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DEVELOPMENT, VALIDATION AND REAL LIFE APPLICABILITY OF NEW BIOANALYTICAL METHODS IN THE ASSESSMENT AND PREVENTION OF CHEMICAL RISK IN HEALTHCARE ENVIRONMENTS

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RATIONAL

Routine hospital operations may expose healthcare workers to a variety of chemical agents that are potentially hazardous for their safety and health. Occupational exposure in hospital settings can adversely affect health and quality of life of workers, and can be different based on the specific hospital department involved. In fact, depending on the work performed, chemical pollution may originate from multiple sources, such as the use of sterilizing agents, disinfectants, detergents, solvents, heavy metals, dangerous drugs and anesthetic gases. Chemical exposure can occur as an acute intoxication or be the result of chronic and prolonged exposure of workers, also at low doses of contaminants. It can lead to damage to the nervous, hematopoietic or reproductive systems and in specific cases, such as the exposure to antiblastic agents, may be related to the onset of neoplastic pathologies. All healthcare and service personnel, including laboratory technicians, cleaning and maintenance personnel, are potentially exposed to different types of chemicals every day. These substances, including drugs, are various in chemical nature and mechanism of action, with a wide range of harmful effects on organs and tissue.

The problem of chemical risk for workers in hospitals has been recognized for over thirty years and different approaches are currently used to verify working conditions. In healthcare, the exposure of workers to chemical agents potentially harmful to health is regulated by specific legislation. Health surveillance, intended as the set of interventions to ensure workers safety in the hospital environment, must deal with the management of exposure and the assessment of potential health risks. Italian legislation provides that the employer is responsible for the protection of all workers from the undue effects of exposure to dangerous chemical agents ("Legislative Decree 9 April 2008, n. 81 Consolidated Law on Health and Safety at Work", published in the Ordinary Supplement no.108 / L to the Official Gazette no.101 of 30 April 2008). The implementation of the regulatory framework requires the adaptation of technical and organizational measures. The improvement of prevention procedures and the constant monitoring of the presence and amount of potentially toxic substances, both in the workplace (environmental monitoring) and in workers biological fluids (biological monitoring), may significantly reduce the risk of exposure and contamination. In this

framework, pharmacological and clinical research activities, which often involve the use of compounds or methodological procedures that may be harmful for the workers involved, should also be included. In this dissertation, methodological aspects for the detection and dosage of some of the most common chemical pollutants in healthcare settings and the related strategies used to improve the management of chemical risk in potentially contaminated environments are discussed.

1. INTRODUCTION

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 - BM) and in working environments (environmental monitoring - EM), might significantly reduce the risk of exposure and contaminations.

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]. 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[2] and a potential relationship with neoplastic pathologies has been recently underpinned[3]. Environmental monitoring of working areas and BM of workers are among the most effective tools for the appropriate management of chemical risk assessment.

1.1 Workplace safety legislative evolution: a focus on antineoplastic agents and formaldehyde

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), created in accordance with the "Occupational Safety and Health Act" signed on 29th December 1970[4]. 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[5]. In addition, the Environmental Protection Agency (EPA) an independent executive agency of the United States government, 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[6].

The first and most complete text recognized at an international level concerning risk assessment in work environments, was developed in 1986 by the OSHA[7]. The investigations carried out on the work settings responsible for the preparation and administration of antineoplastic agents (AAs), highlighted a lack in standardization procedures in controls and personal protective equipment (PPE), as well as insufficient information and training of all the staff involved in AAs manipulation. Based on these data, the OSHA drafted the first recognized official guidelines, underlining two essential elements: the information and training of all the staff involved in the manipulation of the AAs and the use of class II laminated flow hoods, also called Biological Security Chambers (BSC, Biological Security Cabinet)[8] (**Figure 1**). In this document a synthesis of the risks associated to the manipulation of AAs were provided (adverse effects in short and long time), along with a list of technical-operational procedures useful to reduce the risk of operators contamination, a description of the devices necessary for containment exposure and a list of drugs in use.

In 1995, an updated version of these guidelines became the reference point for international regulations. Compared to the previous one, this version took into consideration a wide

number of drugs or dangerous substances (Hazardous Drugs, HDS) and described more in detail the PPE and collective protection devices, recommending in particular the type of hoods that should have been used. Currently, the most comprehensive and updated international document is the NIOSH Alert, published in September 2004. The current update of 2016 introduced new drugs and a review of all attachments[9].

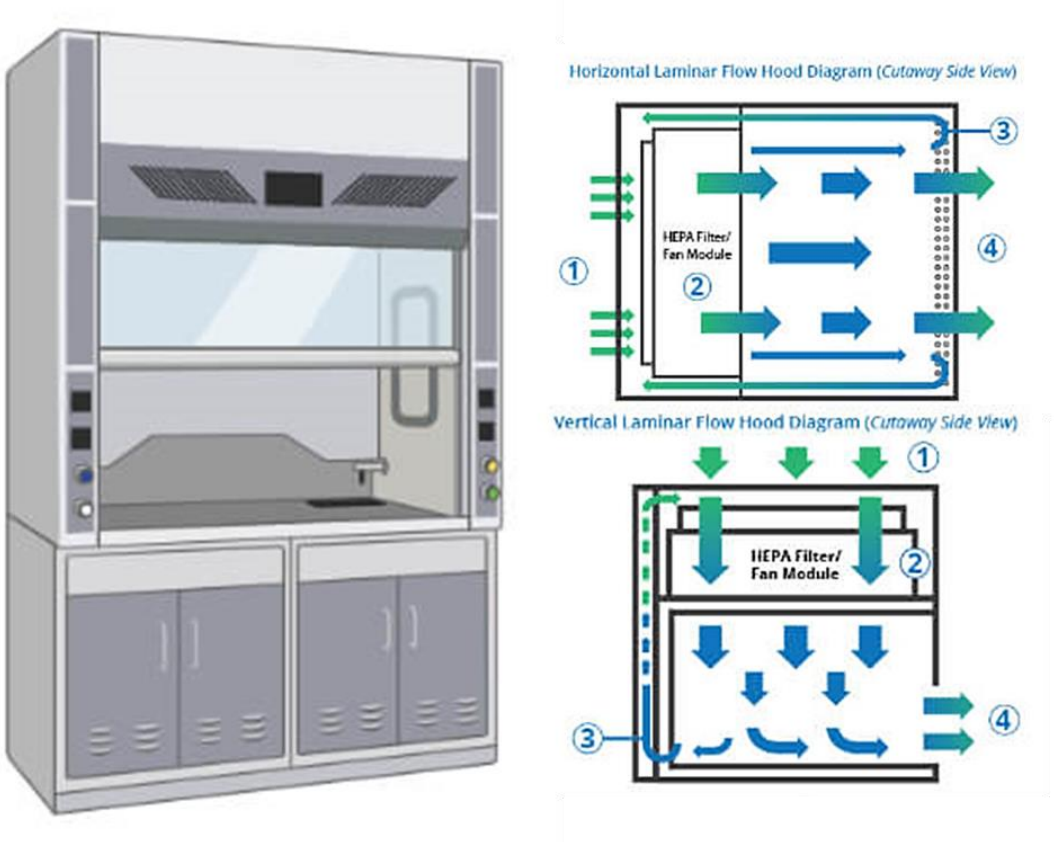


Figure 1. Laminar flow hood with HEPA filters

In the European Union (EU), the European Agency for Safety and Health at Work (EUOSHA) was established in 1994, with the aim of improving European workplaces safety, productivity and health[10]. An important milestone in chemical risk management in workplaces was set by EU regulation n. 1907/2006, concerning the Registration, Evaluation,

Authorization and restriction of CHemical products (REACH)[11]. This latter deals with the production and use of chemicals and their potential impacts on human health and environment. Preventive measures for workers must refer to the Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP)[12,13]. 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. The analysis of the specific risk factors (physical, chemical, biological) was described in the already mentioned REACH and its updates EU Regulation (CLP - Classification Labeling Packaging)[14]. Italian regulation about safety in workplaces was first comprehensively described in Legislative Decree (Lgs. D.) n. 626[15], which follows the EU specific directives. Later, Lgs. D n. 626 was updated by the European reference legislation, which provides general guidelines for the management of workers health prevention. With the implementation of Directive 97/24/EC on the "Protection of health and safety of workers against the risks deriving from toxic chemical agents", the Italian and European legislation has been conformed. Although numerous compounds have been recognized by the International Agency of Research on Cancer (IARC) and by other authoritative international agencies such as carcinogenic substances for humans, the rules of Title VII of Legislative Decree n. 626/94 "Protection from carcinogenic agents" do not apply to all of these compounds. For example, since the AAs are classified as “drugs”, they are not subjected to the Directive 67/548/EC and, therefore, the R45 label "can cause cancer" or the label R49 "can cause inhalation cancer" are not applied to them. Even so, current data make it possible to affirm that AAs must be considered, also for professionally exposed workers, as potentially carcinogenic substances, although it is not possible to exactly predict the extent of the risk associated with their exposition. To date, Italian legislation regarding safety in the

workplace is enclosed in the "Consolidated Law on Health and Safety at work" - Legislative Decree 9 April 2008, n. 81,coordinated Text with Legislative Decree 3 August 2009, n. 106, Consolidated Law on Occupational Health and Safety (last version: January 2023)[16].

1.2 Principal pollutants in healthcare facilities

In hospitals and other healthcare facilities, the attention is usually focused on preventing the biological risk to avoid nosocomial diseases and accidental infections. However, healthcare workers are frequently exposed to several types of harmful compounds and, among them, chemical risk is often underestimated[17] (**Figure 2**).

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[18]. 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 anesthetic gases as an example) routinely used in healthcare settings and clinical laboratories (Figures 2 and 3) may cause adverse health effects on exposed workers[19], which are different according to inter-individual variabilities that may influence pollutant absorption, distribution, metabolism 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[21].

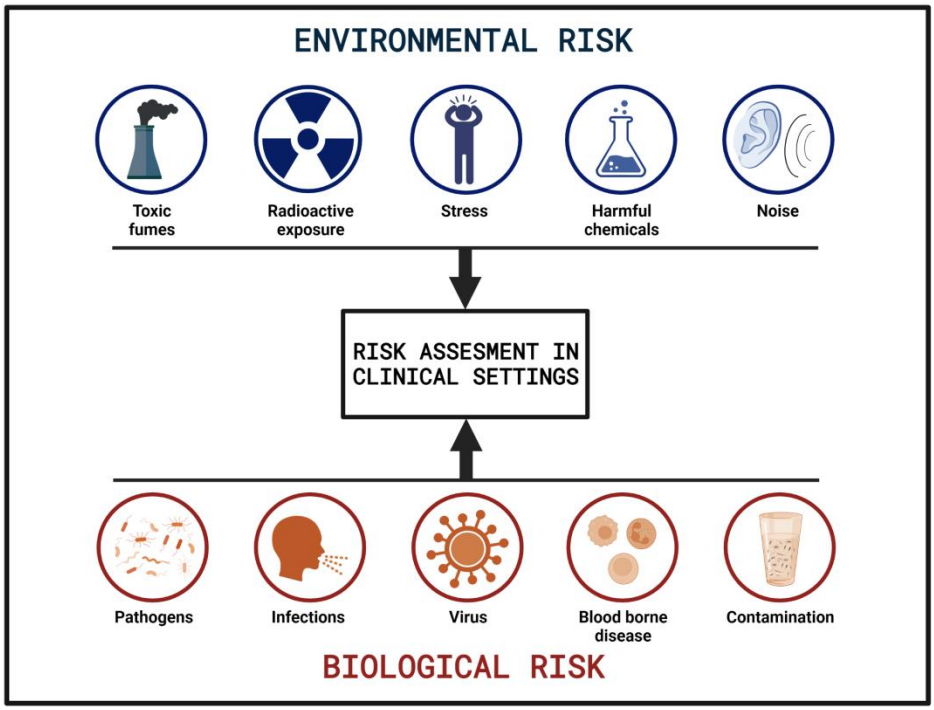


Figure 2. Primary chemical and biological risk factors in clinical practice.

1.2.1 Formaldehyde

Formaldehyde is a colorless, 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[22]. 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[23]. The Occupational Exposure

Limits (OELs) for environmental FA are set at 0.3 part-per million (ppm; 0.368 mg/m^3) by the American Conference of Governmental Industrial Hygienists (ACGIH)[24]. However, several studies demonstrated that these limits are often unattended during many procedures such as autopsies. Bono and co-workers showed that during histological samples preparation, workers were exposed to air concentrations of FA above 53.74 ppm, which seemed to be related for the increase in malondialdehyde-deoxyguanosine adducts (M1-dG), a biomarker of oxidative stress and lipid peroxidation[25]. 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[26]. Starting December 2009, it has become mandatory to limit FA exposure levels to protect workers health[27]. As a result, FA exposure has been further limited by setting two-time limit values: the short-term OELs (15-min reference period) at 0.2 ppm and the 8-h working day OELs at 0.4 ppm by European Chemicals Agency (ECHA)[28]. Several Biological Exposure Indicators (BEI) 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. Quantification of urinary FA at the end of the work shift remain still the most common test used in the investigation of the possibility that inhaled FA may be present in biological fluids in significant concentrations[29].

