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A study protocol for the assessment of nurses internal contamination by antineoplastic drugs in hospital centres: a cross sectional multicentre descriptive study

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Abstract

Introduction: Antineoplastic drugs (AD) are potentially carcinogenic and/or reprotoxic molecules. Healthcare professionals are increasingly exposed to these drugs and can be potentially contaminated by them. Internal contamination of professionals is a key concern for occupational physicians in the assessment and management of occupational risks in health care settings.
Objectives: to report AD internal contamination rate in nursing staff and identify factors associated with internal contamination.
Methods and analysis: this trial will be conducted in two French hospital centres: University Hospital of Bordeaux and IUCT-Oncopole of Toulouse. The target population is nurses practicing in one of the fifteen selected care departments where at least one of the five studied AD is handled (5-fluorouracil, cyclophosphamide, doxorubicin, ifosfamide, methotrexate). The trial will be conducted with the following steps: (1) development of analytical methods to quantify AD urine biomarkers, (2) study of the workplace and organization around AD in each care department (transport and handling, professional practices, personal and collective protection equipments available) (3) development of a self-questionnaire detailing professional activities during the day of inclusion, (4) nurses inclusion (urine samples and self-questionnaire collection), (5) urine assays, (6) data analysis.
Ethics and dissemination: The study protocol has been approved by the French Advisory Committee on the Treatment of Information in Health Research (CCTIRS) and by the French Data Protection Authority (CNIL). Following the opinion of the Regional Committee for the Protection of Persons, this study is outside the scope of the provisions governing biomedical research and routine care (n°2014/87). The results will be submitted to peer-reviewed journals and reported at suitable national and international meetings.

Trial registration number: NCT03137641, April 28, 2017

Strengths and limitations of this study:

The analytical methods used needs to be specific and highly sensitive, which is essential for reliable detection and to reduce the number of misclassifications as uncontaminated.

Exposure biomarkers of five antineoplastic drugs will be analysed in each urine sample.

The care departments of the study are selected among different medical specialties.

The data from the self-questionnaires coupled with the results of the urine assays will serve to identify factors associated with internal contamination.

This study will only assess the internal contamination of nurses.

Introduction:

The number of cancer cases is constantly increasing worldwide and consequently, the administration of antineoplastic drugs (AD) is more and more widespread. In France, more than 320,000 people were treated with AD in 2015.[1] This leads to an increase in the use of these products by health professionals in terms of frequency and quantities handled and therefore to an increase in occupational exposure to these substances. According to the Sumer survey conducted with occupational physicians in 2010, more than 49,400 employees were potentially exposed to these drugs in France[2] and more than 5.5 million employees in the United States in 2003.[3]

Several professions are concerned by this exposure, including pharmacist technicians, pharmacists, couriers, nurses, assistant nurses, hospital agents, doctors, etc. Several international studies conducted between the 1980s and 2003 report that pharmacist assistants and nurses handling these drugs were contaminated, with rates exceeding 75% or even 90% of staff in some studies.[4-6] The best approach to measure internal contamination is biomonitoring, *i.e.* AD detection in urines of exposed healthcare professionals.

More than one hundred AD are currently marketed in France.[7] Most are on the list of "dangerous to handle" medicines issued by the US National Institute for Occupational Research and Safety (NIOSH) in 2004[3] because of their carcinogenic, mutagenic and/or reprotoxic effects (CMR). Thirty-eight AD have been evaluated by IARC: 13 are classified as a human carcinogens (group 1), 11 as probably carcinogen (group 2A), 7 may be carcinogenic (group 2B), and 7 are not classifiable for human carcinogenicity (group 3).

Since the 1970s, epidemiological studies conducted with nurses handling AD have shown an increase in risk of cancers[8 9] such as leukemias[8] and/or reprotoxic effects. The reported reprotoxic effects are: spontaneous abortions,[10-16] fetal malformations,[9 17-20] decreased fertility,[16 21 22] risk of uterine growth retardation and prematurity.[22] In the absence of reference biological value for occupational AD exposure, the long-term effects of occupational low-intensity exposure to these CMR products should lead to a reduction in exposures to the lowest possible level.

