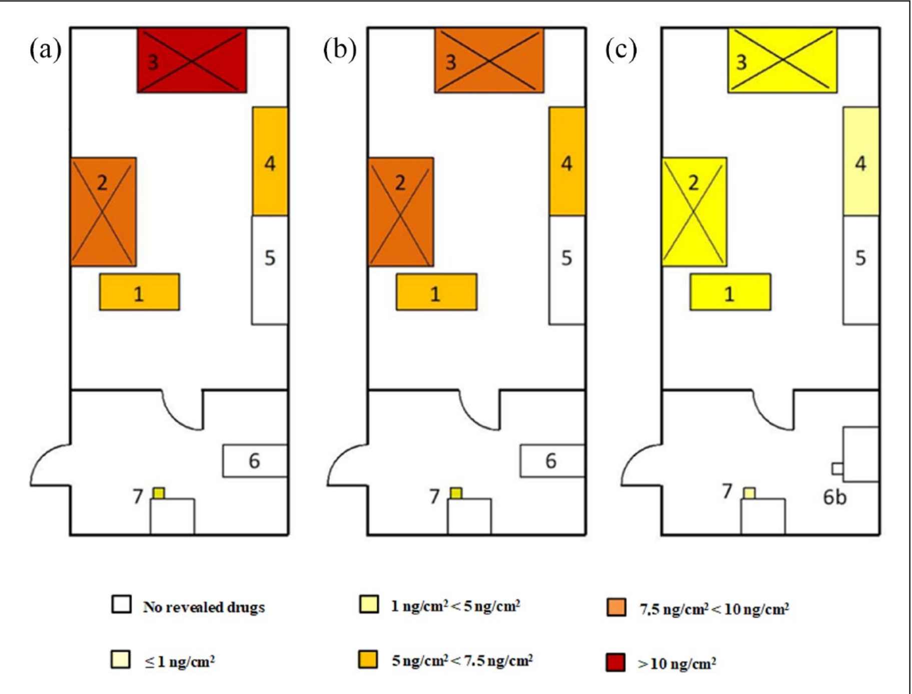

Figure 5. Whole contamination levels detected in the UCDP areas: (a) drug concentration in the different areas in the first sampling, (b) drug concentration in the different areas after cleaning, and (c) drug concentration in the different areas after laboratory deep cleaning and reorganization.

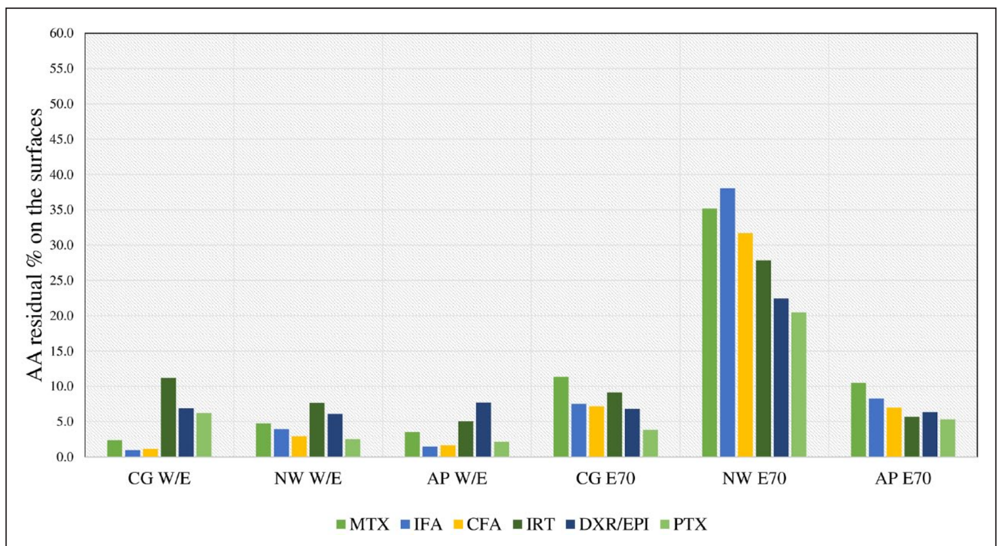

Figure 6. Antineoplastic drugs residual percentage on surfaces as a result of different cleaning procedures. E70 (water 70:30 v/v); W/E (water/ethanol); NW (nonwoven cleaning cloth); CG (compressed gauze); AP (absorbent paper).

addition, in some cases the drug concentration detected on working surface increased after cleaning procedures. This may be related on routinely cleaning operations unable to efficiently remove all the contaminants, which could therefore accumulate in corners or dead spots and be withdrawn during following sampling procedures.

Based on the results obtained, the efficacy of different surfaces cleaning methods was investigated. Antiblastic molecules routinely used in UCDPs belongs to diverse chemical classes, characterized by different polarities. Therefore, the use of a single washing solution for cleaning all types of AAs from the surfaces may be ineffective. Following this hypothesis, two washing approaches were tested: the first (E70) consisted of a single cleaning procedure carried out using a mixture ethanol: water 70:30 (v/v) whereas the second involved a washing step performed with pure deionized water followed by a cleaning step with pure ethanol (W/E). The efficacy of these approaches was assayed using three different removal supports: nonwoven cleaning cloth (NW), compressed gauze (CG), and absorbent paper (AP). The possible combinations of solvents and supports (NW-E70, CG-E70, AP-E70, NW-W/E, CG-W/E, and AP-W/E) were then used to clean surfaces previously polluted with known quantities of the different AAs and the percentage quantity of each drug remaining on the surface was assessed. The results obtained (Figure 6) highlighted that the use of a single washing solution (E70) does not provide good cleaning results for the tested analytes. Indeed, by using that solvent, high amounts of all AAs were detected and the less efficient combination was NW-E70. The sequential use of pure water and ethanol, instead, provided more satisfying results, regardless of the support used, although the higher efficacy for all AAs was achieved using AP.

The persistence of several chemotherapeutic drugs may be the consequence of their accumulation on non-homogeneous work surfaces, worn out by time and characterized by the presence of microfractures and material alterations not visible to the unaided eye. Removing these residues can take quite a long time, even using the right cleaning strategy. A further reason for chemotherapeutics persistence can be due to a lack of a regular control of hood filters, which can be responsible for an uncontrolled release of toxic molecules. Therefore, routine monitoring of UCDPs contamination represents a fundamental tool to verify the presence of risk situations that are not always manageable and avoidable and to ensure healthcare professionals safety.

Conclusions

To date, the challenge of protecting workers health is persisting and expanding, with an increasing number of publications showing that contamination of AAs is still present on work surfaces after cleaning procedures are concluded.[29–33] Considering the risk that workers have of coming into contact with these substances, the routinely use of environmental monitoring as an important tool contributing to the risk assessment in exposed healthcare workers is evident, along with the improvement of the performance of cleaning tools and the correct education of the personnel dedicated to the handling of these compounds. One of the major challenges in the development of an efficient protocol for environmental monitoring is the use of a fast and sensitive method for the determination of contaminants along with the optimization of sampling procedures. It is indeed of utmost importance that the approach used allows withdrawing most of the compounds effectively present on

the contaminated surface, regardless of their chemical and physical properties. There are at least two main difficulties related to the set-up of an analytical procedure aimed at detecting traces of hazardous compounds on workbenches and other critical surfaces: first, drugs used for the chemotherapeutic regimen are chemically heterogeneous and may strongly differ in terms of physical-chemical properties.[18] In addition, the selection of the monitored working areas is not always straightforward. An ideal methodological procedure should allow to simultaneously detect and accurately quantify a subset of AAs large enough to be representative of most of the compounds commonly used in a UCDP. Moreover, all surfaces where AAs traces may be retrieved should be checked, paying a particular attention to those areas that might be touched by un-protected personnel, such as handlers or desks.

The procedure that we have developed and validated allowed to efficiently carry out environmental monitoring of workplaces used for preparation and manipulation of AAs, drugs that significantly differ in chemical structures, volatility and hydrophobicity.

The monitoring approach proposed was used in the UCDP of an Italian hospital. The possibility of using this procedure to obtain accurate data on the contamination levels of the working-spaces following the different cleaning operations, was essential to allow a critical assessment of the efficiency of the procedures adopted and to prevent risks derived from chemical contamination. Criticisms revealed by these analyses played a pivotal role in the optimization and reorganization of the working environment, leading to a significant improvement of the protection of potentially exposed workers.