1.2.2 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[30]. 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 edema and respiratory arrest[31]. Long-term exposure can produce anemia, thrombocytopenia and leukopenia, cardiac abnormalities with electrocardiogram modifications, dyspnea, and cyanosis[32]. In pregnant women, exposure to xylene increases the probability of spontaneous abortions[33]. Short-term OELs are set at 100 ppm, and 8-hour OELs at 50 ppm[14].

1.2.3 Anesthetic gases

The first gaseous anesthetic agent used was nitrous oxide in 1844. Later, 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 nitrous oxide[34]. Nowadays, new inhalation anesthetics such as isoflurane, desflurane, and sevoflurane are used, alone or in combination with nitrous 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. Since 1967, several studies showed that exposure to anesthetic agents, including halogenated ones, can cause adverse effects on exposed workers[35]. Occupational exposure to residual anesthetic gas concentrations may produce headache, dizziness, lethargy, fatigue, memory problems, and neuro-behavioral changes[36]. Studies performed on animal models indicate that chronic exposure to anesthetic gases can lead to miscarriages and congenital malformations. 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 nitrous oxide can interfere with vitamin B12 and irreversibly deactivate methionine synthase in the central nervous system[37]. Some studies also report effects of teratogenicity or increase in miscarriages in nitrous 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[38]. For these reasons, it is important to monitor workers exposed to anesthetic gases by both EM of chemicals in air and BM of compounds present in urine and exhaled air. Some studies have highlighted a good correlation between measured amounts of unmodified anesthetic gases in urine and in breathing air[39]. 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. The ACGIH set the Threshold Limit Values (TLVs) for nitrous oxide at 50[24], while the Italian Department of Health, with Circular n. 5 of March 14 1989, 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[40]. To the best of our knowledge, no TLVs are currently present for sevoflurane or other halogenated anesthetic gases. **Figure 3** shows the chemical structures of some of the most used VOCs in hospitals setting.

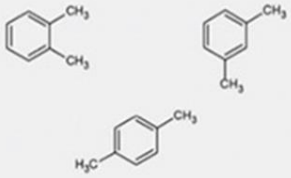
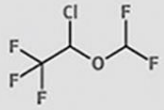
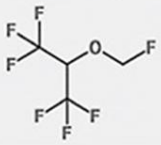
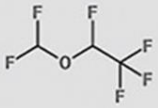
Molecules	Structures
Formaldehyde	
Benzene	
Toluene	
Xylenes (o- , m- , p-)	
Isoflurane	
Sevoflurane	
Desflurane	

Figure 3. Chemical structures of common volatile organic compounds used in clinical settings.

1.2.4 Antineoplastic Agents

Tumors are the second leading cause of death in developed countries after cardiovascular disease[41]. About 98% of neoplasms are treated pharmacologically, and about 85% of medical treatment involves the use of AAs. Based on this consideration, the total number of operators in oncology services can be reasonably estimated at 5,000 units. Antineoplastic agents include

a wide range of drugs, heterogeneous in structure and activity, capable of inhibiting proliferation and cellular replication, and used for their properties in the treatment of patients with tumors. However, the specificity and selectivity of their therapeutic activity are limited and these molecules also exert their effects on healthy cells[41]. Indeed, all AAs have toxicity against cells, tissues and organs. Some AAs show mutagenic, teratogenic and carcinogenic properties as cell growth inhibitors. They induce DNA or cell division alterations with consequent death of active replicating cells[42].

The most frequent paths of involuntary or accidental absorption are the transdermal penetration and inhalation. Although technical support, PPE and handling protocols are continuously improved to reduce contamination[43], accidental exposure of the operators cannot be completely eliminated. Therefore, a significant reduction in risk could undoubtedly be obtained by limiting environmental contamination and the airborne of drugs during the manipulation. Most common used AAs are very different from each other in terms of chemical and toxicological characteristics, but they share the frequency of use, the high quantities prescribed to patients and the toxic health effects.

The exposure to AAs has been well-known since the early 1970s as a potential risk to healthcare professionals. In 1979, Falck and coworkers described for the first time a significant increase in the risk of mutagenicity in urine samples collected by a staff of nurses assigned to the preparation and administration of AAs[44]. Since then, several studies have confirmed the occurrence of adverse health effects for workers exposed to AAs, including the impact on the pregnancy rate[45], chronic[46]and acute effects[47]. Although numerous AAs have been recognized by the IARC as carcinogenic, or probable carcinogenic to human, no prevention rule is applied to these substances, since they are used for therapeutic purposes only. Considering this, some among the above mentioned national and international bodies,

responsible for worker safety, have drawn up documents aimed at providing precise guidelines for the reduction of risk during AAs manipulation. Following national and international guidelines, AAs must be treated into specific Operating Units called "units for the preparation of cytotoxic drugs" (UCDP). These must comply with specific requirements and are usually composed of three different rooms (**Figure 4**):

- *Protected area:* this is the first room, accessed from the pharmacy service corridor. This area is intended for support to laboratory activities and must have a pressure of 0 Pa, a controlled temperature, and no openable windows. The pressure of all rooms must be monitored by differential air pressure gauges.
- *Airlock or filter zone:* these areas are intended for material inlet and outlet and for personal dressing. The area must be equipped with filtered air inlet (HEPA H14 filters, according to UNI EN 1822). There must be a pressure difference of approximately 10-15 Pa between the two areas.
- *Operating area:* area for sterile products mixing under a unidirectional vertical laminar flow hood, equipped with three BIOHAZARD HEPA Class II filters, bio-safe, according to EN 12469).

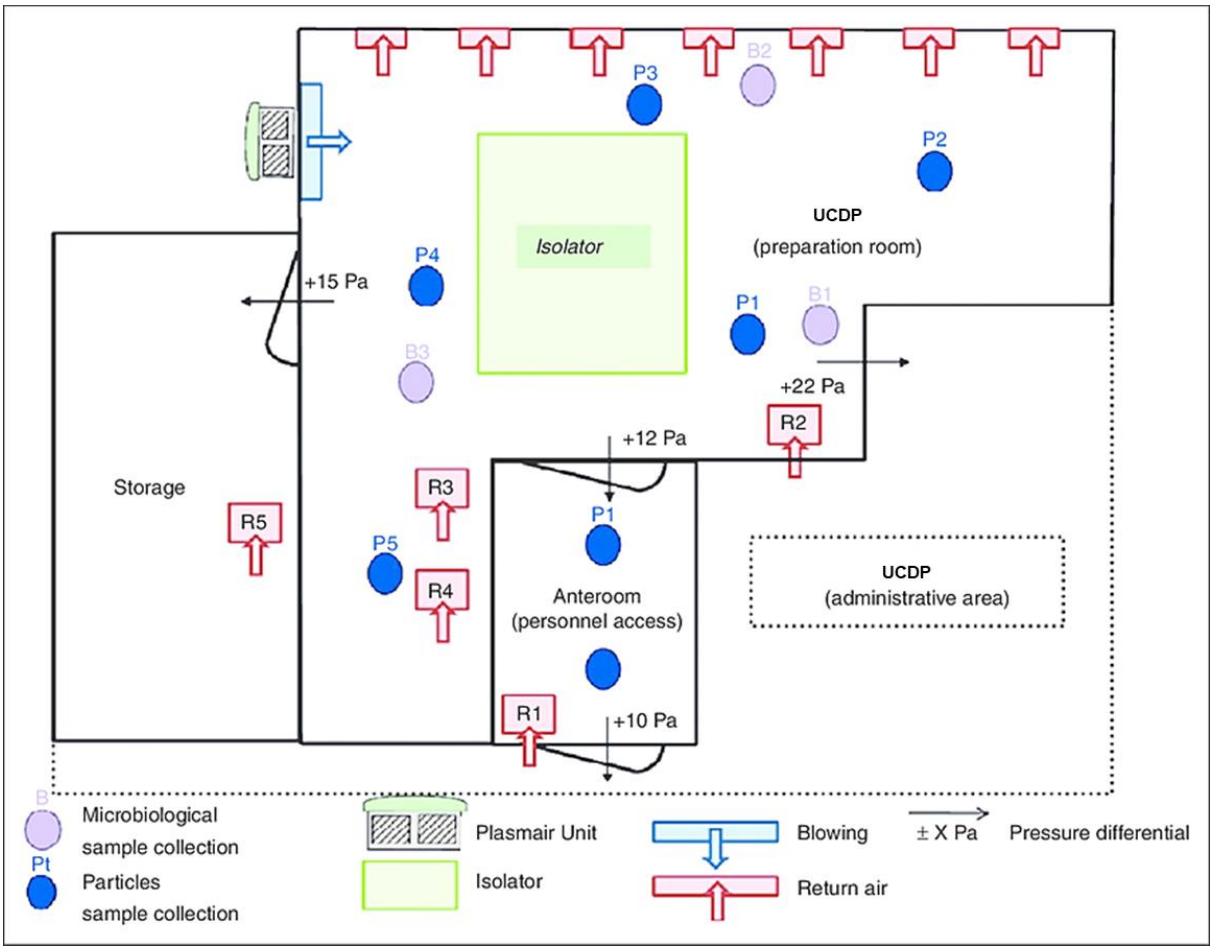


Figure 4. Units for Cytotoxic Drugs Preparation (UCDP) and aeraulic plan.

To date, epidemiological data related to adverse health effects of personnel professionally exposed to AAs are not fully available, and therefore it is not possible to identify with certainty symptoms deriving exclusively from occupational exposure to these drugs. However, several studies in the literature report adverse events occurring in UCDP, where operators described the occurrence of symptoms such as nausea, headache, dizziness, skin rash, skin paraesthesia, hair loss; these symptoms seemed to occur mainly when handling drugs took place in unprotected conditions[48]. However, it is hard to accurately define a cause - effect relationship between the extent of exposure and the symptoms reported. Therefore, an estimate of the effects resulting from exposure to AAs can be obtained by observing the

reactions in patients treated with AAs. In these patients, in fact, therapeutic effect is frequently accompanied by toxicity, particularly in tissues characterized by high cell turnover (bone marrow, epithelia, gonads). Undesirable effects can be distinguished into acute, early, delayed and late toxic effects (**Figure 5**)[42]:

- *Acute toxic effects:* nausea, vomiting, fever, skin and mucosal ulcerative lesions, hemorrhagic cystitis, renal failure and allergic reactions. They typically occur shortly after drug administration.
- *Early toxic effects:* resulting from the myelotoxic action of AAs, they include leukopenia, thrombocytopenia, even alopecia, diarrhea, stomatitis, pulmonary infiltrates, alterations of the acoustic nerve, hypercalcemia, kidney damage, cerebellar ataxia, psychosis.
- *Delayed toxic effects:* anemia, hepatocellular lesions, peripheral neuropathy, hypogonadism and decreased libido, amenorrhea and, less frequently, azoospermia, pulmonary fibrosis, heart failure and hormonal dysfunction.
- *Late toxic effects:* infertility, liver fibrosis, encephalopathy and osteoporosis may occur.

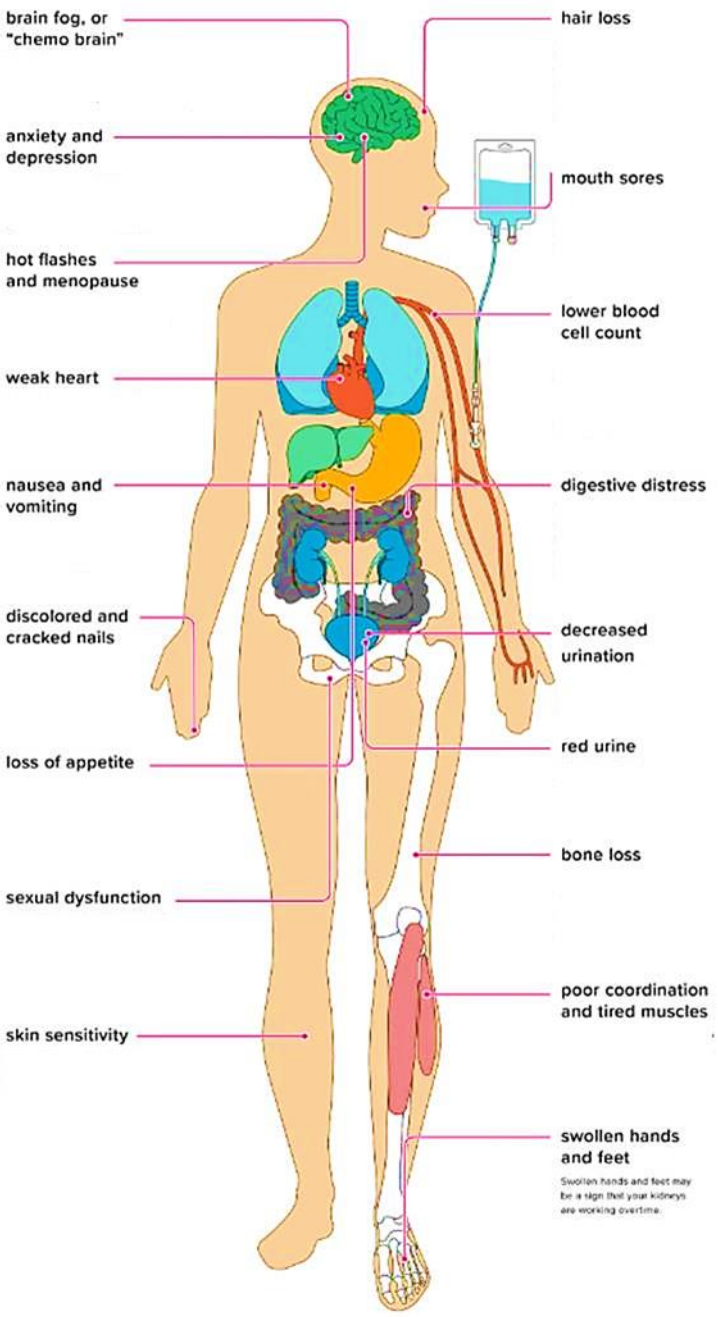


Figure 5. Antiblastic chemotherapeutic agents side effects.