During occupational exposure, the contamination can take place by the respiratory and/or cutaneous and/or oral route.[23] It can occur directly during the reception, preparation, transport, injection of the drug and the handling of waste or indirectly through the patients and their excreta (vomit, urine, stool, sweat), sheets and soiled linen. [23 24] In order to limit these exposures and to guarantee the safety of employees, centralized reconstitution units for chemotherapies have been created in healthcare establishments and recommendations have been drawn up by government agencies and other occupational health organizations.[3]

Despite the recommendations and the improvements made in terms of safety on the handling and transport of these drugs, several recent studies show that the problem of contamination is still relevant, both in the working environment[23 25 26] and for the professionals themselves.[25 27-29] These internal contamination data show that preventive measures are not sufficiently controlled. It is thus necessary to understand the determinants of exposure.

Very little current data are available on the internal contamination of French healthcare professionals exposed to AD. The CACIES protocol detailed in this paper, aims to collect data on AD internal contamination in nurses and understand factors associated with this contamination.

Objectives:

The main objective of the CACIES protocol is to evaluate the rate of internal contamination by AD in nurses administering AD and/or taking care of patients treated with these molecules, in two French hospitals. This rate will be described globally and then stratified by care department.
The secondary objectives are: (I) to describe for each studied AD the rate of internal contamination among the nurses in the study, and the concentrations associated with this contamination; (II) to identify factors associated with internal contamination in this study (exposure characteristics and use of protective equipments by nurses).

Methods and analysis:
CACIES is a cross-sectional, descriptive, prospective multicentre study conducted in two French hospitals (University Hospital of Bordeaux and IUCT- Oncopole of Toulouse).
Eleven hospitals care departments, having an activity in the management of cancer patients treated with any of the following AD: cyclophosphamide, ifosfamide, methotrexate, 5-fluorouracil and/or doxorubicin, were chosen for this study.
The target population is nurses occupationally exposed to the studied AD.

Eligibility criteria
The three following inclusion criteria are required: (1) be a nurse practising in one of the selected care departments where at least one of the five studied AD is handled; (2) handle at least one of the five studied AD and/or take care of a patient treated with one of the five studied AD on the day of study participation (i.e day of urine samples collection); (3) agree to participate in the study and sign the participation consent form.
The exclusion criteria are: (1) be a student nurse; (2) be treated with one of the five studied AD or have been treated with any in the year prior to the day of study participation; (3) have at home a person treated with one of the five studied AD, in the month before the day of study participation.

Study design
The study will be conducted in six steps.

Step 1: Development of analytical methods for quantification of AD urine biomarkers
Analytical methods will be developed in the Pharmacology and Toxicology Laboratory of the Bordeaux University Hospital. These methods use an ultra-high-performance liquid chromatography system coupled with tandem mass spectrometry (UHPLC-MS/MS) characterized by high sensitivity and high specificity (5500 QTrap, Sciex®). AD urine biomarkers will be the AD themselves with the exception of 5-fluorouracil, which is not detectable in urine. For this molecule, its urinary metabolite, alfa-fluoro-beta-alanine (FBAL), will be assayed to assess internal contamination. Two methods have been already validated[30] but the limit of quantification (LOQ) will be improved. Two other methods will be developed for this study for the determination of doxorubicin and 5-fluorouracil urine biomarkers. These methods will be robust and highly sensitive with LOQ adapted to this type of study: i.e. very low LOQ values allowing detection of urine AD traces of the order of ng/L.

Step 2: Study of the workplace and organization around AD in each care department
A hygienist of the Occupational Medicine department will observe the activities around AD in each selected care department at the end of the urine sample and self-questionnaire

collection. Collective and individual protection equipment available in each department as well as the professional practices observed will be reported in this study of the workplace. A description of the complete organization around AD and excreta of treated patients within each care department will also be carried out: AD reception in the department, administration to patients, disposal of waste. All these observations will be collected and reported in a standardized way for each care department.

Step 3: Development of a self-questionnaire

A self-questionnaire will be built with the aim to collect several data: socio-demographic and occupational data (table 1), data concerning AD handling on the day of inclusion (table 2), data concerning take care modalities of AD treated patient (table 3), and personal protective equipment (PPE) worn the day of inclusion (table 4).