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Significance for public health section

To date, the challenge of protecting the health of workers exposed to antineoplastic agents (AAs) is a major concern in hospital settings. The routinely use of environmental monitoring as an important tool contributing to the chemical risk assessment is of utmost importance. The LC/MS-MS based procedure that we have developed and validated allowed to efficiently carry out environmental monitoring of workplaces used for the preparation and manipulation of chemically different AAs. The monitoring approach proposed was used in consecutive campaigns in an Italian public hospital. Criticisms revealed by these analyses played a pivotal role in the optimization and reorganization of the working environment, leading to a significant improvement of the protection of potentially exposed workers.

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Article

Environmental and biological monitoring of formaldehyde inside a hospital setting: A combined approach to manage chemical risk in workplaces

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Abstract

Background: The safety of healthcare workers exposed to formaldehyde remains a great matter of concern for healthcare management units. This work aimed at describing the results of a combined monitoring approach (environmental and biological) to manage occupational exposure to formaldehyde in a hospital setting.

Design and Methods: Environmental monitoring of working spaces and biological monitoring of urinary formaldehyde in 16 exposed healthcare workers of the Anatomic Pathology Unit of a University Hospital in Southern Italy was performed on a four-year timescale (2016-2019).

Results: Values of aero-dispersed formaldehyde identified were on average low; although workers' urinary formaldehyde levels were also minimal, the statistical analysis highlighted a slight weekly accumulation.

Conclusions: Our data confirm that both environmental and biological monitoring are important to identify risk situations, in particular when values of hazardous compounds are below the accepted occupational exposure levels.

Introduction

Formaldehyde (FA) is a widespread chemical substance having formula HCHO, commercially available as an aqueous solution known as formalin, containing 30-50% FA with methanol as a stabilizer to prevent its polymerization. Formaldehyde solution is a clear colourless liquid with a pungent and irritating smell[1] and is mainly used in the canning industry, leather tanning, embalming, fabric manufacture, and as a biocide in the food industry.[2] Formaldehyde is endogenously produced in living organisms as a by-product of serine, glycine, methionine, and various other amino acids metabolism. Endogenous levels of metabolic FA production range from 3 to 12 ng/g of tissue;[3] plasmatic concentration of FA in humans is estimated to be *ca*. 2.5 ppm. Exogenous FA does not accumulate in the body and is rapidly eliminated from human plasma; a biological half-life of this molecule of only 1-1.5 min seems to be responsible for preventing FA systemic distribution in the human body.[4,5] Indeed, no increase in FA blood concentration has been observed in either humans, rats, or monkeys after acute exposure at concentrations of 1.9 ppm (2.3 mg/m^3), 6 ppm (7.2 mg/m^3), and 14.4 ppm (17.3 mg/m^3) of gaseous FA, respectively. This can be explained with both formaldehyde main deposition in the respiratory tract and rapid metabolism.[6]

Possible ways of exposure to exogenous FA are ingestion, inhalation, skin absorption, and blood exchange. Once absorbed, FA is quickly metabolized by almost all body tissues and converted into a non-toxic chemical compound called formate, which is then expelled with urine. Formaldehyde can also be converted into carbon dioxide and exhaled out of the body via pulmonary expiration (Figure 1).

Despite the rapid elimination from the human body, the exposure to exogenous FA sources, both indoors and outdoors, poses a significant threat to human health, and the interest in this topic has been boosted by the current legal statement that has labeled FA as a "Carcinogen for man" - category 1.[7,8]

Workers employed in industries producing FA or FA-containing substances, along with laboratory technicians and certain healthcare professionals, may be exposed to higher levels of this molecule compared to the general public. These workers can suffer from harmful effects from breathing FA gas or vapor or by absorbing liquid containing FA through the skin. The site of direct contact (eyes, nose, throat, and skin), in fact, quickly reacts with FA, which can destroy protective skin oils causing dryness, flaking, and dermatitis. High levels of FA (5-30 ppm) can severely irritate the lungs, causing chest pain and breathing problems. Humans perceive FA smell at a concentration of about 0.5-1.0 ppm, followed by sensory irritation (>2.0 ppm) of nose, throat, and eyes, with eye irritation accepted as the most sensitive endpoint. Both hepatotoxicity and neurotoxicity have also been considered as potential effects of formaldehyde exposure.[9,10]

In hospital units, FA in aqueous solution, or formalin, is used for collection and transport of tissues derived from surgical interventions and biopsies in operating rooms and biopsy sampling clinics (endoscopy, radiology, *etc*.) and as a fixative in pathologi-

Significance for public health

Health workers' regular exposure to formaldehyde may be responsible for long-term health issues; unfortunately, threshold limits of this compound are not harmonized between different government agencies. The combination of environmental and biological monitoring thus becomes an invaluable tool to preserve worker's safety and effectively assess chemical risk in hospital settings.

cal anatomy for its unique properties of preserving cell and tissue morphology. National and international guidelines recommend the use of buffered formalin for histological, immunohistochemical, and molecular (gene mutation) examinations. In addition, all validated protocols related to histochemical, immunohistochemical, and molecular investigations are currently standardized on formalin-fixed tissues.[11] Thus, monitoring environmental FA levels to which health workers are exposed through the use of an efficient methodology to measure biological levels of FA is of crucial importance to prevent organism overload and potential multi-organ damage.

The main goal of this work was to describe operational strategies aimed at reducing the risk related to FA exposure in hospital settings by using an approach combining Environmental and Biological Monitoring. FA measurements based on this combined method were performed on a four-year timescale at a University Hospital in Southern Italy, where environmental and urinary FA levels of 16 employees working at the Anatomic Pathology Unit were evaluated.

Design and Methods

Building and facilities

The Anatomic Pathology Unit is located on the ground floor of the clinical building and has five rooms for specimen treatment, a corridor, and physicians' rooms. In all rooms, a ventilation system is installed, consisting of down flown ventilation with conditioned air flowing into the room from the ceiling and extraction units in the walls; openable windows to the outside of the building are present. Formaldehyde is mainly used in a 23 m^2 room, equipped with a chemical hood and an aspirated cupboard for the storage of anatomical samples.

At the beginning of our monitoring activity (2016), sampling concerned all department rooms to evaluate FA dispersion also in locations not directly affected by the processing of histological samples. In the second, third, and fourth monitoring campaigns (2017, 2018, 2019) only the processing room was analyzed.

Biological monitoring of urinary formaldehyde

Workers' urinary FA was measured using High Performance Liquid Chromatography (HPLC) coupled with a UV-Visible detector (HPLC-UV). A CE-IVD (European Certification - *in vitro* Diagnostics) kit provided by the "Eureka Lab Division" was used. According to the manufacturer, urinary samples were derivatized with a chemical reagent supplied with the kit and incubated at 70°C for 15 min. Then, five-hundred microliters of HPLC-grade water were added to the sample and 50 μl of the resulting mixture was directly injected into the HPLC system.

The chromatographic separation was achieved by RP-HPLC, performed on a Waters 1525 Model Binary Pump System equipped with a multi λ Fluorescence detector (Model 2475), a Photodiode Array detector (Model 2998), and an Autosampler (Model 2707) (Waters, Milford, MA, USA). Samples in the autosampler were at RT and a column oven was used to maintain column temperature at 30°C. Breeze software 2.0 (Waters) was used for peak analysis, integration, and to calculate the linear regression of the calibration curve. Chromatographic analysis was carried out on a Poroshell 120 EC-C_{18} (50×4.6 mm, 2.7 μm) column (Agilent). The mobile phase was included in the commercial kit. The separation was