Generally, effects of AAs in exposed workers are qualitatively the same as those found in treated patients, even if the extent of exposure and, therefore, of the risk is undoubtedly different: the doses that can potentially be absorbed at work, in fact, are far lower than those

used in therapy. In professionally exposed subjects, the effects can be distinguished in: acute, carcinogenic, teratogenic and early biological[46].

In hospital staff, exposure to AAs can occur mainly through the skin and / or the respiratory route. To quantify the exposure it is necessary to determine the airborne levels using body and area samplers. To this purpose, suitable collection devices are used, which are subsequently extracted and analyzed for the detection of active compounds[49]. The presence of AAs in the environmental particulate can be due to the presence of airstream near the vertical laminar flow hood, to its incorrect use (including lack of maintenance and / or periodically replace of the HEPA filters) or by procedural errors. Differences in the chemical structure and composition of chemotherapeutic agents preparations, can also affect their permanence in the manipulation areas environment. Cyclophosphamide (CP), as an example, is characterized by a low vapor pressure, but sufficient to allow the dispersion of vapors in the air at room temperature. Furthermore, CP molecules in the vapor phase are much smaller than the pore size of the HEPA filter, and would therefore not be retained by it. In this thesis work it will be highlighted how the CP molecule is recalcitrant to cleaning, even after several cycles of environmental decontamination[49].

It has also been confirmed that the use of gloves and gowns is not sufficient to completely exclude the risk of skin absorption of AAs. The permeability of latex gloves to certain AAs, such as CP, 5-fluorouracil (5-FU) and methotrexate (MTX), is also not negligible[44]. One of the most used methods to quantify the presence of AAs on surfaces and objects is the wipe test[50]. This technique consists in using supports of different materials (mostly cellulose-based) and specifically wetting solutions, for the recovery of the analytes from the surfaces. Beyond this, the accidental episodic way of contamination, such as unintended punctures, vials or

bottles breaking, or due to the disposal of patient excreta or cleaning operations must be also considered. In view of the above, the necessity of having monitoring strategies to assess skin exposure of operators and environments is evident. Since operators are generally exposed to a broad spectrum of compounds, usually different indicators are monitored, which can be identified among the AAs most frequently used in that ward, or selected from a range of substances in the attempt to cover the wider range of polarity. This procedure allows to obtain an overall picture of the working environment conditions and to address, if necessary, subsequent specific monitoring interventions. Usually, in the monitoring of professionally exposed personnel, a limited number of AAs are used as tracers (mainly CP, MTX, 5-FU, platinum derivatives). When it comes to BM, the matrices usually used are blood and urine, both for the ease of collection and for the information they can provide on exposure levels[43]. Objective of BM and EM are:

1. Need to identify the methods of dissemination of AAs in the workplace
2. Definition of the pathways of AAs absorption in exposed healthcare personnel.
3. Verification of the effectiveness of type and individual protective devices

1.3 Chemical risk assessment and management

Workers are generally exposed to relatively high doses of only one, or at most a few, genotoxic agents present in the workplace. Independently from the kind of the exogenous chemical substance used, these latter are distributed in the various organs and tissue of human body in accordance with their half-life. The biotransformation of the xenobiotic produces active or inactive metabolites which are re-distributed in the body and then excreted, mainly through feces and urine. Risk (R) can generally be defined as the probability and possibility of

undesirable events occurring in particular circumstances. The model for health risk evaluation from hazardous chemicals can be described by the following equation:

$$R = H \times E$$

Where: hazard (H) is intended as danger level of a substance or mixture without considering the amount of exposure, while the exposure (E) is the level of exposure, considering the specific work activity, the duration of exposure, the way of exposure, the used amount, the effects of the preventive and protective measures adopted[52]. According to Legislative Decree 81/2008 (Art. 28), the risk is "the probability of reaching the potential level of damage in the conditions of use or exposure to a specific factor or agent or to their combination"[16]. The risk assessment, resulting from exposure to genotoxic agents, is performed through monitoring procedures. Monitoring involves a series of analyses, repeated over time, aimed at the quantitative and qualitative determination of xenobiotic substances. Monitoring provides a complete picture of environmental pollution and therefore of the potential health risk. Through the analysis of these data it is possible to make projections about the possibility of risk to which people are exposed (risk assessment) which must also take into account the potential effects of combination[43]. Likewise, data obtained from monitoring give essential information for risk management, like indications to manage the dangers underlined with the risk assessment process (RA), but also about economic, social and policies implications[53]. Risk assessment is requested to ensure or protect workers health and safety; it represents the tool used to define preventive interventions, to plan information and training activities about risks. The National Academy of Sciences (NRC) established the risk assessment and management paradigm in 1983, later adopted by EPA[54]. According to the NRC, risk

assessment and risk management are "two distinct elements" between which agencies should maintain a clear conceptual distinction. Following the NRC paradigm, risk assessment is based on two sequential analyses. The first is hazard identification, which identifies contaminants that may pose health risks at environmentally relevant concentrations and qualitatively describes the effects that may occur in humans. The second analysis is the human health dose-response assessment, which characterizes the relationship between exposure to a pollutant and the resulting health effects. Consequentially, to perform a proper RA, necessary measurement activities include:

- environmental monitoring;
- biological monitoring;
- monitoring of biological effects;
- health surveillance.

In this context, EM and BM evaluation are complementary tools that allow for fundamental information in the risk assessment process as they provide essential information to fully quantify the exposure level of worker in accordance with the paradigm of the NRC (exposure assessment)[54]. The EM is the determination of the external dose of xenobiotics to which workers may be exposed, while, on the other hand, the BM consists in the quantification of the internal dose of the toxic substance actually absorbed, or of one of its metabolites found in biological fluids.

1.3.1 Environmental monitoring

According to the European Environment Agency (EEA), EM is the "measurement, evaluation and determination of environmental parameters and / or pollution levels, periodic and / or continuous in order to prevent negative and harmful effects on the environment"[55]. More specifically, EM involves the quantification of one or more potentially dangerous agents, achieved with standardized methodologies.

Environmental monitoring pursues one or more of the following purposes:

1) check whether the exposure limit values are respected and evaluate the technological progress achieved for their containment;

2) activate the emergency control procedures;

3) observe the environmental trends of exposure and pollution;

4) collect data necessary for the evaluation of health effects, for the development and evaluation of new intervention strategies.

According to the Legislative Decree 81/08 on Safety and Health at Work, the measurement of chemical agents in the workplace must concern those introduced both by inhalation and through the skin, although only the first measurement is commonly carried out[16]. Generally, direct methods for measuring skin contamination are not always validated; therefore, BM is used where possible, although this latter provides an overall indication of both inhalation and dermal exposure. This is a limit on useful information for identifying appropriate preventative measures. Airborne pollutants can be classified based on their physical state, which is the parameter that most affects the absorption by inhalation. Particulate compounds (aerosols) include airborne materials resulting from the drug itself, its treatment and materials previously

deposited on surfaces. 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. For the identification and subsequent quantification of environmental pollutants, two approaches are currently available: direct and indirect measurement methods[56]. 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 that 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 or active sampling on adsorbent cartridges or long-term samplings (time sampling from a few hours to several days), generally performed with diffusive samplers[57]. 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 itself. 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 dependent manner concentration or can be trapped and, at the end of the sampling, the tubes are stored until desorbed and analyzed in laboratory[58]. 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[58]. 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). 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[59].

1.3.2 Biological monitoring

Biological monitoring can be defined as the analysis, single or repeated over time, of biological fluids, or tissues, to assess and quantify the interaction of a physical, chemical or biological agent within the organism[56]. In occupational medicine, the term BM is used to indicate the assessment of exposure to chemical agents present in the working environment by measuring in biological fluids the parent compounds, their metabolites or indicators of their effect. Actual legislation (Legislative Decree 81/2008-Legislative Decree 106/2009) has set a Biological Limit Value (BLV) for most of chemical agents, this values is intended as the maximum concentration of the relative agent, of one of its metabolites or an indicator of effect, acceptable in the appropriate biological matrix[16]. There are, however, other limit values proposed by international institutions, to which the results of BM can be compared, such as those adopted by the ACGIH[24]. Exposure to an analyte or its metabolite is defined through BEIs; these provide information on the extent of worker exposure to the chemical agent, and are not directly related to the presence of adverse effects. Biological Exposure Indicators can be classified according to several criteria:

- tested biological matrix (urine, blood, tissues, exhaled air, etc.);
- organ or tissue in which they are originated or which produced them (kidney, liver, nervous system, etc.);
- chemical-physical characteristics (volatility, hydro / fat solubility, etc.);
- toxicological significance and predictive value attributed to them with respect to the risk factor for which they are indicators.

Based on this latter definition, BEIs are usually divided into three categories:

- exposure indicators (IE)[60];
- response / or effect indicators (IBR)[61];
- susceptibility indicators (IBS)[61].

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[56]. 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 BEI.

Different biological matrixes (e.g., blood, urine, breath) may be selected based on expected molecule concentration, kinetics and the difficulty related to the sample collection (urine and breath are the less invasive samples to collect)[62]. Sampling times are also important and strongly dependent 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.

The analysis of exhaled air (breath test) is an attractive non-invasive technique for the determination of 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[63]. 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. 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 FA 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 BEI are present in the blood for a certain amount of time and data normalization is not

required; nonetheless, venipuncture is an invasive procedure that must be performed only by trained personnel.

1.4 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[59]. 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[57]. In this case, 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[64]. Chromatography has significantly improved both EM and BM. Compared to classic immunometric or radiolabeling techniques, chromatography is faster and cheaper. Many of the most common and recent work on compound monitoring, including AAs, involves high-performance liquid chromatography (HPLC) coupled with different types of detectors, such as Photodiode array, Fluorimeter and other. These equipments, in fact, are available in almost all healthcare facilities and is employed for the simultaneous detection and quantification of many compounds. The use of

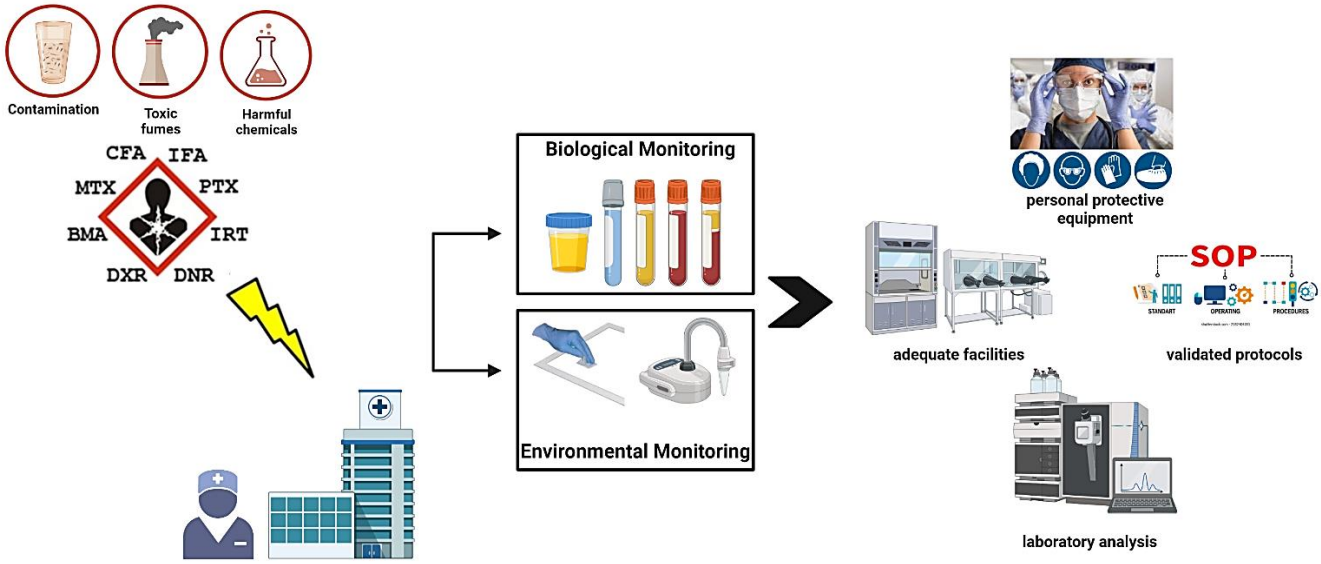
liquid chromatography to monitor compounds potentially harmful to health extends to an ever-increasing number of compounds, such as solvents, commonly used drugs and cytotoxic agents[65]. Ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) has a high selectivity and sensitivity for the simultaneous analysis of several pollutants and chemicals from complex matrices, such as biological fluids, even at very low concentrations. The great versatility and sensitivity of mass spectrometry render this technique suitable for both BM and EM. The high selectivity of UHPLC-MS/MS allows for the simultaneous analysis of a great number of compounds in complex matrices, such as biological fluids, even at very low concentrations[66].