For each task listed in tables 2 and 3, the influence of the questionnaire on the nurse practices on the day of participation and for the future is asked. For each task listed in tables 2 and 3, personal protective equipments (table 4) that the nurse wears the day of inclusion are asked. For each task the PPE list is exhaustive so as not to influence the nurse in the choice of PPE according to the task.

Step 4: Nurses inclusion

For each nurse, the study participation lasts 24 hours. Three urine samples will be collected at different times in less than 24 hours (Figure 1). A document gathering the date and times of urine samples will be attached to the samples. Urine samples will be sent to the pharmacology and toxicology laboratory of Bordeaux university hospital within 72h at +4°C. Then samples will be aliquoted and stored at -20°C until analysis. At the same time, nurses will complete a self-questionnaire concerning their professional activity throughout the AD handling day. Each self-questionnaire will be sent by mail (return postage paid envelopes) to the Coordinating Centre, which will monitor the completed data.

Step 5: Urine assays

For each urine sample, four extraction methods followed by a validated analytical method will be performed. The result will be expressed according to the AD concentration level (ng/L and ng/g of urinary creatinine). Participant will be considered as contaminated when at least one of the five studied AD, is detected in at least one of the three collected urine samples.

Step 6: Data analysis

Statistical analyzes will be performed using SAS® software (SAS Institute, v9.3, North Carolina, USA) by a statistician from the Coordinating Centre.

The rate of internal contamination will be calculated by reporting the number of contaminated subjects by at least one of the studied AD to the total number of subjects included and will be expressed as a percentage. This proportion will be estimated globally then detailed by molecule and department. The extent of the concentration levels achieved will also be described for each sampling time and each drug.

The statistical analysis will include a global descriptive analysis of collected data from the self-administered questionnaire. Then factors associated with internal contamination of nurses will be studied using a multivariate logistic regression model. An univariate analysis will be used to select the variables which will be included into the multivariate model at the significance level of 25%. A step-by-step method will be used to select the significant

variables at the 5% threshold in the final multivariate model. Interactions and confounders will be sought and tested throughout the modeling.

Endpoints:
The primary endpoint will be the absence or presence of internal AD contamination for each nurse. It will be determined in the light of AD urine assays results. A subject will be considered contaminated if at least one of the five AD is detected in at least one of the three urine samples.
Others endpoints will be studied:
- AD internal contamination stratified by drug and by sampling times (S1, S2, S3).
- Descriptions of the studied population from the self-questionnaire data: (I) socio-demographic data; (II) occupational data; (III) AD handling data (IV) take care modalities of treated patients by studied AD. This description will be stratified by centre and by department (stratification conditioned by the number of participants)
Following these descriptions, the factors, described above, associated with internal contamination of nurses will be studied.

Calculation of the number of participants:
The main objective is to estimate the rate of nurse internal AD contamination in two hospitals. Thus, no sample size calculation will be made for the main criterion since it will be estimated from the total eligible population. Given the total number of nurses working in the 11 selected care departments to participate in the study, 300 nurses are potentially eligible.
Since this protocol is not very constraining for participants, with only one day of inclusion and only three noninvasive urinary samples, we expect a participation rate around 75% for the nursing staff. With this participation rate, the number of recruited subjects expected for this study will be about 225 subjects.
Impact of the study:
The impact of this study will be: (1) the assessment of the rate of nurses internal contamination in care departments, (2) awareness of nurses about their contamination, (3) implementation of corrective actions, (4) improvement of AD handling and transport safety, (5) improvement of nurse professional practices and particularly the use of protection equipement, (6) powerful (highly sensitive) analytical tools set up in the laboratory, adapted to the follow-up of professionals exposed to "dangerous handling drugs", and available for occupational physicians.

Patient and public involvement:
The research question and the protocol has been developed without the assistance of exposed nurses. On the other hand, before inclusion some nurses will be observed during their professional activity in the aim to build an adapted self-questionnaire. This will then be tested by nurses before the beginning of inclusion. Representative workers of hospital personnel, managers of the two hospitals, health managers will be informed of the study. Each nurse from the selected care departments will receive a briefing note prior to inclusion and will be invited to participate in an information meeting about CACIES study.