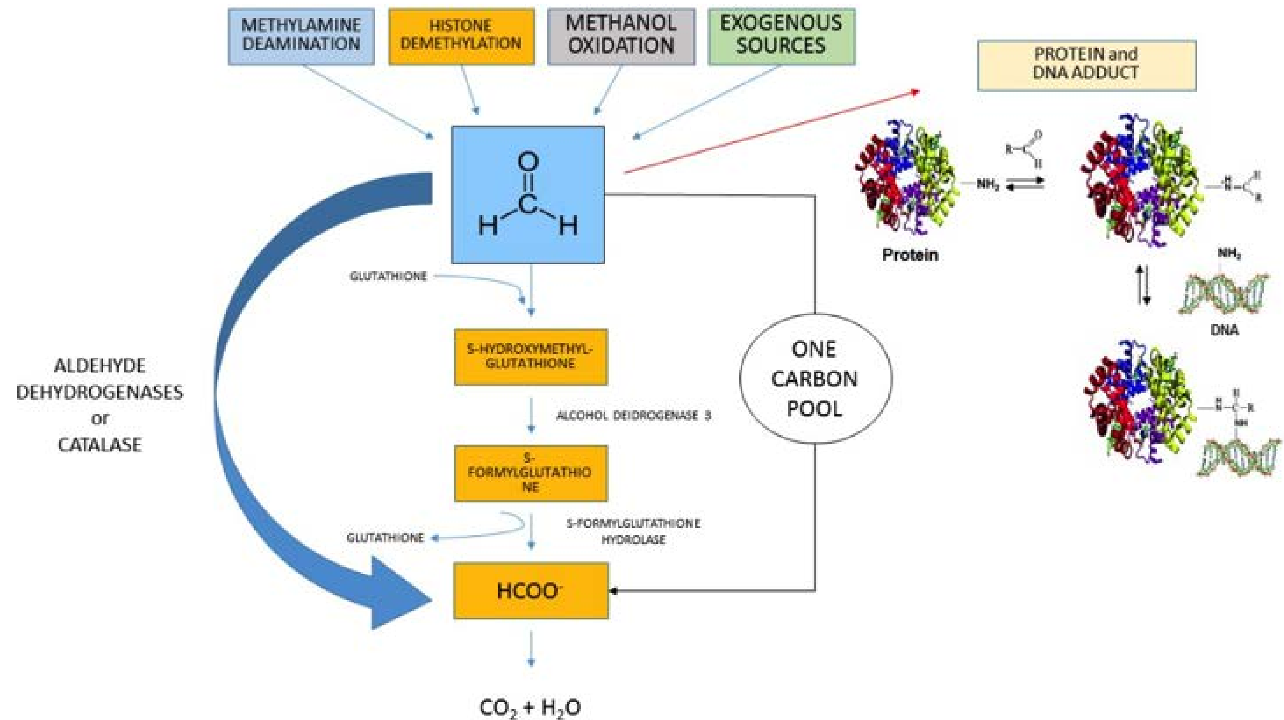


Figure 1. Formaldehyde pathway and metabolism.

achieved by isocratic elution with a flow rate of 1.2 ml min^{-1}. Analytes were revealed with a UV detector set at λ= 385 nm.

Environmental monitoring of formaldehyde

Formaldehyde environmental monitoring was performed on selected fixed locations by both:

1) active sampling using chemo-adsorbent tubes (following NIOSH 2016 method), with a properly calibrated pump, provided by Aquaria srl (Lacchiarella, MI, Italy), and whose flow was set to a constant value of 1L/min throughout the sampling period. The sampling flow rate was checked continuously by a fluxometer.[12,13] Four samples per working day and three measurements for each sample were analyzed and mean values were reported. The sampling procedure requires a known volume of sample air (from 1 to 15 litres) to be passed through an acidified silica gel coated with 2,4-dinitrophenylhydrazine (DNPH). Formaldehyde reacts with the DNPH producing the corresponding hydrazone. After the sampling phase, tubes were closed with caps, transported, and stored in glass containers in refrigerated systems maintained at a controlled temperature.

2) diffusive (passive) sampling, to evaluate average concentration values (TLV-TWA 8 h per working day), by exposing diffusive samplers (RING devices provided by Aquaria srl) containing a silica gel cartridge coated with 2,4-dinitrophenylhydrazine (DNPH) in one selected location. This method is indicated for long-term monitoring.[14-18] Three measurements were realized for each determination and the average value was reported. At the end of the sampling phase, tubes were closed with the appropriate caps and stored in glass containers in refrigerated systems maintained at a controlled temperature. The mass concentration C(μg/m^3) of passive samplers was calculated with the manufacturer's uptake rate of 92 ml/min, and using the following formula:

$$C(\mu g/m^3) = \text{mass}(\mu g) / 10^{-6}\, x\, P(ml/min)\, x\, \text{time}(min)$$

This equation can be directly derived from Fick's first law considering that the mass of the analyte is sorbed by diffusion,[16] time represents the time of exposition of the sampler and P, the diffusive uptake rate, is dependent only on the diffusion coefficient of the given analyte and on the geometry of the diffusive sampler used.

The limit of detection (LOD) of this methodology was three-times the standard deviation of the blank values (as reported in EN 13528-2) and accuracy was calculated as the 2σ deviation of the absolute differences of the individual sample values compared to the mean in triplicate samples.[19]

Formaldehyde sampled with both active and passive samplers was detected according to the method described by NIOSH 2016,[20] which involves an organic extraction with acetonitrile and subsequent analysis by HPLC. Analyses were performed on an HPLC instrument with a PDA detector set at 385 nm supplied by Waters® Corporation and an Ascentis® C18 analytical column (4.6 mm x 150 mm, 3 μm); chromatographic elution conditions consisted in a mobile phase composed of 45% acetonitrile/55% water (v/v) and a flow rate of 1.0 ml/min. For data analysis, Breeze® 2.0 software, supplied by Waters®, was used. Extraction was performed by adding 3 ml of acetonitrile to the vial.

Analytic grade acetonitrile and 99.9% pure formaldehyde-2,4-dinitrophenylhydrazone (FA-2,4-DNPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). For calibration, a known amount of FA-2,4-DNPH was weighed and diluted in acetonitrile; from this stock, dilutions in acetonitrile were prepared for calibration in a range of 0.23 to 37 μg per sample.

RING diffusion samplers and tubes for active sampling were provided by Aquaria® srl. Water and acetonitrile (ACN) were purchased from Romil® (Waterbeach, Cambridge, UK) and were all HPLC grade.

Statistical analyses

Data were expressed as means ±SD per year, as median and range of values (min - max); comparisons between the different years of collection were analyzed and comparisons of the values of the different work shifts were analyzed. These analyses were performed considering the differences between FA levels measured during working days (intra- and inter-day backlog) and basal values (start of weekly work shift).

Results

Environmental monitoring

To maintain a safe and healthy workplace for employees working with hazardous chemicals such as FA, it is important to minimize exposure to this compound. To this purpose, the maximum air concentration of the chemicals that may still be considered safe has been defined as time-weighted averages measured over 8 h (TWA) and short-term exposure limits (STEL) for a 15 min period (TLV-Ceiling). However, these occupational exposure limits (OELs) can be set at World level, at European level, at national level or by companies themselves and, therefore, regulations for setting OELs may deeply vary. Moreover, OEL can be estimated by different methods, which may result in a variety of OELs. Table 1 reports the main Threshold Limit Values for FA proposed by different

Table 1. Threshold limit values for formaldehyde provided by main government agencies.

Organization/legal of countries	Type	Concentration (ppm)
Occupational Safety and Health Administration (OSHA-USA)	TWA	0.75
	STEL	2.0
American conference of Governmental Industrial Hygienists (ACGIH-USA)	TWA	0.1
	STEL	0.3
National Institute for Occupational Safety and Health (NIOSH)	TWA	0.016
	STEL	0.1
World Health Organization (WHO)	STEL	0.08
Scientific Committee on Occupational Exposure Limits UE (SCOEL)	TWA	0.3
	STEL	0.6

TWA, time-weighted averages measured over 8 h; STEL, short-term exposure limits.

international organizations.

It is important to underline that these limits do not constitute a clear dividing point between non-dangerous and harmful concentration, but they only indicate the concentrations of the airborne substances to which it is believed that most workers can remain exposed for eight-hours daily, forty hours a week, forty-eight weeks a year, without suffering of adverse health effects.

The environmental monitoring campaign in the Anatomic Pathology Unit was aimed at evaluating the amount of airborne FA in the various environments, and the ability of FA to diffuse between adjacent rooms. Twenty sampling areas were selected in the structure to evaluate the concentration of FA and identify the possible diffusive path of FA in the environments adjacent to the histological sample processing room. In Table 2 the results of four monitoring campaigns at the Anatomic Pathology Unit of the Hospital are reported. During the first campaign (2016), we randomized the sampler location to identify the sampling sites that could be representative of the general pollution conditions, whereas sampling was focused on a fixed location during the next campaigns (2017-2018-2019). Sampling was performed by both passive and active methods, as described in the Materials and Methods Section to verify compliance with both TWA and STEL limits.

As expected, the active sampling method returned a higher concentration than the passive method. However, all active and passive measurements showed compliance with the OSHA and ACGIH exposure limits, but not always with the lowest NIOSH REL. Only a few active measurements exceeded NIOSH STEL, mainly in correspondence with the sampling site located near the open trash bin next to the fume hood.

These results were in agreement with the inaccurate and erroneous workers' practice to throw in the open bin gloves and paper towels used to clean FA contaminated surfaces, thus leading to the evaporation of residual formaldehyde in the environment.

Moreover, since it is well known that environmental factors such as temperature and relative humidity may influence the performances of passive samplers, we always checked these parameters using a calibrated instrument for measuring environmental parameters; values are reported in Table 1 and show minimal variations for each sampling period. In particular, the temperature was in the range of 25-28°C and the relative humidity was in the range of 38-52%. In these conditions, no adverse effect on recovery is expected.