2. AIM OF THE WORK

Environmental 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. In this framework, it becomes particularly important to have robust and sensitive methodologies to monitor workers and work environment, to minimize the risk of accidental exposure and consequent damage to health. Our attention has been focused on the occupational exposure to FA and/or AAs, whose common and harmful exposure remains a major concern for health surveillance. In this work, the results of a combined (environmental and biological) monitoring approach to manage occupational exposure to FA and AAs in the hospital setting are described. For this latter, a new wipe-test based EM method, suitable for UHPLC-MS/MS analyses, has been developed. This method allows monitoring of different kind of AAs widely used in cancer therapy, such as MTX, CP, ifosfamide (IFO), doxorubicin (DXR) and epirubicin (EPI), irinotecan (IRT) and paclitaxel (PTX). All methods were validated following the current European Medicines Agency (EMA) guidelines on Bioanalytical Method Validation[67]. These methodological approaches were used for the chemical risk evaluation of workers either at the UCDP or at the Anatomic Pathology Unit at the "San Giovanni di Dio and Ruggi d'Aragona" University Hospital of Salerno (Italy). The working procedures, adopted to guarantee the safety of workers, were assessed, highlighting some critical issues both in the work protocols and in the organization of the workspaces. Appropriate adjustments were carried out based on the results obtained from the various monitoring campaigns, showing a significant improvement in the exposure conditions of the personnel employed in those Units.

Part of the work here presented, involving the set-up and validation of a wipe test-based monitoring method for the evaluation of AAs contamination on surfaces, obtained the Italian patent[68]. The patented procedure was used for the development of a new commercial kit and for the creation of a university spin-off called MaTOX s.r.l. A prototype of the kit was used to carry out additional collection campaigns at the UCDP of the "ASL Napoli 3 Sud, Gragnano" Hospital in Naples (Italy) for the determination of environmental AAs contamination.

GRAPHICAL ABSTRACT



3. MATERIALS AND METHODS

3.1 Buildings and facilities

The Anatomic Pathology Unit is located on the ground floor of one of the buildings of the University Hospital and consists of five rooms for samples treatment, a corridor and physicians rooms. All rooms are equipped with a descending ventilation system with conditioned air introduced into the room from the ceiling and air outlet units in the side walls. In all rooms there are windows that open towards the outside of the building. Formaldehyde is mainly used in a room of 23 m^2, equipped with a fume hood and a safety storage cabinets with exhaust system for storing anatomical samples. At the beginning of our monitoring activity, all the rooms of the Unit were considered in the sampling procedures, to evaluate FA dispersion even within sites not directly involved in the histological samples processing. In subsequent monitoring campaigns, only the processing room was analyzed.

Monitoring campaigns carried out in the UCDP of the "San Giovanni di Dio e Ruggi d'Aragona" University Hospital were performed at the end of the working shift at the end of the working week (Friday), taking care to obtain two samples in two different areas for each point, one prior to normal cleaning operations, and the other after the last set had been performed (**Figure 6**).

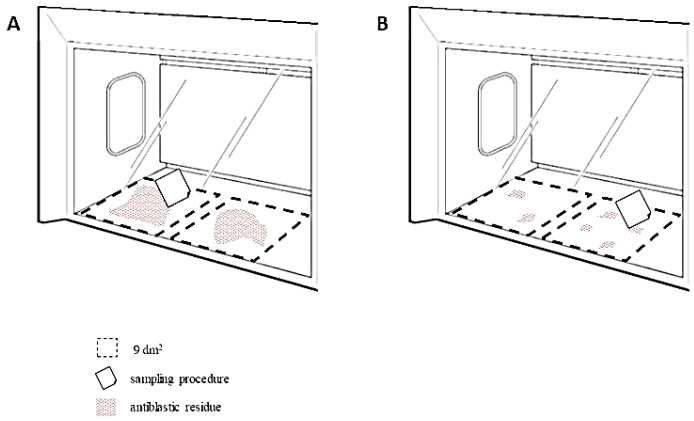


Figure 6. Simplified representation of the procedure used to evaluate hood surface contamination: (A) Sampling during working time performed on a 30x30 cm^2 surface, (B) Sampling after cleaning procedure, performed on the adjoining area (30x30 cm^2).

Based on the UCDP plans, that consists of three rooms, the attention was focused on the operating area and on the filter area (air-lock) (**Figure 7**):

- operating area**:**

1. side table near to the primary hood (collection area 30 x 30 cm);
2. primary hood, in use at the time of sampling (collection area 30 x 30 cm);
3. secondary hood, not in use at the time of sampling (collection area 30 x 30 cm);
4. support trolley in front of the primary hood (collection area 30 x 30 cm);
5. side table for saline solution (collection area 30 x 30 cm);
6. operating area door handle (collection area 10 x 30 cm).

- filter area (air-lock):

7. desk top surface in the filter area (collection area 30 x 30 cm);
8. medical fridge handle 4°C (collection area 10 x 60 cm).

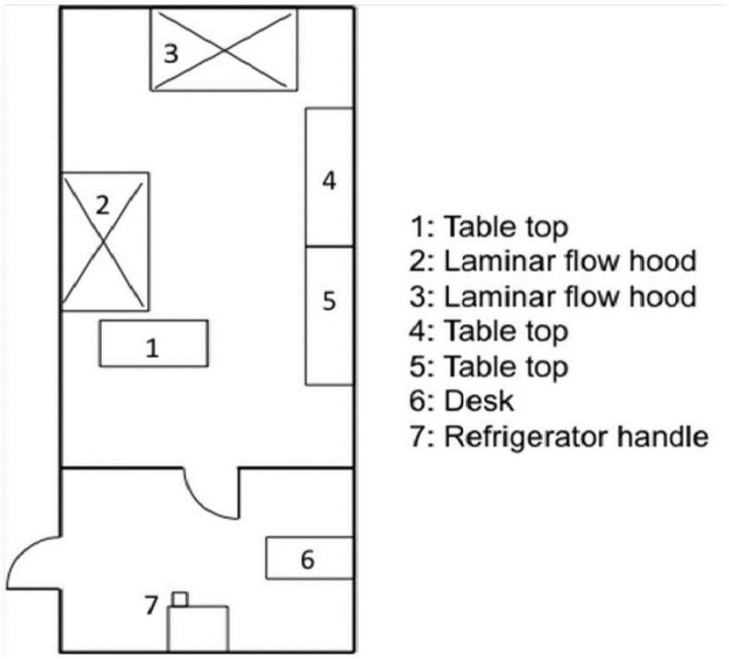


Figure 7. Schematic map of the monitored working areas in the UCDP.

3.2 Monitoring sampling strategy

Environmental monitoring campaigns, carried out at the Pathological Anatomy Unit, were aimed at evaluating the quantity of airborne FA in the various rooms and the FA capability to spread between adjacent rooms and were performed in collaboration with the research groups of Prof. Oriana Motta and Prof. Francesco De Caro at the Department of Medicine, Surgery and Dentistry of the University of Salerno. Twenty sampling points were selected in the analyzed facility to evaluate the concentration of FA and identify the potential diffusion pathways in the rooms adjacent to the histological sample processing room.

For what concern FA BM, each campaign included three sampling, specifically on Monday morning, at the beginning of the weekly working shift (T0), Monday evening at the end of the daily shift (T1), and Friday evening at the end of the weekly shift (T2). This timing made it possible to evaluate both the intraday exposure and the weekly backlog (interday exposure). The importance to analyze the urinary FA concentrations at the beginning of the working week is to evaluate whether an anomalous FA level was related to occupational rather than accidental exposure, for example during the weekend or due to diet for example. Biological monitoring was repeated every six months, in parallel with FA EM.

Environmental samples potentially contaminated with AAs, was collected from UCDP work areas using paper-wipes and analyzed through a multi-residual UHPLC-MS/MS-based analytical method. Taking into account frequency of use, theoretical harmfulness (in terms of biological effects and bio-availability), and ease of analysis in a single run of several species at the same time, six different chemotherapeutics as common markers of contamination were selected (MTX, IFA, CFA, DXR, IRT and PTX).

3.3 Environmental monitoring of formaldehyde

Formaldehyde EM was performed using detectors installed in previously selected fixed locations. Two different types of detectors were used:

1) active sampling, consists of chemo-absorbent test tubes. Tubes were used with a suitably calibrated pump, supplied by Aquaria s.r.l. (Lacchiarella, MI, Italy), and flow was set at a constant value of 1 l/min throughout the sampling period. The sampling rate was continuously monitored by a flowmeter. Four samples per working day and three measurements for each

sample were analyzed and mean values reported. The sampling procedure requires that a known volume of sampled air (1 to 15 l) be passed through an acidified silica gel coated with 2,4-dinitrophenylhydrazine (DNPH). Formaldehyde reacts with DNPH to produce the corresponding hydrazone. After the sampling phase, the test tubes were closed with caps, transported and stored in glass containers kept at controlled refrigerated temperature.

2) diffusive sampling (passive), by air diffusive exposure to samplers such as "RING devices" supplied by Aquaria s.r.l., containing a silica gel cartridge coated with DNPH. This method is indicated for long-term monitoring to evaluate the average concentration values (TLV-TWA 8 h per working day). At the end of the sampling phase, the test tubes were closed with the appropriate caps and stored in glass containers in refrigerated systems maintained at a controlled temperature. For each determination, three measurements were made and the mean value was reported. The mass concentration C ($\mu g/m^3$) of the passive samplers was calculated with the manufacturer's absorption rate of 92 ml/min and using the following formula:

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

This equation can be directly derived from Fick's first law, assuming that analyte mass was absorbed by diffusion, time representing the sampler exposure time and P the diffusive absorption rate. 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 the precision was calculated as the 2σ deviation of the absolute differences of the individual sample values from the mean in triplicate samples[69]. Formaldehyde sampled with active and passive devices was evaluated according to the method described by NIOSH 2016[70], which involves an organic extraction with acetonitrile (ACN) and subsequent analysis by HPLC. Analyses were performed on the HPLC instrument previously described using the PDA detector set at 385

nm, and an Ascentis® C18 analytical column (4.6 mm x 150 mm, 3 μm); the chromatographic elution conditions consisted of a mobile phase composed of 45% ACN / 55% water (v/v) and a flow rate of 1.0 ml/min. Breeze® 2.0 software, supplied by Waters®, was used for data analysis. The extraction was performed by adding 3 ml of ACN to the vial. Analytical grade ACN and 99.9% pure formaldehyde-2,4- dinitrophenylhydrazone (FA-2,4-DNPH), Sigma-Aldrich (St. Louis, MO, USA). For calibration, a known amount of FA-2,4-DNPH was weighed out and diluted in ACN; from this stock, dilutions in ACN were prepared for calibration ranging from 0.23 to 37 μg per sample. The RING diffusion samplers and the active sampling tubes were supplied by Aquaria® s.r.l.

3.4 Biological monitoring of urinary formaldehyde

Urinary FA of exposed workers was measured by 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, urine samples were derivatized with a chemical reagent supplied with the kit and incubated at 70°C for 15 min. Then, 500 μl of HPLC grade water were added to the sample and an aliquot of 50 μl of the resulting mixture was injected directly into the HPLC system. Chromatographic separation was achieved by RP-HPLC using a GENESIS C18 (4.6 x 150 mm, 4 μm) column supplied of a pre-column filter, performed on a Waters model 1525 binary pump system equipped with a photodiode array detector (model 2998), and autosampler (model 2707) (Waters, Milford, MA, USA). Samples in the auto-sampler were at RT and a column oven was used to maintain the column temperature at 30°C.

Breeze 2.0 software (Waters) was used for peak analysis, integration, and to calculate the linear regression of the calibration curve. The mobile phase was included in the commercial kit. The separation was achieved by isocratic elution with a flow rate of 1.2 ml min^{-1}. Analytes were detected with a UV detector set at λ = 385 nm.