Ethics and dissemination:

The study protocol has been approved by the French Advisory Committee on the Treatment of Information in Health Research (CCTIRS) and by the French Data Protection Authority (CNIL).
Collected data will be subject to a computerized treatment in the Coordinating Centre of this study (Research Platform in Pharmacoepidemiology, BPE, CIC Bordeaux CIC1401) in compliance with law n ° 78-17 (January 6, 1978) relating to data processing, files and freedoms modified by the French law 2004-801 (August 6, 2004). Collected data will be kept during five years.
The results from this study will be submitted to peer-reviewed journals and reported at suitable national and international conferences or workshops.

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Contributors
MCR, MM designed the initial study concept,
CVE, AV, IB, SMP, BM, contributed to the design of the study and development of the protocol,
MCR, CVE, EB, AV, MR, participate to develop the self-questionnaire,
AV, MCR wrote the manuscript with the contributions of others authors for each work packages.
All authors have taken part in the academic discussions of the manuscript's content, and in revising the article.
All authors read and approved the final manuscript.
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Competing interests
The authors declare that they have no competing interests.

Patient consent for publication
Not required

Ethics approval
Following the opinion of the French Regional Ethic Committee for the Protection of Persons (CPP n° 2014/87), this study is considered as outside the scope of the provisions governing biomedical research and routine care. Indeed, CACIES is an observational study which does

not modify working methods and/or the use of protective equipment. The study protocol has been approved by the French Advisory Committee on the Treatment of Information in Health Research (CCTIRS) and by the French Data Protection Authority (CNIL).
This study is registered on the ClinicalTrials.gov website, under the number NCT03137641.

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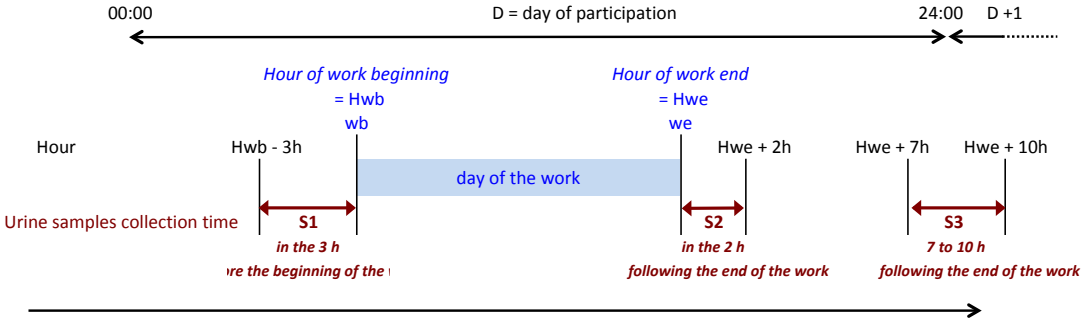

Figure 1: urine samples collection time

Table 1 : general collected data from the self-questionnaire administered to nurses

Socio-demographic data	- sex, - month and year of birth, - pregnancy, - smoking - onychophagia
Occupational data	- diplomas and specializations : type and years of obtaining, - seniority at the workplace : number of years, - number of years of AD handling and/or taking care patient treating by AD, - current status, - care department, - establishment, - received information on the risks related to AD and years of the information - received awareness on the risk related to AD handling and years of the awareness - level perception on AD exposing tasks, AD handling risks, the individual protective equipments, the action to be taken in AD accidental exposure cases. - data on AD accidental exposures during their career.