Noteworthy, the percentage of non-compliance, calculated as the number of measurements above the OEL, decreases from the first to the last monitoring campaign from 50% to 7% for the active measurements and from 12.2% to 0% for the passive one. This marked improvement in environmental conditions mainly depended on the employees who, despite no structural or technical changes in the work environment had been realized, had perceived the importance of their actions in limiting the spread of formaldehyde, and devoted greater attention to the actions they carried on daily.

Biological monitoring

Each biological monitoring campaign included three withdrawal times, more specifically on Monday morning at the beginning of the weekly shift (T0), Monday evening at the end of the daily shift (T1), and Friday evening at the end of the weekly shift. This timing allowed evaluating both the intra-day exposure and the weekly backlog (inter-day exposure). By analysing urinary FA concentrations at the beginning of the working week, it was also possible to evaluate whether any abnormal level of FA was related to an occupational rather than an accidental exposure, for example during the weekend. Biological monitoring started in Fall 2016 and was repeated every six months, in parallel with environmental monitoring. Urine samples were collected, labeled, and stored at -20°C until analysis.

A statistical comparison of the values obtained in the four years analysed (Figure 2), shows that the highest average value was measured in 2018 (0.75 mg/L). The analysis of variance (ANOVA) shows that in 2018 the values deviate less from the mean value. Medians reflect this trend, with higher values in 2018 and lower in the second half of 2017.

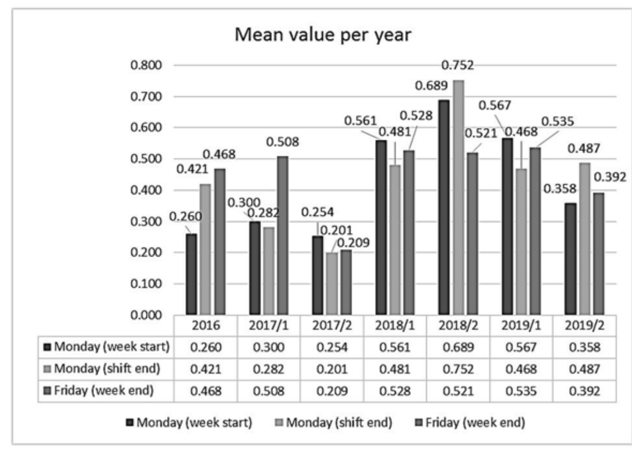


Figure 2. Average values of the three sampling times obtained for each monitoring campaign.

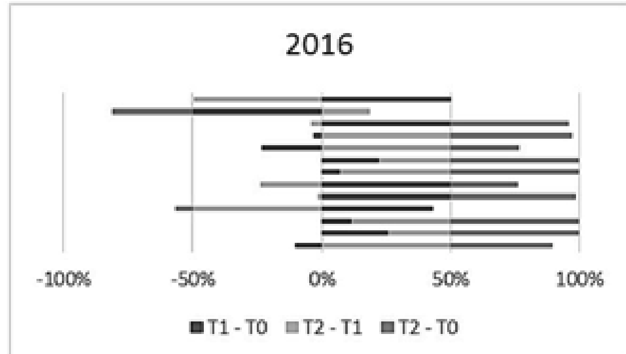
Table 2. Formaldehyde concentration (ppm) detected in the Anatomic Pathology Unit of the University Hospital "San Giovanni di Dio e Ruggi d 'Aragona" in Salerno (Italy) in four years.

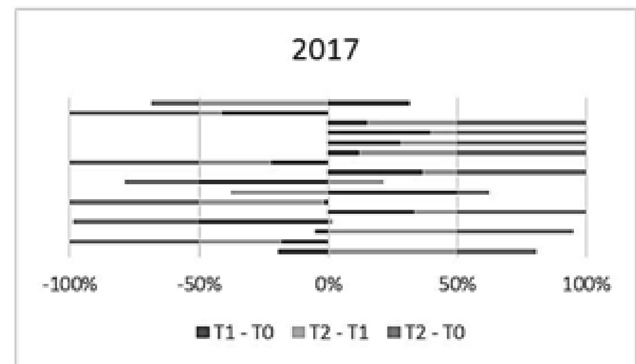
Year	RH (%)	Temperature (°C)	Sampling method (active/passive)	n. samples	Averages (ppm)	Range (ppm)	Non-compliance (%)
2016	46±6	25.7±1.0	Passive	15	0.0098	0.0033-0.0400	12.2
			Active	24	0.0790	0.0220-0.1390	50.0
2017	42±4	25.2±0,8	Passive	30	0.0013	0.0004-0.0050	0
			Active	48	0.0430	0.0290-0.1587	22.2
2018	45±7	25.8±2.0	Passive	27	0.0006	0.00014-0.00105	0
			Active	56	0.0380	0.0134-0.1250	10.0
2019	44±7	27.8±2.0	Passive	20	0.0005	0.00018-0.00098	0
			Active	52	0.0280	0.0060-0.1050	7.0

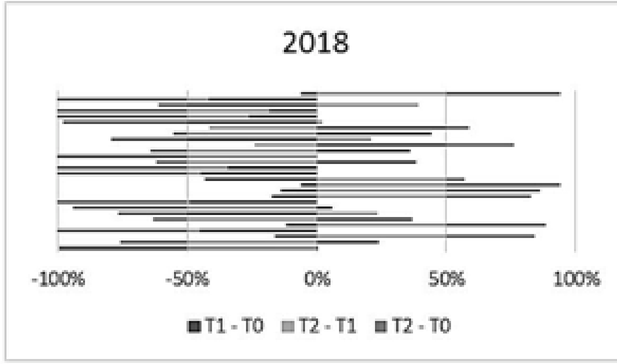
Table 3 shows the average values obtained in each single monitoring campaign. The reference value used to identify potential risk for the workers is, according to the guidelines of the Italian Association of Industrial Hygienists (AIDII) (https://www.aidii.it/), 5.6 mg/L. As evident from Figure 2 and Table 3, values were always far below the accepted threshold value. However, it is worth noting that in 2016 and the first campaign of 2017, the average of the values recorded on Friday evening was higher than that of Monday end shift, thus suggesting the tendency, albeit minimal, to a weekly stack.

In the second half of 2017 and in 2018, the three sampling times report comparable values; this can be attributed to exposure of workers to outside FA sources, not imputable to hospital workspace, or, likely, to a subjective endogenous level of FA.

An analysis focusing on single workers' values trend, measured over the four years, has been shown in Figure 3. In 2016, 61.5% of the values, measured on Friday at the end of the working shift (T2), increased compared to the values measured on Monday evening at the end of the working shift (T1), while the intra-day measurements of the first working day (T0 *vs* T1) highlighted an increase in FA values in 69.2% of workers. In 2017, the trend was similar. In 2018, we observed an increase in T1 *vs* T2 for 50% of workers, with values more than doubled in 18.7% of cases. In the T0 *vs* T1 measurements, on the other hand, an increase in FA values was found in only 25% of workers.







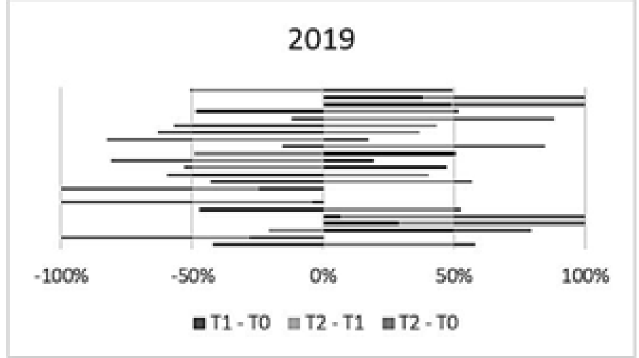


Figure 3. Analysis performed over four years of values trend for each worker. Values expressed in mg/l.

Table 3. Formaldehyde values measured in each biological monitoring campaign for workers in the Anatomic Pathology Unit of the University Hospital "San Giovanni di Dio e Ruggi d 'Aragona" in Salerno (Italy). Values expressed in mg/l.