3.5 Biological and environmental monitoring of antineoplastic drugs

Pure antineoplastic molecules for method setting up and calibration were purchased from Sigma-Aldrich® Corporation. Deuterated and non-deuterated Internal Standards (ISs) selected for method development were: Methotrexate (MTX), Ifosfamide (IFA), Cyclophosphamide (CFA), Irinotecan (IRN), Bendamustine (BND), Doxorubicin (DXR), Paclitaxel (PTX), 7-ethyl-10-hydroxy-camptothecin (SN-38), Methotrexate-d3 (MTX-d3), Cyclophosphamide-d4 (CFA-d4), Irinotecan-d10 (IRT-d10), Bendamustine-d5 (BMA-d5), Daunorubicin (DNR) and Paclitaxel (PTX-d5). The solvents used as mobile phases, such as methanol (MeOH), ACN and water were all super pure ultra-gradient quality; other reagents used were formic acid (HCOOH), dimethyl sulfoxide (DMSO) and were all purchased from the Romil® company.

For the preparation of blood and urine samples for BM, columns for solid phase extraction (SPE) type HR-X Chromabond® and a vacuum manifold model Chromabond® were used. Supernatant were dried using a Thermo Scientific Savant® DNA 120 SpeedVac Concentrator. For the LC-MS/MS analysis, a system consisting of an Ultra-High-Performance-Liquid-Chromatography (UHPLC) "UltiMate 3.000 Dioneex®" by Thermo-Fisher Scientific™ coupled with a Thermo Scientific TSQ Endura™ mass spectrometer triple quadrupole analyzer equipped with electrospray source was used (**Figure 8**).

Figure 8. Thermo-Fisher Scientific™ UHPLC-MS/MS system.

For method performance evaluation pure DXR was used, but, when the method is applied on UCDP working surfaces, it is worth noting that it is not possible to discriminate between DXR and its isomer Epirubicin (EPI). For this reason, the DXR / EPI notation was used in the tables shown, even when only one of the two drugs was used during the EM campaign. Selected reaction monitoring (SRM) mode was used and two transitions were selected for each analyte (**Table 1**).

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	544	397*	11	3.49	52-58
		361§	25		
PTX	854	569*	11	5.82	55-61
		286§	15		

* quantifier ion; § qualifier ion. CE= collision energy

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

3.6 Procedure and analysis of AAs in environmental samples

For the development of the AAs EM method, single stock solutions of the seven selected analytes (MTX, IFA, CFA, IRN, BND, DXR and PTX) were prepared, starting from the solid form, at a concentration of 100 μg ml^{-1} in DMSO. The individual stock solutions of each analyte were then diluted into a unique working solution containing all the seven analytes at a final concentration of 10 μg ml^{-1} in DMSO. All the "parent" and working solutions were then stored at -20°C until use. For the development, validation and calibration of the method, a work surface pollution was simulated by spreading solutions of analytes at known concentrations directly on the surfaces, leaving them to dry at room temperature and then carrying out the environmental measurements. The recovery efficiency of the molecules was initially evaluated, using different solvents, volumes and drying procedures, to develop a method able to collect molecules of different chemical nature from the surfaces. A manual cleaning procedure with wipes ("wipe-sampling") was used.

Samplings were carried out randomly on different days of the week, so that the relative contamination was as representative as possible of the overall workload of the facility. The study of the planimetry and the visive inspection of the working rooms allowed to identify the sensitive work areas preserving homogeneity in sampling points distribution. Wiping procedures were carried out on all the surfaces considered "hot spots", such as hoods, workbenches, medicine cabinets, but also points where there could have been accidental buildup of AAs, such as door handles, refrigerators, telephones and computers. Analyses were performed on the LC-MS/MS instrument previously described.

3.7 Procedure and analysis of AAs in biological samples

For the BM of AAs, a stock solution of ISs at a final concentration of 1 μg/ml in 50% MeOH was prepared which contained: MTX-d3, CFA-d4, IRT-d10, BMA-d5, DNR and PTX-d5. Five μL of this solution were added to 200 μL of sample (blood or urine), to obtain a final concentration of ISs equal to 25 ng/ml in each sample. Daunorubicin was the only non-deuterated molecule used as IS, due to the fact that since this molecule was not manipulated by the personnel monitored, it is not present in workers biological fluids. Paclitaxel d-4 was considered only in plasma analyses, since taxanes, due to their chemical and pharmacokinetic characteristics, are not eliminated with urines.

Samples prepared as described above were loaded onto the columns for SPE and extracted according to the following protocol:

- conditioning and activation of the column by the sequential addition of 3 ml of 100% MeOH, and subsequently of 3 ml of water
- loading of the sample which is completely adsorbed on the column bed
- washing with 2 ml of a solution containing 2% MeOH and 0.1% acetic acid,
- elution in safe-lock tubes using 1 ml of pure MeOH.

Eluate was dried in a Speed-Vac and the pellet suspended in 200 μL of 50% MeOH (for both urine and plasma). The sample was centrifuged for 1 minute at 13,300 rpm and transferred to a vial with insert for subsequent LC-MS/MS analysis.

3.8 UHPLC-MS/MS analyses

3.8.1 Chromatographic conditions

Ten microliters of extracted samples were injected on a Phenomenex® Luna-Ω, C18 column (50 x 1.0mm, 1.6 μm) to achieve chromatographic separation. The column was maintained at 50°C for entire run. Mobile phases flow rate was 0.06 ml min^{-1} and the separation of the analytes in chromatography was performed by a binary gradient so composed: 0.1% formic acid solution in water (phase A) and 0.1% formic acid solution in ACN (phase B), ranging from 20 to 45% B in 5 minutes. Four minutes were used for column wash and reconditioning, so taking into account the ramping steps, the entire analysis of each sample took 10 minutes.

3.8.2 Mass spectrometry parameters

The LC-MS/MS analysis was performed in Selected Reaction Monitoring (SRM) mode, using an electrospray ionization source. SRM analysis is a particular type of mass spectrometry in which only species with a pre-selected molecular mass and known fragmentations are monitored. Specifically, in the SRM acquisition mode, only ions with a preselected *m/z* ratio (called "precursors") are accumulated in the first quadrupole (analyzer), while the others are excluded from the analyzer. Precursor ions are then collided in the second quadrupole (collision cell), allowing only some ions (called "products") to accumulate in the third quadrupole (analyzer). No spectrum was acquired for other ions not selected by the analyzer. This allows for maximum accuracy and specificity, avoiding the possibility of false signal. For the development of this method, pure analytical standards were preliminarily injected in solvent directly into the mass spectrometer, without chromatographic separation; this made it

possible to select two specific transitions for each analyte, one transition for the quantification and the other -the qualitative- for the confirmation of the chemical identity. The transitions of each analyte are reported in **Table 2**. The data obtained were analyzed using the Xcalibur 4.0 software.

Analytes	Polarity	Precursor (m/z)	Product (m/z)	Collision energy (eV)
5-fluorouracil_1	*Negative*	*129*	*42*	*15*
5-fluorouracil_2	*Negative*	*129*	*86*	*12*
Bendamustine_1	*Positive*	*357,9*	*228*	*30*
Bendamustine_2	*Positive*	*357,9*	*304*	*40*
Bendamustine_d5	*Positive*	*363,1*	*233*	*36*
Cyclophosphamide_1	*Positive*	*261*	*140*	*21*
Cyclophosphamide_2	*Positive*	*261*	*233*	*16*
Cyclophosphamide_d4	*Positive*	*265,3*	*140*	*21*
Daunorubicin	*Positive*	*528,1*	*397*	*12*
Doxorubicin_1	*Positive*	*544,1*	*397*	*11*
Doxorubicin_2	*Positive*	*544,1*	*361*	*25*
Ifosfammide_1	*Positive*	*261,1*	*154*	*21*
Ifosfammide_2	*Positive*	*261,1*	*182*	*16*
Irinotecan_1	*Positive*	*587,2*	*502*	*30*
Irinotecan_2	*Positive*	*587,2*	*458*	*35*
Irinotecan_d10	*Positive*	*597,2*	*502*	*30*
Methotrexate_1	*Positive*	*455,1*	*175*	*30*
Methotrexate_2	*Positive*	*455,1*	*308*	*20*
Methotrexate_d3	*Negative*	*456,1*	*327*	*20*
Paclitaxel_1	*Positive*	*854,4*	*286*	*11*
Paclitaxel_2	*Positive*	*854,4*	*569*	*11*
Paclitaxel_d5	*Positive*	*859,4*	*569*	*11*

Table 2. Selected transitions for the identification/quantization (1) and confirmation (2) of all studied analytes. Internal standards are highlighted in grey.

3.9 Statistical analysis

Formaldehyde environmental and biological data were expressed as means ±SD per year and as median and range of values (min - max). Comparisons between the different years of collection were taken into consideration and comparisons of the values between the different work shifts were analyzed. Paired Student t-test and One-Way ANOVA were used for data interpretation. For the AAs data presented in this work, a Shapiro-Wilk test was used to analyze the distribution and, therefore they were compared using also the Wilcoxon paired test. The p-values obtained was considered significative at >0,05. For data analyses, Excel and R software were used.

4. RESULTS

4.1 Method validation

Method validation consists in a sequence of experimental tests to certify the performance, in terms of suitability and reliability, of the analytical procedure. This is necessary to confirm that the results obtained on unknown samples, through the application of a specific analytical procedure, have scientific value and can be reported.

For what concerning the procedure of AAs environmental detection, one of the main critical points to ensure the high accuracy of the measurements, was the method of sampling collection from the environment. In this case, only a partial validation to verify the performance of the patented methodology was carried out (see Materials and Methods for details). 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 3**) and values ≤ 0.10 ng/cm^2 were obtained for all six compounds.

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

Table 3. Lower limits of detection (LLOD) and lower limits of quantization (LLOQ) evaluated for each compound.

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 9**).

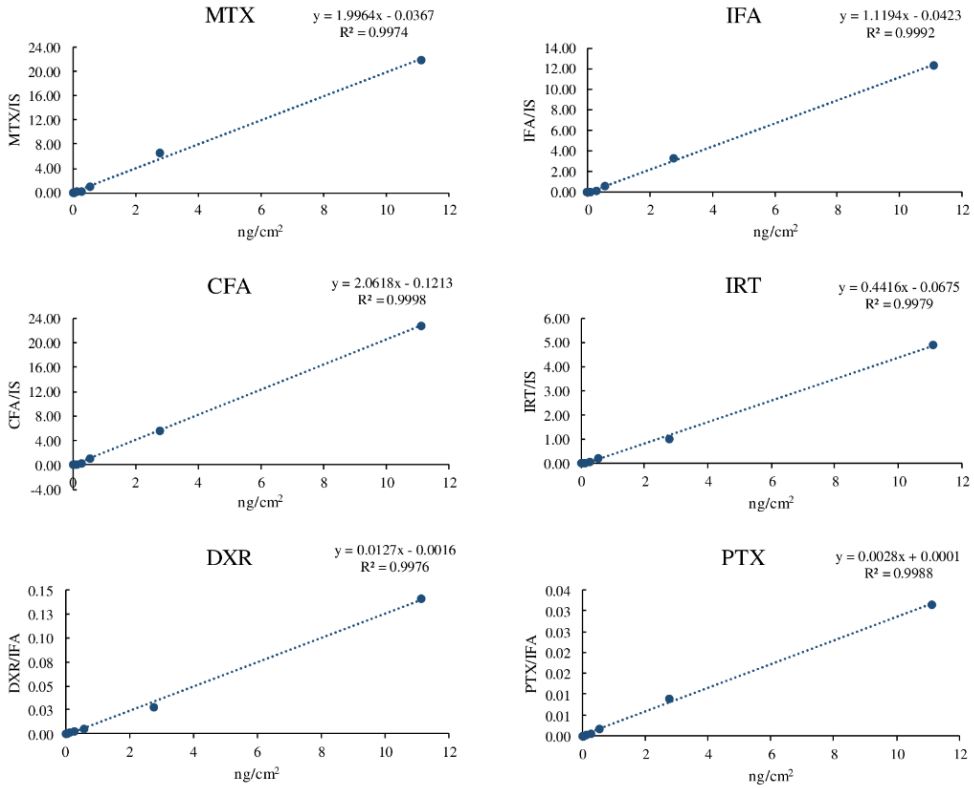


Figure 9. Linearity of the analytical method, evaluated (n=6) for each of the six compounds, recovered from spiked surfaces.

Intraday and interday 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 4**). Considering the 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 over 24 hours and the response obtained for all analytes was substantially unmodified (CV ≤ 7%).

	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

Table 4. Intra- and inter-day precision and trueness of the method evaluated at three different concentrations. LL (Low Level) = 0.39 ng/cm^2; ML (Medium Level) = 0.78 ng/cm^2; HL (High Level) = 3.89 ng/cm^2.

For what concerning FA determination in urine samples, as already underlined in the Materials and Methods section, a commercial CE-IVD kit was used, therefore no validation was performed on our instrumentation and the methodological procedure was followed according to the manufacturer's indications. On the other hand, for AAs identification and quantification, an "in-house" procedure was developed and validated according to the current EMA guidelines[67].