Table 2 : collected data from the self-questionnaire administered to nurses concerning AD handling the day of inclusion (the day of urine sample collection)

Day of AD handling Work schedule the previous 7 days	- Day of sample urine collection - Work / no work (detailed for each seven days)
Work shift	- Hour of the beginning - Hour of the end
Exposure / manipulation to any of the five AD *	Name of AD handling
Performed tasks (for each task the number of task and AD nature are specified) :	- AD infusion bags reception, - opening of the package of AD infusion bags, - AD infusion, - possible use of secure administration equipment (Luer-Lock type), - tubing purge, - adjustment of the tubing flow, - tubing disconnection, - unscrewing needle, - deposit of AD waste in bin, - bin evacuation,
Total handled amount (in mg)	Detailed data for each AD
Route of administration for each AD:	- IV, - IM, - oral, - dermal, - intrathecal.
Perception of each participant on the department activity	
Accidental exposure event *, ** (ex: needlestick, reversal or leakage of pockets ...),	- event nature and number of events - AD concerned by this event - associated clinical symptoms - declared event to occupational physician

Table 3 : collected data from the self-questionnaire administered to nurses concerning take care modalities of AD treated patients the day of inclusion (the day of urine sample collection)

Number of treated patients who received an studied AD (number and AD nature) that nurse has taking care the day of participation	- patient treatment on the day of participation - patient treatment within the seven days before the day of participation
Performed tasks:	- direct contact with treated patients (help to wash, handling of treated patient), - handling of treated patient excreta (vomit, urine, faeces, expectoration, soiled sheets), - participation in cleaning chemotherapy treatment room, - cleaning room of treated patient - cleaning sanitary facilities of treated patient - insertion or removal of an urinary catheter - change of drape or bed repair of a treated patient - deposit of treated patient excreta in bin, - bin evacuation,

* data will also be collected for the 7 days prior to the day of study participation

** data will also be collected for all the career

Table 4: collected data from the self-questionnaire administered in nurses concerning personal protective equipment (PPE) wearing the day of inclusion*

Wearing and type of clothing	- hat - plasticised apron - short sleeve gown - long sleeve gown
Wearing and type of mask	- surgical mask - FFP2 mask - FFP3 mask
Wearing and type of eye protection	- protective eyewear - visor
Wearing and type of gloves	- latex/vinyl/nitrile/PVC - simple pair or double pairs of gloves - short or long sleeve
Performed procedure of hand washing after gloves removal (gloves used after AD handling)	- nothing - hand sanitizer use - wash of hands with water only - wash of hands with water and soap

* PPE list proposed to each nurse for each performed task
** for each item the use frequency is ask (never, sometimes, systematically)

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A study protocol for the assessment of nurses internal contamination by antineoplastic drugs in hospital centres: a cross sectional multicentre descriptive study

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Abstract

Introduction: Antineoplastic drugs (AD) are potentially carcinogenic and/or reprotoxic molecules. Healthcare professionals are increasingly exposed to these drugs and can be potentially contaminated by them. Internal contamination of professionals is a key concern for occupational physicians in the assessment and management of occupational risks in healthcare settings. Objectives of this study are to report AD internal contamination rate in nursing staff and to identify factors associated with internal contamination.

Methods and analysis: this trial will be conducted in two French hospital centres: University Hospital of Bordeaux and IUCT-Oncopole of Toulouse. The target population is nurses practicing in one of the fifteen selected care departments where at least one of the five studied AD is handled (5-fluorouracil, cyclophosphamide, doxorubicin, ifosfamide, methotrexate). The trial will be conducted with the following steps: (1) development of analytical methods to quantify AD urine biomarkers, (2) study of the workplace and organization around AD in each care department (transport and handling, professional practices, personal and collective protection equipments available) (3) development of a self-questionnaire detailing professional activities during the day of inclusion, (4) nurses inclusion (urine samples and self-questionnaire collection), (5) urine assays, (6) data analysis.

Ethics and dissemination: The study protocol has been approved by the French Advisory Committee on the Treatment of Information in Health Research (CCTIRS) and by the French Data Protection Authority (CNIL). Following the opinion of the Regional Committee for the Protection of Persons, this study is outside the scope of the provisions governing biomedical research and routine care (n°2014/87). The results will be submitted to peer-reviewed journals and reported at suitable national and international meetings.

Trial registration number: NCT03137641, April 28, 2017

Strengths and limitations of this study:

- For reliable detection and to reduce the number of misclassifications as uncontaminated, the analytical methods used will to be specific, highly sensitive, will use isotopic internal standard to normalise urine matrix effect and the AD urine stability during storage will be studied.
- Exposure biomarkers of five antineoplastic drugs will be analysed in each urine sample and AD concentration will be expressed in ng/L and in ng/g of urinary creatinine to account for urine dilution.
- The care departments of the study are selected among different medical specialties.
- The data from the self-questionnaires coupled with the results of the urine assays will serve to identify factors associated with internal contamination.
- This study will only assess the internal contamination of nurses and the environmental contamination of working surface will be performed separately in an other study.