Year	Mean ±SD	Median	Range (min-max)	
2016	0.39±0.23	0.33	0.03	0.93
2017/1	0.34±0.25	0.27	0.06	0.99
2017/2	0.22±0.14	0.22	0.03	0.80
2018/1	0.52±0.17	0.48	0.22	1.04
2018/2	0.65±0.39	0.56	0.13	1.89
2019/1	0,52±0,23	0.45	0.06	0.91
2019/2	0.41±0.32	0.38	0.04	1.31

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Discussion

Starting January 2016, FA re-classification as a “carcinogenic substance” has urged employers to find solutions to limit workers’ exposure to this harmful compound. Doubts and fears among workers due to the risk associated with FA professional exposure, are rightful. Short-term health effects are variable depending on the subject’s sensitivity to the compound and include irritative pathologies mainly affecting upper and lower airways.[21]

In Italy, a maximum exposure limit for FA in working and living environments was initially set, as early as 1983, at a concentration of 0.1 ppm (124 μg/m^3).[22] European Union has definitively classified FA as a carcinogenic substance (category 1B) with the EU regulation No. 895/2014, concerning the registration, evaluation, authorization, and restriction of chemical substances (REACH). Italy has fully adopted this classification and included it into Legislative Decree 81/08, which specifies the employer’s obligations to replace the carcinogenic compound, when possible, and to reduce the exposure to the lowest technically possible level. According to these guidelines, a risk assessment must be reconsidered every three years, or in case of modifications of the workflow routine. It is important to underline that the regulation foresees that the employer also measures the presence of carcinogens or mutagens to verify the efficacy of the measures adopted.[23]

This work aimed at providing an example of a combined approach of environmental and biological monitoring carried out at a University Hospital setting in Southern Italy. Values of aerodispersed FA, resulting from environmental analyses, were on average very low, except for some environments, in which the concentrations slightly overcame the limit values established by NIOSH (TLV = 0.1 ppm), with a maximum value of 0.16 ppm (macroscopic room, where bioptic samples were analysed). Accordingly, analyses performed on workers’ urinary samples showed low values of FA, far from accepted TLV.

Statistical analysis of biological samples suggested a potential FA weekly accumulation. Although a different number of samples was analysed in each campaign, due to the turnover of department personnel, it is worth underlining that in 2018-2019 we noticed a lowering, albeit minimal, of FA accumulation levels between the beginning and the end of the working week.

However, it should be emphasized that the measurement of urinary formaldehyde levels in the long-term has severe limits, due to its very short half-life; therefore, other markers should be investigated to evaluate biological long-term backlog. Furthermore, AIDII guidelines consider as professional exposure limit values that are compatible with manufacturing companies, but not with environments such as hospitals, where the use of FA is used at much lower concentrations, but still potentially harmful. This led us to use the limit suggested by NIOSH, as it is the lowest. Nevertheless, our results clearly show that the concomitant analysis of air quality and actual workers’ exposure is a key tool to allow optimization of work safety procedures, which must become routinely in hospitals. Combining environmental and biological monitoring is necessary to understand the state of workplaces, the efficacy of individual protection disposables, and the air filtering system. This is also important to assess the adherence of workers to good laboratory practices and preserve their health status.

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Contributions: OM, VI, conceptualization; BC, AC, CP, methodology; BC, FDR, GM, formal analysis, OM, VI, MC, FDC, AB, investigation; FDC, AF, AB, OM, VI, resources; BC, AC, FDR, data curation; GM, CP, original draft preparation; OM, VI, review and editing.

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Ethics approval and consent to participate: this study falls within the framework of controls that workers perform routinely as part of the health surveillance programs and is not a clinical study. The data presented in this manuscript were produced by the San Giovanni di Dio and Ruggi d'Aragona hospital in Salerno, as part of the routine program of preventive medicine for monitoring workers exposed to organic solvents, as reported by the current law on workers (Legislative Decree 81 of 9 April 2008), and common good laboratory practices (GLP). No further withdrawals were made for the analyses reported in this study. For this reason, the opinion of the Ethics Committee was not necessary as it is not a clinical study or pharmacological treatment. The workers had already been informed by written communication that these samples were part of the normal practice of assessing exposure to chemicals and that the only analytes investigated in these procedures were those indicated in compliance with the aforementioned law. Workers data have been processed in accordance with the current laws on the protection of privacy and in accordance with the General Data Protection Regulation (GDPR) procedures n. 2016/679. Informed consent was not acquired from the workers as this procedure falls within the parameters indicated by the law for the protection of personal data Published in the Official Gazette no. 72 of 26 March 2012 (Register of measures n. 85 of 1 March 2012).

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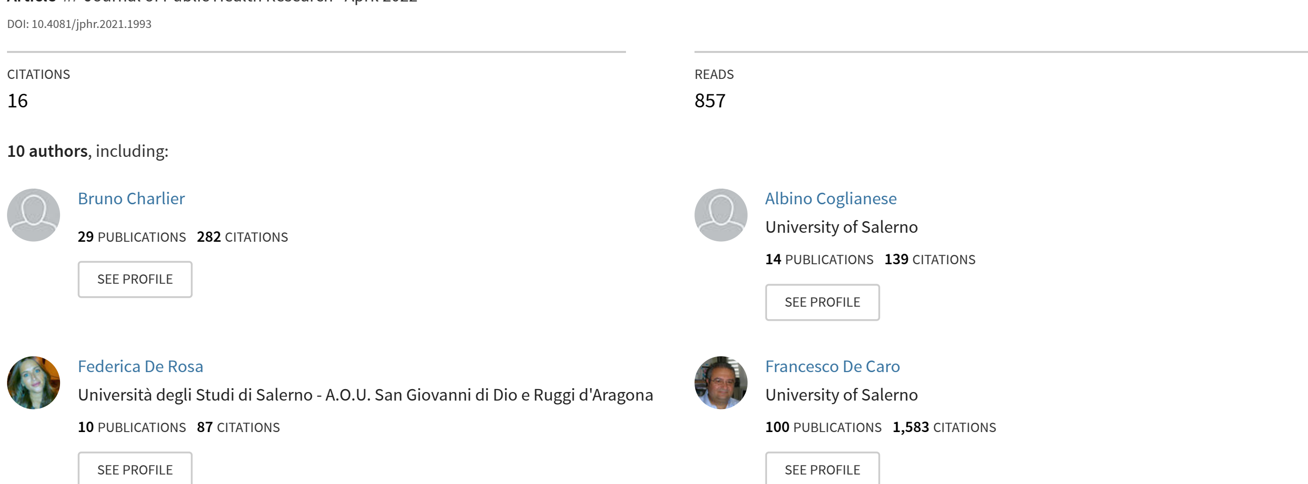

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Review

Chemical risk in hospital settings: Overview on monitoring strategies and international regulatory aspects

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Abstract

Chemical risk in hospital settings is a growing concern that health professionals and supervisory authorities must deal with daily. Exposure to chemical risk is quite different depending on the hospital department involved and might origin from multiple sources, such as the use of sterilizing agents, disinfectants, detergents, solvents, heavy metals, dangerous drugs, and anesthetic gases. Improving prevention procedures and constantly monitoring the presence and level of potentially toxic substances, both in workers (biological monitoring) and in working environments (environmental monitoring), might significantly reduce the risk of exposure and contaminations. The purpose of this article is to present an overview on this subject, which includes the current international regulations, the chemical pollutants to which medical and paramedical personnel are mainly exposed, and the strategies developed to improve safety conditions for all healthcare workers.

Introduction

Occupational exposure of healthcare workers to hazardous chemicals in hospital settings may negatively affect health and quality of life, and greatly differs depending on the type of clinical unit and specific job involved.[1,2] Chemical exposure in hospital environments may occur as acute intoxication or be the result of chronic and time-extended exposure of workers to low doses of contaminants. It can lead to damage to the nervous, hematopoietic, or reproductive systems,[3] and a potential relationship with neoplastic pathologies has been recently underpinned.[4]

In recent years, increasing attention has been focused on chemical risk prevention, which includes strategies aimed at protecting operators from both accidental and chronical exposure. Biological monitoring (BM) of workers and environmental monitoring (EM) of working areas are among the most effective actions that can effectively improve chemical risk management. Aim of this article is to provide an overview of the most common chemical pollutants to which medical and paramedical staff can be professionally exposed to, and the different strategies that can be used to improve chemical risk management.