For the biological AAs monitoring, the analytical procedure was fully validated for plasma and urine. No interference from endogenous compounds was observed regardless of the biological fluid analyzed, and no interference from any drug with the channels of other analytes was detected. Injection of highly concentrated samples (up to 10 μg/ml) followed by the analysis of blank samples showed that carry-over was substantially absent for all the investigated compounds. Lower limits of detection (LLOD) and quantification (LLOQ) were measured for the drugs in plasma and urine (**Table 5**). LLODs in the range 2.5–15 pg/ml and 2.5–5 pg/ml were obtained, respectively, in plasma and urine. LLOQs ranged, independently

from the matrix used, from 5 to 15 pg/ml for all analytes, whereas PTX showed a quantification limit in plasma samples of 50 pg/ml.

Compound	Plasma		Urine	
	LLOD (pg/ml)	**LLOQ (pg/ml)**	**LLOD (pg/ml)**	**LLOQ (pg/ml)**
MTX	2.5	5	5	15
IFA	10	15	5	10
CFA	10	15	2.5	5
IRT	2.5	5	2.5	5
DXR	2.5	5	5	10
BND	10	15	5	10
PTX	15	50	n.a.	n.a.

Table 5. Lower limits of detection (LLOD) and lower limits of quantification (LLOQ) measured for each compound in plasma and urine.

For each compound, linearity of the response was evaluated over a concentration range going from 20 pg/ml to 2 ng/ml (n = 6) in both plasma and urine, and the mean correlation coefficients obtained ranged from 0.99725 to 0.99954. In the case of PTX, a good linearity was observed from 50 pg/ml to 2 ng/ml (n = 5) in plasma (R^2= 0.99854). Intra- and interday precision and accuracy of the method were evaluated for all compounds at four different concentrations, including LLOQ and the highest tested concentration (2 ng/ml), in plasma and urine. Results shown in **Table 6** were satisfying, as coefficient variants (CV) lower than 8% and relative standard errors (RSE) lower than 6% were observed in both biological matrixes.

Compound	Plasma															
	%CV intraday				%CV interday				%RSE intraday				%RSE interday			
	LLOQ	LL	HL	ULOQ	LLOQ	LL	HL	ULOQ	LLOQ	LL	HL	ULOQ	LLOQ	LL	HL	ULOQ
MTX	6.5	5.6	4.8	5.0	6.9	7.3	6.5	6.0	4.3	2.5	3.0	3.6	4.0	3.5	3.1	3.4
IFA	5.5	4.9	4.8	4.4	6.3	5.8	4.3	4.6	4.6	3.2	3.5	3.9	5.1	3.6	2.9	3.0
CFA	6.1	6.3	5.5	5.6	6.4	6.1	5.1	5.4	5.2	3.1	2.7	4.2	4.6	4.1	3.8	4.2
IRT	3.9	2.9	3.6	4.1	4.4	3.8	3.2	3.0	3.9	2.0	2.6	3.7	4.4	3.9	3.8	3.6
DXR	4.3	5.2	2.8	3.7	4.6	3.5	4.9	5.1	5.6	3.1	3.8	2.8	4.1	4.3	4.0	4.5
BND	5.2	3.5	3.2	3.4	4.5	4.1	3.0	4.9	4.8	2.6	1.9	2.0	3.2	2.3	2.7	3.1
PTX	7.2	6.5	6.3	6.0	6.3	7.0	5.8	6.5	3.6	4.0	3.8	4.3	5.5	5.1	4.3	4.0

Compound	Urine															
	%CV intraday				%CV interday				%RSE intraday				%RSE interday			
	LLOQ	LL	HL	ULOQ	LLOQ	LL	HL	ULOQ	LLOQ	LL	HL	ULOQ	LLOQ	LL	HL	ULOQ
MTX	5.8	4.7	5.3	4.1	5.5	6.5	6.9	5.8	3.9	2.9	2.5	20	3.3	4.2	3.8	1.5
IFA	4.9	5.5	4.5	3.8	6.0	5.2	5.5	5.8	4.4	2.7	2.2	2.6	4.0	2.5	3.1	3.7
CFA	5.7	5.9	5.5	4.4	7.1	5.3	6.6	5.0	4.2	3.7	2.0	2.0	4.0	3.2	3.0	3.6
IRT	3.6	2.5	2.1	2.0	3.8	3.5	4.0	3.3	5.0	3.3	1.9	2.5	3.5	2.2	2.0	45
DXR	4.3	5.8	3.2	3.4	4.5	4.1	4.4	4.0	4.6	3.3	2.7	3.6	3.0	2.9	3.7	4.9
BND	4.2	4.0	3.7	4.8	6.2	4.9	5.2	5.5	3.5	2.8	2.5	1.8	4.8	3.3	2.1	4.5

Table 6. Intra- and interday precision (%CV) and accuracy (%RSE) achieved in plasma and urine for the analyzed antiblastic compounds. LLOQ (Lower Limit of Quantization), see Table 2; LL (Low Level)=0.1 ng/ml; HL (High Level)=1 ng/ml; ULOQ (Upper Limit of Quantization)=2 ng/ml.

To define the maximum storage time of the samples, the stability of the eight compounds was evaluated in plasma and urine under several conditions. All the analytes were stable in both biological fluids at room temperature for more than 24 h, at least 3 days at 4°C and were not affected by three consecutive freeze–thaw cycles. Processed samples were stable at 5°C (autosampler temperature) for more than 15 h. Stock solutions were stable for more than 1 month at -80°C.

4.2 Formaldehyde environmental monitoring

Minimizing exposure to volatile contaminants, such as FA, play a pivotal role in ensuring employee safety in the workplace. For this purpose, the maximum limits for the presence of

these substances in the airborne have been defined; these limits were defined as the air concentration of the chemicals measured over an 8 hours work shift (TWA) and as short-term exposure limits (STEL) for a period of 15 minutes (TLV Ceiling). However, these OELs can be set by global, European and national bodies or by different companies for the same country and, therefore, the regulations for setting OELs can widely differs. Furthermore, OEL can be evaluated with different methodologies, leading to a many OELs even for the same compound. **Table 7** shows the main Threshold Limit Values for FA proposed by various international organizations.

Organization/legal of countries	**Type**	**Concentration (ppm)**
Occupational Safety and Health Administration (OSHA-USA)	TWA STEL	0.75 2.0
American Conference of Governmental Industrial Hygenists (ACGIH-USA)	TWA STEL	0.1 0.3
National Institute for Occupational Safety and Health NIOSH	TWA STEL	0.016 0.1
World Health Organization (WHO)	STEL	0.8
Scientific Committee on Occupational Exposure Limits UE (SCOEL)	TWA STEL	0.3 0.6

TWA: Time Weighted Average measured over 8 h; STEL: short-term exposure limit

Table 7. Threshold limit values for formaldehyde provided by main government agencies.

It is important to underline that these limits do not provide a clear dividing line between non-hazardous and harmful concentrations, but only indicate the concentrations of airborne substances to which it is believed that most workers may be exposed for 8 hours a day, 40 hours a week, 48 weeks a year, without undergo adverse effects on health. **Table 8** shows the results of four monitoring campaigns at the Pathological Anatomy Unit of the University Hospital.

Year	RH (%)	Temperature (°C)	Sampling method	N° of samples	Average (ppm)	Range (ppm)	Noncompliance (%)
Year 1	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
Year 2	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
Year 3	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
Year 4	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 8. 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 consecutive years.

During the first campaign, sampling points were randomized to identify the sites that might have been representative of the overall pollution conditions, while sampling was concentrated on a fixed location during subsequent campaigns. Sampling was performed by both passive and active methods, as described in the Materials and Methods section, to verify compliance with the TWA and STEL limits. Additionally, as environmental factors such as temperature and relative humidity, are known to affect the performance of passive samplers, these parameters have been constantly checked using suitable instruments: more specifically, the temperature was in the range of 25-28°C and the relative humidity was in the range of 38-52%. Under these conditions, there should not be negative impact on sampling recovery and reproducibility. As it was easy to foresee, the active sampling method showed a higher concentration value respect to the passive harvest. Despite this, all active and passive sampling measurements showed compliance with the maximum levels defined by OSHA and the OELs dictated by ACGIH, but not always concordant with those of NIOSH, thus highlighting the discrepancy between the different agencies. In fact, only a few active measurements have exceeded NIOSH STEL, mainly at the sampling site near the open waste

bin near to the fume hood. These was due to workers inaccurate and erroneous practice to discard gloves and paper towels used to clean FA-contaminated surfaces into the open bin, leading to evaporation of residual FA into the environment; the values are reported in **Table 8** and show minimal variations for each sampling period. Noteworthy that percentage of non-compliance, calculated as the number of measurements above the OEL, ranges from the first to the last monitoring campaign from 50% to 7% for active measurements and from 12.2% to 0% for the passive one. This marked improvement in the environmental conditions was mainly due to the employees who, despite no structural or technical changes in the working areas had been made, had understood the importance of their action in limiting the spread of FA, and paid more attention to daily working and waste disposal procedures.

4.3 Formaldehyde biological monitoring

Urine samples were collected, labeled and stored at -20°C until analysis. A statistical comparison of the values obtained in the four years analyzed (**Figure 10**), shows that the highest average value was measured in year 3 (0.75 mg/L). The analysis of variance (ANOVA) shows that in year 3 the values deviate less from the mean value. The medians reflect this trend, with higher values in year 3 and lower values in the second half of year 2.

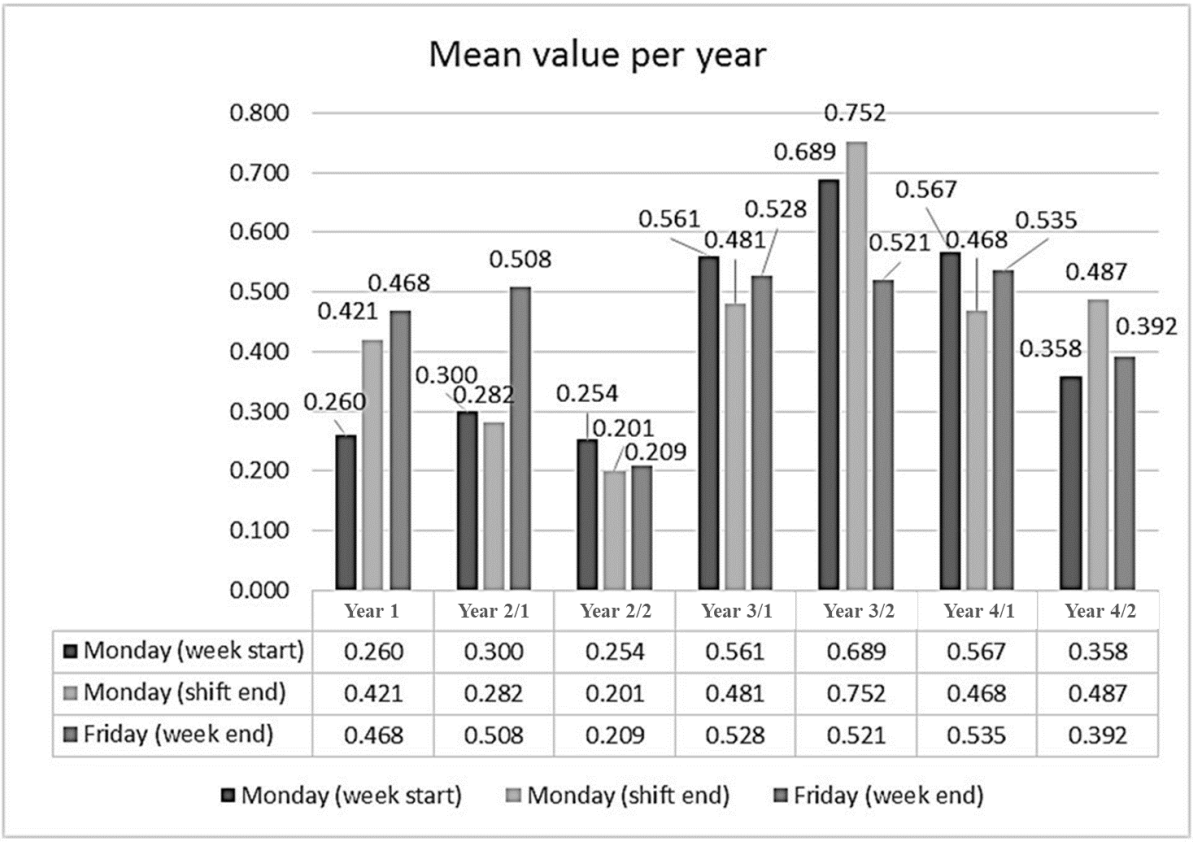


Figure 10. Average values of the three sampling times obtained for each monitoring campaign.

Table 9 shows the average values obtained in each single campaign monitoring. The reference value used to identify the potential risk for workers is, according to the Italian guidelines of the Industrial Hygienists Association (AIDII) (https://www.aidii.it/), 5.6 mg/L.