Introduction:

The number of cancer cases is constantly increasing worldwide and consequently, the administration of antineoplastic drugs (AD) is more and more widespread. In France, more than 320,000 people were treated with AD in 2015.[1] This leads to an increase in the use of these products by health professionals in terms of frequency and quantities handled and therefore to an increase in occupational exposure to these substances. According to the Sumer survey conducted with occupational physicians in 2010, more than 49,400 employees were potentially exposed to these drugs in France[2] and more than 5.5 million employees in the United States in 2003.[3] Several professions are concerned by this exposure, including pharmacist technicians, pharmacists, couriers, nurses, assistant nurses, hospital agents, doctors, etc.

More than one hundred AD are currently marketed.[4] Most are on the list of "dangerous to handle" medicines issued by the US National Institute for Occupational Research and Safety (NIOSH) in 2004[3] because of their carcinogenic, mutagenic and/or reprotoxic effects (CMR). Thirty-eight AD have been evaluated by IARC: 13 are classified as a human carcinogens (group 1), 11 as probably carcinogen (group 2A), 7 may be carcinogenic (group 2B), and 7 are not classifiable for human carcinogenicity (group 3).

Since the 1970s, epidemiological studies conducted with nurses handling AD have shown an increase in risk of cancers[5 6] such as leukemias[5] and/or reprotoxic effects. The reported reprotoxic effects are: spontaneous abortions,[7-13] fetal malformations,[6 14-17] decreased fertility,[13 18 19] risk of uterine growth retardation and prematurity.[19]

Several international studies conducted between the 1980s and 2003 report that pharmacist assistants and nurses handling these drugs were contaminated, with rates exceeding 75% or even 90% of staff in some studies.[20-22] Moreover, numerous studies show surface contamination of workplace.[23]

Surface sampling is a useful tool in order to identify sources of environmental contamination, to help in the implementation of corrective measures, to verify the effectiveness of the surface decontamination process and to insure a monitoring of these surfaces. Surface sampling are complementary to biomonitoring which is the best approach to measure internal contamination, *i.e.* AD detection in urines of exposed healthcare professionals. Indeed, unlike metrology of surface contamination, biomonitoring allows to take into account at the level of each individual, all exposure pathways (respiratory, dermal, oral), the wearing or not of the protective equipment, the effectiveness of the type of protective equipment, gestures and professional practices, personal hygiene and quantities handled. Several analytical methods have been published for surface metrology of AD [24-28] and for AD urine biomonitoring.[29-32] More than 17 AD or their urine metabolites can be detected with these methods. Detection limit value in urine, for six of them, is from 0.01ng/L[32-34] to 0.02ng/L.[35] For the others, the LOD value in urine is from 0.05 to 1ng/L.[36]

In the absence of reference biological value for occupational AD exposure, the long-term effects of occupational low-intensity exposure to these CMR products should lead to a reduction in exposures to the lowest possible level.

During occupational exposure, the contamination can take place by the respiratory and/or cutaneous and/or oral route.[23] It can occur directly during the reception, preparation, transport, injection of the drug and the handling of waste or indirectly through the patients and their excreta (vomit, urine, stool, sweat), sheets and soiled linen.[23 37] In order to limit

these exposures and to guarantee the safety of employees, centralized reconstitution units for chemotherapies have been created in healthcare establishments and recommendations have been drawn up by government agencies and other occupational health organizations.[3 38] Despite the recommendations and the improvements made in terms of safety on the handling and transport of these drugs, several recent studies show that the problem of contamination is still relevant, both in the working environment[23 39-43] and for the professionals themselves.[33 35 39 44-46 47-51] Currently, scientific reviews report that there is no significant correlation between AD surface monitoring and AD urine monitoring.[40] In this context, there is no disadvantage in conducting both studies separately.