International regulations

The European Agency for Safety and Health at Work (EU-OSHA) was established in the European Union in 1994, with the aim of improving European workplaces safety, productivity and health.[5] An important milestone in chemical risk management in workplaces was set by European Union regulation n. 1907/2006, concerning the Registration, Evaluation, Authorization and restriction of CHemical products (REACH).[6] This latter deals with the production and use of chemicals and their potential impacts on human health and environment. The REACH is also considered as a model in non-European countries such as South Korea. The Act on Registration, Evaluation, Authorization and restriction of CHemicals, established in 2015 and named K-REACH, is the Korean version of the regulation and aligns with the European model.[7]

Preventive measures for workers must refer to the Good Manufacturing Practice and Good Laboratory Practice.[8,9] In these guidelines, specific suggestions for each class of compounds are continuously updated, according to novel classifications. Formaldehyde (FA), as an example, has changed from "suspected causing cancer" agent to "may cause cancer" on January 2016.[10]

Italian regulation about safety in workplaces was first described in Legislative Decree (Lgs. D.) n. 626,[11] which follows the European Union (EU) specific directives. Later, Lgs. D n. 626 was updated by the European reference legislation,[12] which provides general guidelines for the management of workers health prevention. The analysis of the individual risk factors (physical, chemical, biological) was described in the already mentioned REACH and its last update EU Regulation n. 1272/2008 (CLP - Classification Labeling Packaging).[13]

In the United States, workers safety is managed by two federal agencies, the National Institute for Safety and Health (NIOSH), and the Occupational Safety and Health Administration (OSHA),

Significance for public health

Chemical risk in hospital settings is a growing concern that health professionals and supervisory authorities must deal with daily; acute and chronic exposure to commonly used compounds such as formaldehyde, organic solvents, anesthetic gases and anticancer drugs may lead to severe health effects for medical and paramedical personnel. This paper describes several aspects of chemical risk assessment in hospital settings by focusing both on regulatory aspects and monitoring strategies.

created in accordance with the "Occupational Safety and Health Act" signed on 29 December 1970.[14] OSHA is a regulatory agency that periodically revises safety and health standards, while NIOSH was established to help ensure safety and healthy working conditions, especially for what concerns the development of guidelines for work injuries prevention and related diseases.[14,15] In addition, the Environmental Protection Agency,[16] an independent executive agency of the government of the United States, deals with regulations concerning environment appraisal and protection. EPA, through the Toxic Substances Control Act (TSCA or TOSCA) issued in 1976, regulates the introduction of new or existing substances by assessing their chemical risk.[17]

Environmental and biological monitoring

Occupational exposure to chemical agents should be evaluated, when possible, by a combined approach involving both environmental monitoring (EM) and biological monitoring (BM).[18]

Environmental monitoring involves the collection of one or more measurements aimed at identifying and quantifying the presence, in a specific environment, of potentially harmful pollutants.[19,20] EM allows evaluating workers effective exposure to chemicals and building a risk map by: i) Quantifying exposure to chemical hazards and evaluating the most advanced methodologies available to limit their dispersion; ii) Activating emergency control procedures to contain and mitigate the effects of acute exposure events; iii) Observing trends of exposure and pollution in workspaces; iv) Developing safety strategies, based on scientific data presented in literature.

Quantitative data achieved in the EM should be critically evaluated based on the threshold limit values (TLVs) defined for each pollutant. TLVs are the maximum environmental concentrations of a compound to which a person can be subjected without adverse health effects, even in case of a prolonged exposure, and are established combining data derived from both epidemiological data related to the industrial field, and experimental research. However, it should be underlined that EM and TLVs fail to evaluate the effective amount of chemicals that permeate through skin, airways or epithelia, which may be responsible of causing acute or chronic harmful events in healthcare workers.[21]

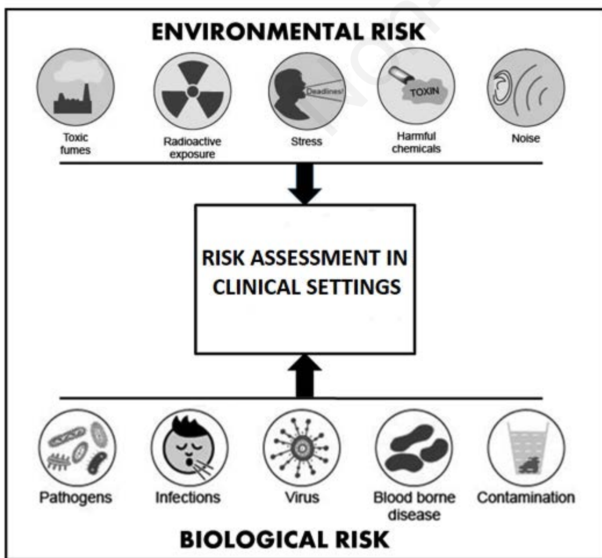

Figure 1. Main aspects contributing to environmental and biological risk in clinical settings.

TLVs for airborne pollutants should consider the dimensional mass fraction of the compound analyzed, which can be classified as follows:

- inhalable fraction, collected in any part of the respiratory tract.
- thoracic fraction, collected in the lung and along gas exchange region.
- respirable fraction, collected in the gas exchange region.

Nowadays, many standardized methods for the measurement in work settings of toxic chemical agents are available; conversely, direct methods to evaluate trans-dermic contamination are not always validated and few standardized procedures for the direct measurement of dermal exposure have been discussed so far.[19] Therefore, when possible, BM is a critical tool to evaluate the effective absorption of chemical toxic compounds.

BM can be defined as "a systematic continuous or repetitive activity for collection of biological samples for analysis of concentrations of pollutants, metabolites or specific non-adverse biological effect parameters for immediate application, with the objective to assess exposure and health risk to exposed subjects, comparing the data observed with the reference level and — if necessary — leading to corrective actions".[22] In BM, it is possible to analyse specific biological indicators (BI), which may be considered direct markers of a real or potential exposure condition.[23]

BIs can be different according to the biological matrix (urine, blood, tissues, exhaled air, *etc*.), organ or tissue in which they originate and/or accumulate (kidney, liver, nervous system, *etc*.) and depending on the specific chemical-physical characteristics (volatility, hydro-liposolubility, *etc*.) of the compound/s of interest.

BIs can be divided into:

- Biological exposure indicators (BEIs)
- Biological response (or effect) indicators (BRIs)
- Biological susceptibility indicators (BSIs).

A BEI can be an exogenous compound, its metabolite, or a product of its interaction with a target molecule or cell. BEIs are specific and often allow comparing values measured in exposed workers with those of a non-exposed population.[23-25] Biological response indicators rely on the identification and quantification of the biological effects that are produced in target tissues, such as chromosomal aberrations or genetic mutations in somatic cells.[23-25]

Biological Susceptibility Indicators are biomarkers related to mechanisms of susceptibility to chemical agents and can be divided into toxic-kinetic and toxic-dynamic BSI. These indicators relate on the single organism reaction to an exogenous compound.[23-25]

Limit values have also been set for BIs. Noteworthy, these values are generally defined by the Conference of American Governmental Industrial Hygienists (ACGIH)[26] and were initially established relying on the levels of chemical pollutants present in the chemical industry.

However, they may not be eligible to assess actual exposure to lower concentrations used for example in hospital settings, which can still be harmful for exposed healthcare workers.

Principal pollutants in healthcare facilities

In hospitals and other healthcare facilities, the attention is usually focused on preventing the biological risk to avoid nosocomial

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diseases and accidental infections. However, healthcare workers are frequently exposed to several types of harmful compounds and, among them, chemical risk is often underestimated (Figure 1). Some studies showed a higher frequency of pathologies in hospitals where air quality was judged unsatisfactory compared to those where air quality standards were respected.[27] Nevertheless, the guarantee of high air quality in hospitals remains a poorly developed field, both nationally and internationally.

Volatile organic compounds (VOCs) and several other toxic chemicals (*i.e.,* chemotherapeutic agents, xylenes and anaesthetic gases as an example) routinely used in healthcare settings and clinical laboratories (Figures 2 and 3) may cause adverse health effects on exposed people,[28] which are different according to inter-individual variabilities that may influence pollutant diffusion, neutralization and excretion.[20] Airborne pollutants, based on their physical status and mass, are classified in aeriform and particulate, which leads to different absorption rates by inhalation, lung retention times, and alveolar diffusion.[19]

Formaldehyde

Formaldehyde (FA) is a colourless, acrid-smelling VOC used for tissue fixation in anatomic pathology laboratories, and can cross-link with several endogenous organic compounds, such as proteins and nucleic acids, causing irreversible modifications of these molecules.[29-32] At room temperature FA is gaseous and, consequently, mostly absorbed by inhalation and deposited in the upper respiratory tract. In addition, as an aqueous solution of formalin, skin exposure is also possible. The great reactivity of FA toward lipids, proteins and nucleic acids is responsible for most observed toxic effects. Acute or chronic toxic effects are strictly time- and concentration-dependent: exposure to 0.3-1 ppm (part-*per*-million) in the environment cause skin, ocular and upper respiratory tract irritation, as well as headache, sleep disorders and fatigue, while exposure up to 4 ppm is associated to serious respiratory tract irritations. Asthma or nasopharyngeal cancers have also been reported.[33] The Occupational Exposure Limits (OELs) for formaldehyde are set at 300 part-per billion (ppb; 0.370 mg/m^3) by the ACGIH.[26] However, several studies demonstrated that these limits are often unattended during many procedures, such as autopsies.[34-36] Bono and co-workers showed that during histological samples preparation, workers were exposed to air concentrations of FA above 66 $\mu g/m^3$, which seemed to be responsible for malondialdehyde-deoxyguanosine adducts (M1-dG), a biomarker of oxidative stress and lipid peroxidation.[36] Costa and co-workers have screened by comet assay the presence of chromosomal aberrations and DNA damage in human peripheral blood lymphocytes of 84 workers exposed to FA. The data obtained showed a potential health risk at concentrations higher than 0.38 ppm of FA.[37] Starting December 2009, it has become mandatory to limit FA exposure levels to protect workers health. As a result, formaldehyde exposure has been further limited setting two-time limit values: the short-term OEL (15-min reference period) at 0.2 ppm and the 8-h working day OEL at 0.4 ppm.[21]