Year	Mean ± SD	Median	Range (min-max)	
Year 1	0.39±0.23	0.33	0.03	0.93
Year 2/1	0.34±0.25	0.27	0.06	0.99
Year 2/2	0.22±0.14	0.22	0.03	0.80
Year 3/1	0.52±0.17	0.48	0.22	1.04
Year 3/2	0.65±0.39	0.56	0.13	1.89
Year 4/1	0,52±0,23	0.45	0.06	0.91
Year 4/2	0,41±0,32	0.38	0.04	1.31

Table 9. 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 are expressed in mg/l.

As evident from **Figure 10** and **Table 9**, the values were always far below the accepted threshold value. However, it is worth noting that in the first (year 1) and in the first half of the second campaign (year2/2), the average of the values recorded on Friday evening was higher than the end shift on Monday, thus suggesting the trend, albeit minimal, towards the weekly stack. In the second half of the second year and in the third, the three sampling campaigns report comparable values; this can be attributed to worker exposure to external sources of FA, likely not related to the hospital work area or, possibly, to a subjective endogenous level of FA. An analysis showing the trend of the values in individual workers, measured over the four years, is shown in **Figure 11**.

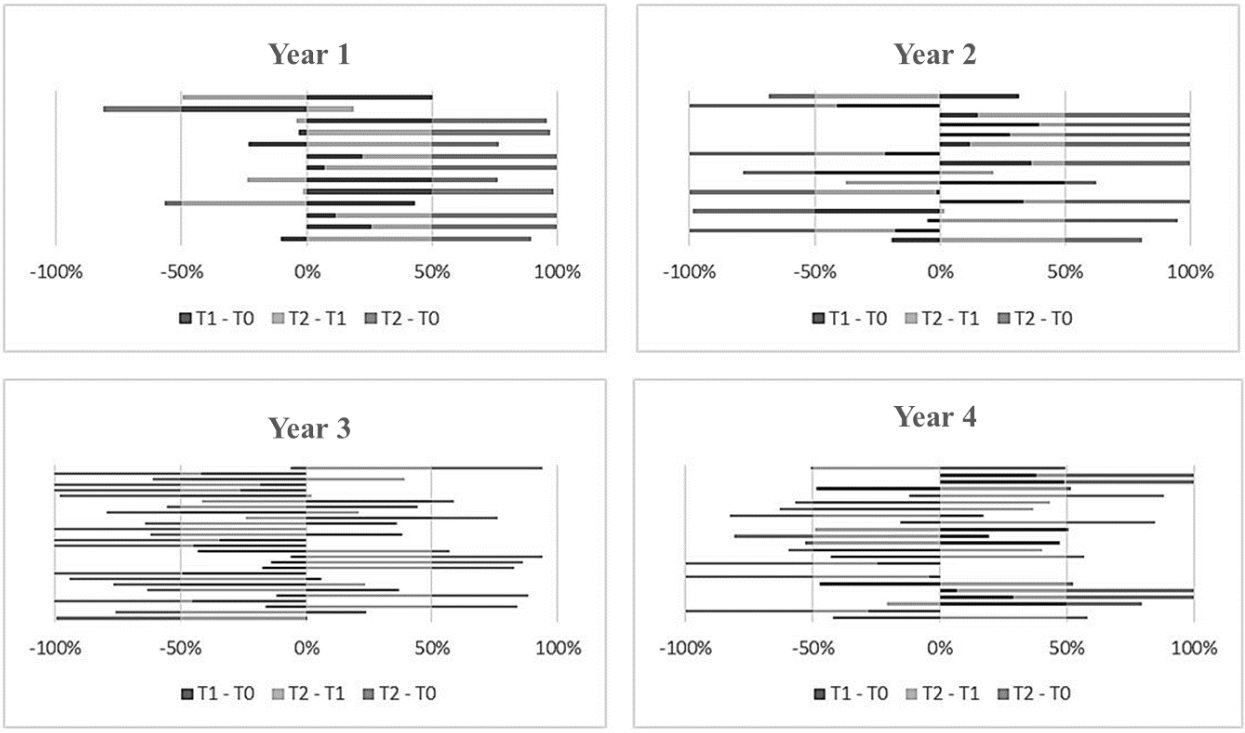


Figure 11. Analysis performed over four years of values trend for each worker. Values expressed in mg/l.

In year 1, 61.5% of the values, measured on Friday at the end of shift processing (T2), were higher compared to the values measured on Monday evening at the end of the working shift (T1), while the intraday measurements of the first working day (T0 vs T1) showed an increase in AF values in 69.2% of workers. In year 2, the trend was similar. In year 3, we observed an increase in T1 over T2 for 50% of workers, with values more than doubling in 18.7% of cases. In the T0 vs T1 measurements, however, an increase in FA values was found in only 25% of the workers.

4.4 Results of the UCDP environmental monitoring campaigns

Environmental samples collected from work areas potentially contaminated with AAs using paper-wipes were analyzed through the described multi-residual UHPLC-MS/MS-based analytical method. As previously mentioned, working areas were analyzed before and after cleaning, to verify the efficacy of the routinary cleaning operations. Areas 6, 7 and 8 were not sampled a second time, as the whole surface area was analyzed only once and they were not subjected to cleaning operations. Results obtained from the surfaces analyzed in the first run, prior to cleaning, showed rather high traces of CFA and IFA, especially under the fume hood. In one of the campaigns, a very high IRT value was detected under the hood in use (zone 2); it should be noted that this result is probably due to the use of IRT during the sampling day and to a spill which occurred under the hood during the dilution process that was reported only after the analyses had been performed. In addition, the IRT stock was in very concentrated particulate form; this might lead to the possibility of splashes during the preparation of the dilutions with consequent fallout of the contaminating material on the work surfaces.

The results obtained from the post-cleaning harvest, on the other hand, highlight other several important aspects. In particular, IFA and CFA, already present in the same areas analyzed in the first round, are found to be present at very high concentrations also during the second sampling. This suggests that the daily cleaning procedures, which consisted of a 5% hypo-chlorite-based washing solution and a sterile gauze for rubbing, even if suitable to contain biological risks or other types of contamination, are quite inefficient in effectively reducing the chemical risk associated with the handling of AAs.

Based on the results obtained, the efficacy of different surfaces cleaning methods was investigated. AA 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 could 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 potential combinations of solvents and supports (NW-E70, CG-E70, AP-E70, NW-W/E, CG-W/E, AP- W/E) were then adopted 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 12**) 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 provided more satisfying results, regardless of the support used, although the higher efficacy for all AAs was achieved using AP.

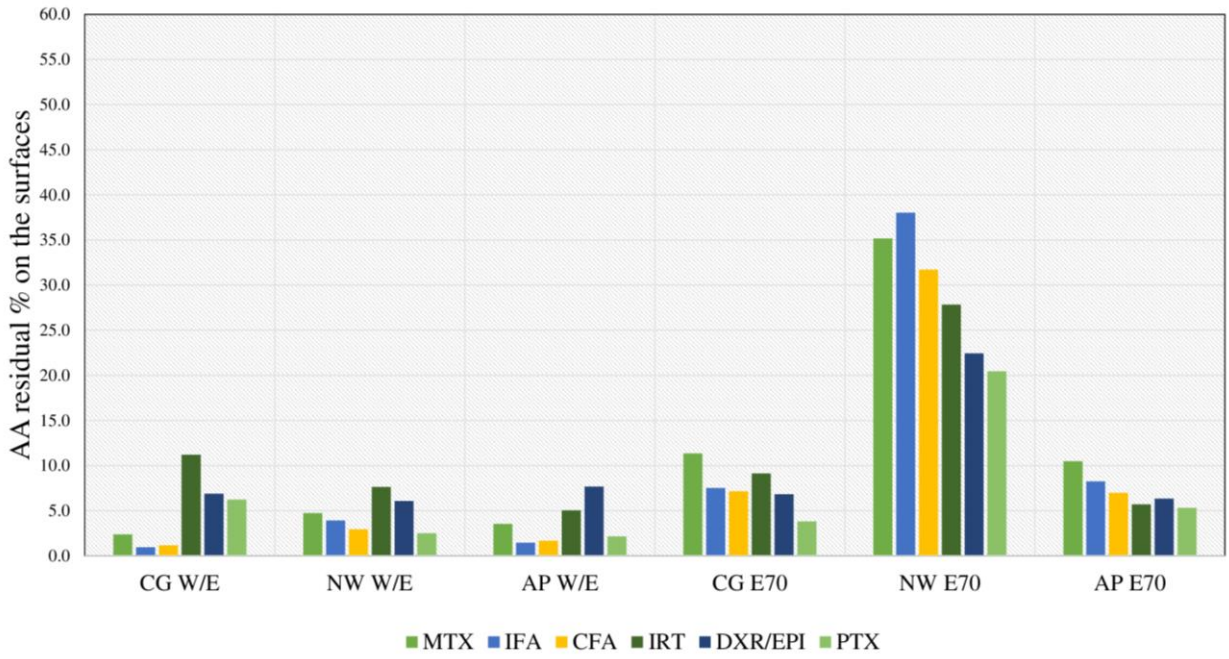


Figure 12. Antineoplastic drugs residual percentage on surfaces as a result of different cleaning procedures.

4.5 Antiblastic agents biological monitoring campaigns

After method optimization and validation, plasma and urine samples of healthcare personnel, daily involved in the AAs handling, were analyzed. Six workers were subjected to all monitoring campaigns. A venous blood and a urine sample were taken for each one. The withdrawals were made on a Friday, at the end of the last weekly working shift. Results obtained on biological samples, showed the presence of IRN in the plasma of a single worker in only one of the campaigns. To have further confirmation of what was observed in plasma, and to exclude the possibility of a false positive, the active metabolite of IRN, 7-ethyl-10-hydroxy-camptothecin (SN-38), was found in the urine of the positive worker. A survey revealed that the “positive” worker had recently been transferred to the UCDP. This probably facilitated the accidental exposure of the worker to chemotherapeutic agents. After an

appropriate training period, the worker was reinstated into routinary work shifts and showed no trace of the two analytes in the subsequent monitoring campaigns (**Table 10** and **11**).

COD	MTX	CFA	IFA	IRN	BND	PTX	DOX	SN-38 (urines only)
M 22	<50pg/ml* <1 ng/ml§	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 23	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	**Positive** <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	**Positive**
M 24	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 25	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 26	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 27	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml

*blood sample LLOQ; §urine sample LLOQ

Table 10. First biological monitoring campaign results

COD	MTX	CFA	IFA	IRN	BND	PTX	DOX	SN-38 (urines only)
M 22	<50pg/ml* <1 ng/ml§	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 23	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 24	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 25	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 26	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml
M 27	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <1 ng/ml	<50pg/ml <50pg/ml	<1 ng/ml <1 ng/ml	<50pg/ml <1 ng/m	<50pg/ml <1 ng/ml	<1 ng/ml

*blood sample LLOQ; §urine sample LLOQ

Table 11. Second biological monitoring campaign results

5. DISCUSSION

The airborne FA values obtained from the EM were very low on average, except for some areas, where the concentrations slightly exceeded the TLV established by NIOSH (= 0.1 ppm), with a maximum value of 0.16 ppm (into the macroscopic room, where biopsy specimens were processed). At the same time, the analyses performed on urinary samples of the same Unit workers, showed very low FA values, far from the accepted AIDII TLV (= 5.6 mg/L). Statistical analysis of biological samples suggested a potential weekly accumulation of FA. Although a different number of samples was analyzed in each campaign, due to the turnover of the staff, it is worth noting that in year 3 and 4 a lowering, albeit minimal, was noted in the levels of FA accumulation between the beginning and the end of the working week. However, it should be remembered that long-term measurement of urinary FA levels has severe limitations, due to the very short half-life of this molecule; therefore, other more suitable markers to evaluate the long-term biological accumulation and the potential damages should be critically evaluated. Furthermore, the AIDII guidelines consider OELs values suitable for manufacturing companies, but not for medical environments, such as hospitals, where FA is used at much lower amount, but still potentially harmful. This suggested the use of the limit prescribed by NIOSH, as it is the lowest available. However, the results obtained in this thesis clearly show that the simultaneous analysis of air quality and biological exposure of workers remains a key tool to allow optimization of occupational safety procedures, which must become routine in hospitals. Combination of BM and EM is necessary to understand the state of workplaces, the effectiveness of PPE, and the air filtration system. This is also important to evaluate workers' adherence to GLP and to preserve their health.