Above reported internal contamination, data show that preventive measures are not currently sufficiently controlled, confirmed by Graeve et al.[52] It is thus necessary to understand the determinants of exposure.

Very little current data are available on the internal contamination of French healthcare professionals exposed to AD. The CACIES protocol detailed in this paper, aims to collect data on AD internal contamination in nurses and understand factors associated with this contamination.

Objectives:

The main objective of the CACIES protocol is to evaluate the rate of internal contamination by AD in nurses administering AD and/or taking care of patients treated with these molecules, in two French hospitals. This rate will be described globally and then stratified by care department.

The secondary objectives are: (I) to describe for each studied AD the rate of internal contamination among the nurses in the study, and the concentrations associated with this contamination; (II) to identify factors associated with internal contamination in this study (exposure characteristics and use of protective equipments by nurses).

Methods and analysis:

CACIES is a cross-sectional, descriptive, prospective multicentre study conducted in two French hospitals (University Hospital of Bordeaux and IUCT- Oncopole of Toulouse).

Eleven hospitals care departments, having an activity in the management of cancer patients treated with any of the following AD: cyclophosphamide, ifosfamide, methotrexate, 5-fluorouracil and/or doxorubicin, were chosen for this study.

The target population is nurses occupationally exposed to the studied AD.

Eligibility criteria

The three following inclusion criteria are required: (1) be a nurse practising in one of the selected care departments where at least one of the five studied AD is handled; (2) handle at least one of the five studied AD and/or take care of a patient treated with one of the five studied AD on the day of study participation (i.e day of urine samples collection); (3) agree to participate in the study and sign the participation consent form.

Some work tasks (table 1) expose workers more than others (table 2) in term of level of AD concentration (AD preparation, patient's urine, washing water after the patient had been washed and cleaning water after a patient toilet had been cleaned, …).[37] However, the industrial sanitary rules (smoking, washing hands, onychophagia…) and the wearing of PPE according to the tasks are not always respected. As a result, some less exposing tasks may cause higher workers contamination level than more exposing tasks. Indeed, Fransman et

al,[37] highlight levels of external hand contamination higher for tasks such as washing treated patients, removing bed sheets and handling urine of treated patients compared to drug preparation and toilet cleaning tasks. Therefore for the second inclusion criteria, all nurses will be included whatever the task done (AD handling and/or take care of AD treated patient) during the day of the participation to the study participation.

Table 1 : collected data from the self-questionnaire administered to nurses concerning AD handling the day of inclusion (the day of urine sample collection)

Day of AD handling Work schedule the previous 7 days	- Day of sample urine collection - Work*/ no work (detailed for each seven days)
Work shift	- Hour of the beginning - Hour of the end
Exposure / manipulation to any of the five AD *	Name of AD handling
Performed tasks (for each task the number of task and AD nature are specified) :	- AD infusion bags reception, - opening of the package of AD infusion bags, - AD infusion, - use of closed system transfer device, - tubing purge, - adjustment of the tubing flow, - tubing disconnection, - unscrewing needle, - deposit of AD waste in bin, - bin evacuation,
Total handled amount (in mg)	Detailed data for each AD
Route of administration for each AD:	- IV, - IM, - oral, - dermal, - intrathecal.
Perception of each participant on the department activity	
Accidental exposure event *, ** (ex: needlestick, reversal or leakage of pockets ...),	- event nature and number of events - AD concerned by this event - associated clinical symptoms - declared event to occupational physician

* data will also be collected for the 7 days prior to the day of study participation

** data will also be collected for all the career

Table 2 : collected data from the self-questionnaire administered to nurses concerning take care modalities of AD treated patients the day of inclusion (the day of urine sample collection)

Number of treated patients who received an studied AD (number and AD nature) that nurse has taking care the day of participation	- patient treatment on the day of participation - patient treatment within the seven days before the day of participation
Performed tasks:	- direct contact with treated patients (help to wash, handling of treated patient), - handling of treated patient excreta (vomit, urine, faeces, expectoration, soiled sheets), - participation in cleaning chemotherapy treatment room, - cleaning room of treated patient - cleaning sanitary facilities of treated patient - insertion or removal of an urinary catheter - change of drape or bed repair of a treated patient - deposit of treated patient excreta in bin, - bin evacuation,

The exclusion criteria are: (1) be a student nurse; (2) be treated with one of the five studied AD or have been treated with any in the year prior to the day of study participation; (3) have at home a person treated with one of the five studied AD, in the month before the day of study participation.