Several BIs have been investigated for the BM of healthcare workers exposed to FA: complete blood counts, evaluation of sister chromatid exchange, comet-assay on blood and buccal swab. While the possibility that inhaled FA may be present in biological fluids in significant concentrations needs to be further investigated, quantification of urinary FA at the end of the work shift is so far the most used test.[38-41]

Xylene

Xylene, a mixture of three organic isomers of dimethylbenzene, is a colorless liquid with a sweet and aromatic odor that can be smelled at 1 ppm, widely used for elimination of paraffin traces from histological samples before DNA staining or extraction.[42] Acute toxicity after exposure to low concentrations of xylene includes skin, eye, and respiratory tract irritation; however, massive inhalation can cause central nervous system depression (from headache to coma and death), pulmonary oedema and respiratory arrest.[43] Long-term exposure can produce anemia, thrombocytopenia and leukopenia, cardiac abnormalities with electrocardiogram modifications, dyspnea, and cyanosis.[44] In pregnant women, exposure to xylene increases the probability of spontaneous abortions. Short-term OELs are set at 100 ppm, and 8-hour OELs at 50 ppm.[45]

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Molecules	Structures
Formaldehyde	
Benzene	
Toluene	
Xylenes (o- , m- , p-)	

Figure 2. Chemical structures of common volatile organic compounds (VOCs) used in clinical settings.

Molecules	Structures
Isoflurane	
Sevoflurane	
Desflurane	

Figure 3. Chemical structures of common anesthetic gases used in clinical settings.

Anesthetic gases

The first gaseous anesthetic agent used was nitric oxide in 1844. Subsequently, the use of diethyl ether and FA was approved for most surgical procedures. In 1950, modern halogenated or fluorinated inhalation anesthetic gases were introduced in the clinical practice. Halothane, a member of this class, is by far the most used anesthetic gas, along with nitrogen oxide.[46] Nowadays, new inhalation anesthetics such as isoflurane, desflurane, and sevoflurane (Figure 3) are used, alone or in combination with nitrogen oxide. Halogenated inhalants cause a rapid induction and recovery of anesthesia and are associated with an early post-operative mobilization because of their low liquid/gas partition coefficient.[47]

Since 1967, several studies showed that exposure to anesthetic agents, including halogenated can cause adverse effects on exposed workers.[48-52] Occupational exposure to residual anesthetic gas concentrations may produce headache, dizziness, lethargy, fatigue, memory problems, neuro-behavioral changes.[53-55] Studies performed on animal models indicates that chronic exposure to anesthetic gases can lead to miscarriages and congenital malformations;[47,53,56-59] Popova and co-workers. reported fetal resorption in rats even at very low concentrations (9 ppm).[60]

Several bio-monitoring studies have suggested the existence of a strong relationship between exposure to halogenated anesthetic gases and the risk of genotoxicity for surgery room staff. It has been observed that nitric oxide can interfere with vitamin B12 and irreversibly deactivate methionine synthase in CNS.[61,62] Some studies also report effects of teratogenicity or increase in miscarriages in nitric oxide exposed women. A higher prevalence of congenital anomalies in the offspring of women professionally exposed during pregnancy and spontaneous abortions in women pregnant with exposed men have also been reported.[63,64] For these reasons, it is important to monitor workers exposed to anesthetic gases by both EM of chemicals in exhaled air and BM of compounds in urine.[65-67] Several studies have highlighted a good correlation between measured amounts of unmodified anesthetic gases in urine and in breathing air; these studies have proposed to use the same OELs for both BM and EM.[68-70] Other authors suggested instead the evaluation of the urinary concentration of anesthetic gases metabolites.[48,66,71,72]

Accepted limits for halogenated substances are 2 ppm when used alone and 0.5 ppm when in combination with nitrous oxide, as suggested by NIOSH.[15] The ACGIH set the Threshold Limit Values (TLVs) for nitrous oxide at 50 ppm[26] while the Italian Health Department established the biological exposure limits at 27 mg/L for urinary nitrous oxide and 3.32 mg/L for urinary isoflurane, equivalent to environmental levels of 50 and 2 ppm, respectively.[73] To the best of our knowledge, no TLV are currently present for sevoflurane or other halogenated anaesthetic gases.

Anticancer drugs

Anticancer drugs (ADs) are used for the treatment of solid and hematologic tumors and are classified based on their action mechanism; however, most of them do not show specific selectivity towards cancer cells and thus have an intrinsic cytotoxicity on normal cells.[74] Consequently, the International Agency for Research on Cancer (IARC) has identified ADs as “potential carcinogens” or “carcinogens” agent for humans.[75]

Anticancer drugs toxicity has been known for decades and include effects such as liver, kidney, gastric, dermatological and haemopoietic damages.[76] Antineoplastic drugs are generally irritating agent for mucous membranes, and they can cause local toxic effects (phlebitis, allergies) and systemic effects (anaphylactic shock and organ toxicity). Cellular necrosis, with lesions that may cause ulcers variable in severity and extension are also reported.[77] According to IARC, it is possible that ADs can cause cancer in patients treated for non-oncologic pathologies; a well-known example of this, is the use of immunosuppressive drugs for organ transplants.[78] Furthermore, new tumors formation, unrelated to primary pathology, has been reported in patients with solid cancers in treatment with ADs, especially in acute myeloid leukaemia.[79] Finally, teratogenic effects on the fetus may occur in ADs exposed uterus.[80]

The risks for healthcare workers exposed to ADs have been known since the 70s, even in case of accidental exposure.[81,82] Chronic exposure to small amounts of ADs in healthcare workers might cause rashes, allergic reactions, or headaches,[83] and long-term effects including genomic instability and increased risk of reproductive dysfunctions.[84-86] Increased AD levels were found in urine samples of nurses from oncology units compared to other wards especially during work shifts.[82] Anticancer drugs can be inhaled or adsorbed through skin; cutaneous absorption has been observed for cyclophosphamide, 5-fluorouracil and methotrexate, also after the use of Personal Protective Equipment (PPE). This might be related to the permeability of latex gloves to these molecules. In addition, cyclophosphamide has a very low vapor pressure and laminar flow hood cannot retain volatilized cyclophosphamide molecules that can pass through the large filter pores.[87] Another source of occupational exposure is the domiciliary intravenously or subcutaneously administration of chemotherapeutic agents (a practice that is currently discouraged). In this case, major risks can arise from air expulsion from the syringe before drug administration and by drug leaking from connectors or vials. For these reasons, EM and BM are both required for the correct management of chemical risk prevention in personnel working in close contact to antineoplastic drugs, such as laboratories, hospitals and pharmaceutical companies. In EM, AD quantification on surfaces and objects is carried out using wipes and pad tests.[88]

Sampling methods in BM

Toxic compounds can be adsorbed by skin contact, inhalation, and/or ingestion; it mainly depends on the chemical-physical properties of the molecules and the type of exposure. These characteristics also influence tissue distribution, as highly water-soluble pollutants distribute in all body fluids, while lipophilic substances will likely concentrate in lipid-rich tissues (*i.e.,* the brain). Tissue characteristics such as composition, pH, permeability and vascularization also influence chemicals absorption in the body.

Compounds can be eliminated, as intact compounds or their metabolites, through various pathways, such as breathing, urine, fecal, and by lactation way. Exogenous compounds undergo metabolic modifications of their chemical structure, such as oxidation, reduction, hydrolysis or a combination of these, often followed by conjugation with an endogenous substrate. Conjugation is a key-step for exogenous substrates excretion and include reaction with glucuronic acid, amino acids, acetylation, sulphate conjugation and methylation. Metabolism and excretion of intact compounds, their metabolites and the ratio between these molecules are influenced by several inter-individual variabilities such as age, diet and health status, presence of known polymorphisms that affect metabolism, body hydration, or time after chemical exposure.[89,90] These variables, together with the pharmacokinetic properties of chemicals, must be taken into consideration during the initial set-up of an analytical method for the BM of a specific BI.