A similar consideration can be made after AAs BM and EM campaigns carried out in the UCDP of the same University Hospital. Following the first monitoring campaigns performed and the evident level of pollution found on the surfaces, a meeting between the U.O.C. of Clinical Pharmacology, the Department of Preventive and Predictive Medicine and the Hospital Pharmacy was organized during which the criticisms emerged from the first campaigns were discussed. A reorganization of the UCDP was then attempted in order to drastically reduce the levels of AAs pollution previously reported. Various changes have been made within the operating unit to improve the performance of the technical devices, the engineering systems and the cleaning procedures. As already mentioned in the Results section, the employee who found positive for the presence of an antiblastic urinary metabolite during the BM campaign was included in a specific training course to improve his handling ability and his perception of the chemical risk. Based on these observations, the laboratory and the anteroom were thoroughly cleaned with 70% ethanolic solution and the working procedures at the UCDP were completely reorganized. Additionally, the anteroom was transformed into a dressing room, where all PPE were worn before routine drug handling began. A closet replaced the desk, and people were only allowed into that room immediately before entering the lab or to strip off any PPE that came out of it. Considering that the high quantity of IFA and CFA found on several surfaces could have been related to the use of solid form of these compounds, one of the first technical intervention performed was to use ready-to-use liquid preparations. Following the methodological and procedural modifications introduced, subsequent monitoring has shown clear results in terms of improvement of the general safety conditions; as a matter of fact, in the last campaigns, no worker had been tested positive for traces of AAs. About a month after this reorganization, the EM of the work areas was repeated. The results of the analyses performed on these samples showed that the AAs

contamination had significantly decreased. These first measures led to an improved situation in the following campaign, even if the contamination was still present, but to a much less evident extent compared to the values found in the previous campaign. Despite this, traces of contamination were still found on areas that should theoretically be “clean”, such as the handle of the door and of the refrigerator where drugs are stored (areas 6 and 8); moreover, since significant quantities of drugs had been detected immediately after the various work surfaces had been regularly cleaned by UCDP workers, this suggested that the standard cleaning procedures were still largely ineffective. At the end of these first sampling cycles, an overview of the total AAs contamination model confirmed the effectiveness of the changes made to the work environment (**Figure 13**).

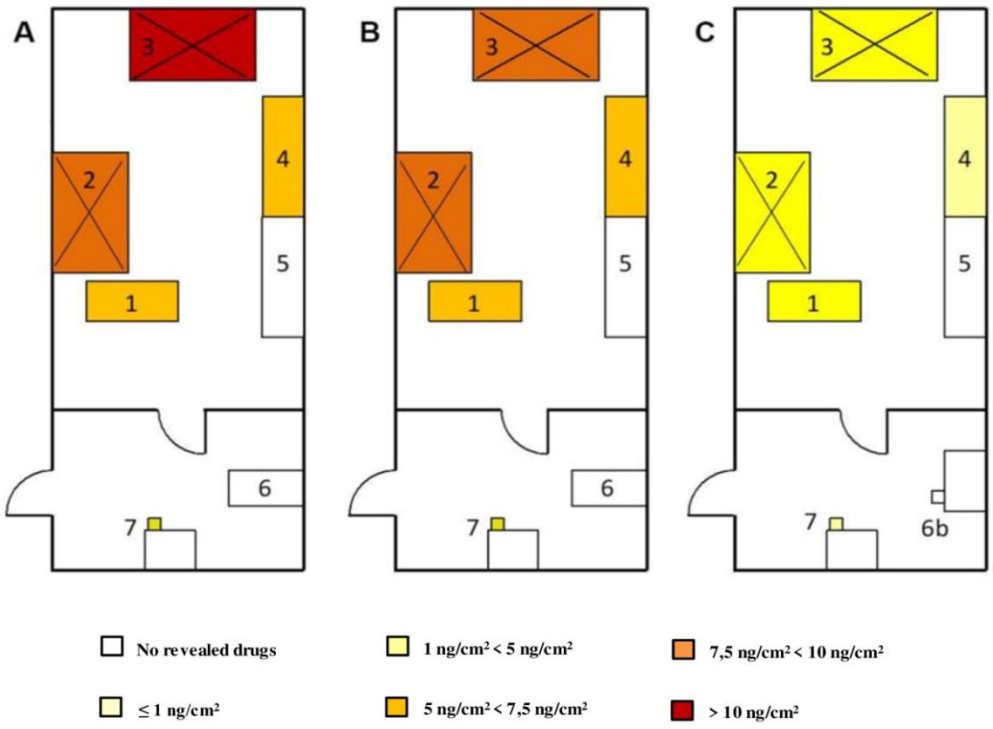


Figure 13. Whole contamination levels detected in the UCDP areas. (A) Drug concentration in the different areas in the first sampling; (B) Drug concentration in the different areas after cleaning; (C) Drug concentration in the different areas after laboratory deep cleaning and reorganization.

In **Table 12** are represented data obtained from campaigns performed before, after routinary cleaning operation and after deep cleaning operation.

		AREA						
Detection phase	**Drug**	**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	0

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

Table 12. Contamination levels (expressed as ng/cm2 of each drug) detected in the monitored areas during working time, after routine cleaning operations and after deep cleaning operations and working procedures reorganization. 1 (table top); 2 (laminar flow hood); 3 (laminar flow hood); 4 (table top); 5 (table top); 6 (desk/door handling); 7 (refrigerator handle).

The data obtained 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, despite improvements in working and cleaning procedures, CFA was still found to be the most persistent compound. In addition, in some cases the drug concentration detected on working surface increased after cleaning procedures. This may be related on routinely cleaning operations, as was also pointed out by the cleaning tests described in the section of the results, using various disposal and solvents, unable to efficiently remove all the contaminants, which could therefore accumulate in corners or dead spots and be withdrawn during following sampling procedures.

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 micro-fractures 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 of an uncontrolled release of toxic molecules. Routine monitoring of UCDPs contamination represents therefore a fundamental tool for verifying the presence of risk situations that are not always controllable and avoidable and to ensure healthcare professionals safety.

6. CONCLUSIONS

In recent years, the interest devoted to the prevention of chemical and biological risk has been growing steadily, diversifying from the simple notion of "protection" and establishing itself as the set of measures and tools aimed to protecting against accidental exposure to chemical and biological risks. To reduce the risk of occupational accidents deriving from chemicals, it is necessary to have prevention processes that include systematic monitoring of the presence and level of potentially toxic substances. Furthermore, attention should be paid to the possible long-term damage caused by chronic exposure to contaminants. Health workers are among the most exposed to occasional or prolonged contamination due to biological factors and chemical hazards. Contaminations are often detected in hospitals, even when trained personnel strictly follow all safety procedures and monitoring practices. For this reason, healthcare facilities need to regularly monitor and continuously improve risk management and protective devices, making monitoring easier, faster, and less expensive. Formaldehyde has been reclassified from January 2016 as a "carcinogenic substance" and this has led to the obligation for the employer to replace the carcinogenic compound, when possible, or to reduce exposure to As Low As Reasonably Achievable level (A.L.A.R.A. idea). This is also true for AAs, for which the danger towards exposed workers has been described. It should also be remembered that the Legislative Decree 81/08 provides that a risk assessment must be reconsidered every three years or in case of workflow changes and that the employer also measures the presence of carcinogens or mutagens to verify the effectiveness of the measures adopted. Therefore, BM and EM in healthcare facilities should be a concrete tool for effective protection of all workers involved in health management. The aim of this project was the development, validation and field testing of methods for the BM and EM of the chemical risk

from exposure to chemical pollutants, in particular FA and AAs. These methods were used to monitor the work areas and the biological samples of personnel employed within the Pathological Anatomy Unit and the UCDP of the University Hospital "San Giovanni di Dio e Ruggi d'Aragona" of Salerno. One of the main challenges in developing efficient monitoring protocols is, in fact, the availability of rapid and sensitive methods for the determination of contaminants, together with the optimization of sampling procedures. Furthermore, the selection of the work areas and biological matrices monitored is not always straightforward. The procedures developed and used in this work have allowed to efficiently perform the BM and EM in a synergistic way, offering a broad overview of the occupational exposure levels of workers, the contamination of the workplace and the capacity of PPE and cleaning operations. The data obtained have shown that, despite the high standard controls adopted in both Units, there was an effective risk of contamination for the personnel. The criticisms highlighted from our analyses played a pivotal role in the optimization and reorganization of the environments and of some working procedures used in the Units under study, leading to a significant improvement in the protection status of potentially exposed workers. Although some problems have not yet been completely resolved, the contamination check periodically performed, has made it effective the possibility to minimize the actual risk of chemical exposure in these hospital units. The work also evaluated the effectiveness of the PPE provided and assisted technical staff in better executing operations in accordance with international guidelines.

SCIENTIFIC PRODUCTION DURING THE PhD PERIOD

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23. De Bellis E., Conti V., Manzo V., Iannello F., Torsiello M., Stefanelli B., **Charlier B.**, Coglianese A., Izzo V., Dal Piaz F., Sabbatino F., Pepe S., Filippelli A. *A genotyping/phenotyping approach to manage the fluoropyrimidines-based therapy: clinical cases.* 40° Congresso Nazionale della Società Italiana di Farmacologia (SIF), digital edition, 9-13 Marzo 2021.

24. De Rosa F., Coglianese A., **Charlier B.**, Izzo V., Mensitieri F., Piazza O., Dal Piaz F., Filippelli A. *Therapeutic drug monitoring of opioids for pain management.* 40° Congresso Nazionale della Società Italiana di Farmacologia (SIF), digital edition, 9-13 Marzo 2021.

25. De Rosa F., Coglianese A., **Charlier B.**, Filippelli A, Piazza O., Izzo V., Dal Piaz F. *Development and validation of a LC-MS/MS based method to monitor opioids plasma concentration in pian management*. 52° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBIOC), virtual edition, 6-8 Ottobre 2020.

26. Coglianese A, **Charlier B.**, Filippelli A., Izzo V., Dal Piaz F. *Adherence to the gluten-free diet: a novel LC-MS/MS method to monitor uribary gluten exposure biomarkers*. 52° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBIOC), virtual edition, 6-8 Ottobre 2020.

27. **Charlier B**, Dal Piaz F, Coglianese A, Pingeon M, Marucci M, Filippelli A, Izzo V. *Volumetric Absorptive Microsampling (VAMS) as a Versatile Sampling Approach for Therapeutic Drug Monitoring in Clinical Settings.* 51° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBIOC), Padova (Italia), 20-22 Novembre 2019.

28. Charlier B, Coglianese A, Motta O, De Caro F, Izzo V, Dal Piaz F, Filippelli A, *Environmental and biological monitoring of formaldehyde inside a hospital setting: a combined approach for risk assessment evaluation and management.* 39° Congresso Nazionale della Società Italiana di Farmacologia (SIF), Firenze (Italia), 20-23 Novembre 2019.

ORAL PRESENTATIONS AND ORGANIZATION OF NATIONAL AND INTERNATIONAL CONGRESS AND WORKSHOPS

22.02.2023 **Oral presentation** entitled *Ruolo chiave del monitoraggio degli ambienti sanitari nella valutazione e gestione del rischio di esposizione professionale a farmaci citotossici.* 21th Congresso SITOX 20-22 Febbraio 2023, Bologna, Italia.

19.09.2022 **Oral presentation** entitled *A novel LC-MS method to quantify cenobamate in young adult patients from university hospital of Salerno.* 20th International Congress of therapeutic drug monitoring and clinical toxicology IATDMCT, 18-21 September 2022, Prague, Czech Republic.

5-7.09.2022 **Member of the Organizing Committee** for the SiBioC (Piano Scuola Permanente di Medicina di Laboratorio, SPML) "*Corso teorico-pratico di spettrometria di massa in medicina di laboratorio: IVD/LTD -verifica/validazione metodi -troubleshooting*". Campus universitario di Baronissi (SA), Dipartimento di Medicina, Chirurgia e Odontoiatria.

21.09.2021 **Oral presentation** entitled *Measurement of UH2/U ratio as prognostic screening of Dihydropyrimidine Dehydrogenase activity in 5-Fluorouracil therapy management*. 19th International Congress of therapeutic drug monitoring and clinical toxicology IATDMCT, 19-22 September 2021, Rome, Italy.

09/03/2021 **Oral presentation** entitled *Perampanel in partial seizures, the importance of a method for measuring blood levels in the adjustment of multi-drug therapy.* 40° Congresso Nazionale della Società Italiana di Farmacologia (SIF), digital edition, 9-13 Marzo 2021.

15.02.2021 **Invited speaker:** "*Impatto della variabilità biologica sugli intervalli di riferimento negli esami di laboratorio*". Meeting "La medicina a servizio della cura dei pazienti di etnie differenti: focus sulle differenze del processo di cura". Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Università degli Studi di Salerno, Baronissi, SA, Italia.

11.12.2019 **Invited speaker:** "*Bioaccumulo e tossicità: quando si supera il "limite?". Meeting "Inquinamento Ambientale e Salute Umana: dalla chimica alla clinica*". Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Università degli Studi di Salerno, Baronissi, SA, Italia.

PATENTS AND TECHNOLOGICAL TRANSFER

19.04.2022- Co-founder of the "Matox s.r.l.", university spin-off specialized in the development of methodologies and diagnostic kits for the environmental and biological monitoring of bio-sanitary workers exposed to chemical risk.

07/04/2021- Granting of the Italian patent 102019000007227 for invention "*Kit e metodo per il rilevamento di contaminanti chimici su superfici*".

PRIZES AND AWARDS

1. **SIF poster award**. De Rosa F., Coglianese A., **Charlier B.**, Izzo V., Mensitieri F., Piazza O., Dal Piaz F., Filippelli A. Therapeutic drug monitoring of opioids for pain management. **40° Congresso Nazionale della Società Italiana di Farmacologia (SIF). *Virtual Meeting* 2021.**

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