Different biological matrixes (*e.g.,* blood, urine, breath) may be selected based on expected molecule concentration, its kinetics and the difficulty related to the sample collection (urine and breath

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are the less invasive samples to collect).[91] Sampling times is also important and strongly depends on the biological half-life of the compound of interest and on the time interval during which the compound has been handled (*e.g.,* sampling before, after or at any time during the working shift). Sampling of molecules with a short half-life may give indications on a recent exposure and should be performed quickly after a potential contact, while monitoring of long half-life indicators can provide information about a chronic exposure.[90] The analysis of exhaled air (breath test) is an attractive non-invasive technique for the determination of volatile organic compounds (VOCs) derived from professional exposure and might be used for many toxic agents. Although exhaled air is a simpler matrix compared to urine and blood, the use of breath test in BM shows some challenges - mainly related for example to the extremely low concentration of the analyzed compounds - which hampers its diffusion into routine practice.[92]

Urine samples are used to measure contaminating chemicals, metals, and hydrophilic metabolites; however, concentrations may vary based on urine volume and composition and this may lead to analytes dilution.[89-91] Therefore, normalization of excreted compounds should be performed by using a molecule present at a constant concentration regardless of the urinary volume collected, such as creatinine. Some volatile chemicals, such as formaldehyde for example, are eliminated in the kidney by diffusion, which is driven by the urine/blood distribution coefficient; thus, in this case, normalization of the concentration value is not required.

Blood is considered as the best biological matrix because the majority of BIs are present in the blood for a certain amount of time and data normalization is not required, but venipuncture is an invasive procedure that must be performed only by trained personnel.

Sampling methods in EM

Environmental Monitoring activities in workplaces are carried out to determine the concentration levels of pollutants, according to their chemical-physical and toxicological characteristics, and to identify the sources of emission. Environmental monitoring responds to: i) surveillance activities following confirmed or potential pollution situations; ii) complaints raised by exposed workers; iii) surveillance activities to evaluate the effectiveness of strategies previously adopted; iv) the need for specific information to facilitate decision-making processes when assessing the exposure of workers with reference to the different residence times in a given environment; v) the verification of compliance through guidelines established by competent authorities.

In this framework, a preliminary qualitative evaluation is required to identify the pollutants or their chemical class. For the identification and subsequent quantization of environmental pollutants, two approaches are currently available: direct measurement methods and indirect measurement methods.[89]

Direct measurement methods use devices and instruments to quantify gases, vapors or aerosols without user manipulation and without sending sample to an external laboratory. These devices allow an immediate evaluation without the need to preserve or manipulate the sample later. Direct measurement systems generally consist of a sampling system, a detector, an electronic processing system, a display and a memory device. Although they allow for an immediate evaluation of the concentration pollutant over time, they suffer of several drawbacks such as measuring range, detection limit, precision, accuracy, resolution, interference and so on. Indirect measurements methods involve collecting air samples in the investigated environment which are then analyzed in laboratory. According to monitoring objectives, short-term samplings (sampling time between a few minutes and several hours) can be planned, generally carried out with canisters[93] or active sampling on adsorbent cartridges[94] or long-term samplings (time sampling from a few hours to several days), generally performed with diffusive samplers.[95]

Canisters are stainless steel containers with a variable volume from 400 ml to 15 L, subjected to an electro-passivation process to reduce the presence of chemically active polar sites and subsequently coated on the internal surface with a thin layer of chemically silica bonded. The canister, after being cleaned, is placed under vacuum and is ready for sampling, which can be instantaneous or mediated. The instantaneous sampling is performed by simply opening the valve placed at the closure of the canister, while the "mediated" one is carried out by applying an orifice calibrated at the opening of the canister.[93] Active sampling, with tubes containing adsorbent materials, is carried out with appropriate systems where the air is first drawn into the tube through a sampling pump, calibrated to the required flow rate. Pollutants react with the specific substrate causing chromatic variations in a concentration-dependent manner or can be trapped and, at the end of the sampling, the tubes are stored until desorbed and analyzed in laboratory. Once the sampling phase is complete, the tubes must be closed with the appropriate caps and stored in glass or metal containers in refrigerated systems maintained at controlled temperature until analysis.

Passive devices are used for long-term measurement through a diffusion air process according to Fick's laws of diffusion. Diffusive samplers have been found to be useful and cost-effective alternatives to conventional pumped samplers. The passive sampler consists of an adsorbent cartridge inserted inside a diffusive body, whereby analyte molecules diffuse along the concentration gradient, from the ambient concentration, which corresponds to the outer part of the sampler, to the effective zero concentration, present on the surface of the absorbent within the sampler.[95] For both active and passive method, at the end of the sampling, the pollutants are chemically or thermally desorbed from the support and transferred for the analytical determination, which can be subsequently carried out using various techniques, such as gas chromatography, ion chromatography or high-performance liquid chromatography (HPLC).[95,96]

The air quality of operating rooms is usually monitored using a real-time or an integrated air sampling. In real-time methods, the concentration of anesthetics is directly measured with a portable gas-chromatograph equipped with a multiple sampling system. In integrated air sampling, air is collected on an appropriate adsorbent tube and analyzed by gas-chromatography in laboratory.[15]

Methods of analysis

The selection of a suitable analytical method is driven by the characteristics of each investigated pollutant. For VOCs, the International Organization for Standardization (ISO) suggests the use of direct measurement instruments equipped with a flame ionization (FID) or photoionization (PID) detector specific for each type of pollutant.[97] For VOCs analysis, "continuous" automatic analyzers are widely used; these all-in-one instruments collect the air sample and perform a real-time analysis. Generally, a specific device is needed for each analyte. For air sampling, it is important to define instrument volumetric flow, time of sampling and sampled air volume. However, gas chromatography coupled with mass spectrometry (GC/MS) remains the gold standard for accurate and simultaneous quantification of a wide range of VOCs. In this case,

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gaseous substances are collected from the environment or adsorbed on special supports, eluted with a gaseous mobile phase and analyzed based on mass/charge ratio for each analyte. For EM of volatile solvents, such as FA, specific and dedicate equipment is also available.[98]

Chromatography has significantly improved both EM and BM. Compared to classic immunometric or radiolabeling techniques, chromatography is faster and cheaper. High-performance liquid chromatography (HPLC) coupled with different types of detectors, such as Photodiode array, Fluorimeter and other, is available in almost all healthcare facilities and is employed for the simultaneous detection and quantification of many compounds. The use of liquid chromatography to monitor compounds potentially harmful to health extends to an ever-increasing number of compounds, such as solvents,[99,100] commonly used drugs[101] and cytotoxic agents.[102] Ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) has a high selectivity and sensitivity for the simultaneous analysis of several pollutants and chemicals from complex matrices, such as biological fluids, even at very low concentrations.[103] The great versatility and sensitivity of mass spectrometry render this technique suitable for both BM and EM. UHPLC-MS/MS high selectivity allows for the simultaneous analysis of a great number of compounds in complex matrices, such as biological fluids, even at very low concentrations.[104]

Inhalation of dust or drug droplets has long been considered the main route of accidental exposure to toxic agent for hospital personnel. On the contrary, recent studies indicate skin direct contact as the main route of exposure, especially through the hands and forearms of nurses and technicians who often wear uniforms with short sleeves. Exposure can occur also through accidental ingestion or for hand-to-mouth contamination.[105] Therefore, wipe test is currently the preferred method to check workspace surfaces and operator gloves. According to this method, surfaces, gloves or even the gowns of the operators are rubbed with wipes impregnated with a solvent. Wipes are then squeezed, and the desorbed solvent is analyzed by previous described chromatographic methods.[88]

Conclusions

In recent years, the interest in prevention has been constantly increasing, diversifying itself from the simple concept of "protection", viewed as the whole set of measures and instruments aimed at protecting from chemical hazard accidental exposure. Prevention processes involving a routine monitoring of the presence and level of potentially toxic substances, can reduce the risk of work injuries derived from chemicals. Furthermore, increasing attention should be focused on the long-term damage caused by chronic exposure to contaminants. Safety and health conditions of all operators operating in health facilities should be the ultimate goal to look for.

Healthcare workers are frequently exposed to accidental biological and chemical risks as occasional contamination or prolonged exposure. Contaminations are often detected in hospitals, even when trained staff rigorously carry out all safety procedures and monitoring practices. For this reason, healthcare facilities need to routinely monitor and continuously improve risk management plans and protective equipment, making monitoring simpler, faster, and less expensive. In addition, the discovery of new-targeted therapies for the treatment of solid and hematologic tumors requires continuous updates in risk management plans and biohazard risk prevention that should be directed not only to operators, but also to all potentially exposed subjects, such as patients' close relatives or volunteers. The long-term monitoring and systematic records could help identifying the risks related to toxic agent exposure. Therefore, EM and BM in healthcare facilities should not only be a project plan merely following national and/or internationally regulations or a solely execution of standard procedures, but a concrete tool for an effective protection of all workers involved in health management.

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