Study design

The study will be conducted in six steps.

Step 1: Development of analytical methods for quantification of AD urine biomarkers

Analytical methods will be developed in the Pharmacology and Toxicology Laboratory of the Bordeaux University Hospital in accordance to the EMEA guideline.[53] These methods use an ultra-high-performance liquid chromatography system coupled with tandem mass spectrometry (UHPLC-MS/MS) characterized by high sensitivity and high specificity (5500 QTrap, Sciex®). AD urine biomarkers will be the AD themselves with the exception of 5-fluorouracil, which is not detectable in urine. For this molecule, its urinary metabolite, alfa-fluoro-beta-alanine (FBAL), will be assayed to assess internal contamination. Two methods have been already validated[33] but the limit of quantification (LOQ) will be improved. Two other methods are developed for this study for the determination of 5-fluorouracil metabolite (FBAL)[35] and doxorubicin urine biomarkers. These methods will be robust and highly sensitive with LOQ adapted to this type of study: i.e. very low LOQ values allowing detection of urine AD traces of the order of ng/L.

For each AD, isotopic internal standard is added in each urine sample to normalize urine matrix effect. Stability of each AD in urine sample is studied under different conditions of storage (+20°C for 24h with and without light, at +4°C for 72h, at -20°C for one month and one year, and after three freeze-thaw cycles in urine). A post-preparative stability was conducted by analysing extracted urine samples kept under auto-sampler conditions (+15°C) for 72h.

Step 2: Study of the workplace and organization around AD in each care department
A hygienist of the Occupational Medicine department will observe the activities around AD in each selected care department at the end of the urine sample and self-questionnaire collection. Collective and individual protection equipment available in each department as well as the professional practices observed will be reported in this study of the workplace. A description of the complete organization around AD and excreta of treated patients within each care department will also be carried out: AD reception in the department, administration to patients, disposal of waste. All these observations will be collected and reported in a standardized way for each care department.

Step 3: Development of a self-questionnaire
A self-questionnaire is built, in the light of literature data, concerning work tasks potentially exposing, risk perception.[49 50 54-58] In addition, we conducted a pilot study in a healthcare unit that enabled us to carry out a study of the complete organization around AD and excreta of treated patients and to collect tasks performed, type and wearing of PPE. During this pilot study, a draft version was pre-tested on a small group of nurses. When it was necessary, questions were changed according to the feedback of the nurses. A final version was elaborated and will be used in the CACIES study.
The aim of this self-questionnaire is to collect several data: socio-demographic and occupational data (table 3), data concerning AD handling on the day of inclusion (table 1), data concerning take care modalities of AD treated patient (table 2), and personal protective equipment (PPE) worn the day of inclusion (table 4).
For each task listed in tables 1 and 2, the influence of the questionnaire on the nurse practices on the day of participation and for the future is asked. For each task listed in tables 1 and 2, personal protective equipments (table 4) that the nurse wears the day of inclusion are asked. For each task the PPE list is exhaustive so as not to influence the nurse in the choice of PPE according to the task.

Table 3 : general collected data from the self-questionnaire administered to nurses

Socio-demographic data	- sex, - month and year of birth, - pregnancy, - smoking - onychophagia
Occupational data	- diplomas and specializations : type and years of obtaining, - seniority at the workplace : number of years, - number of years of AD handling and/or taking care patient treating by AD, - current status, - care department, - establishment, - received information on the risks related to AD and years of the information - received awareness on the risk related to AD handling and years of the awareness - level perception on AD exposing tasks, AD handling risks,

	the individual protective equipments, the action to be taken in AD accidental exposure cases. - data on AD accidental exposures during their career.

Table 4: collected data from the self-questionnaire administered in nurses concerning personal protective equipment (PPE) wearing the day of inclusion***

Wearing and type of clothing	- hat - plasticised apron - short sleeve gown - long sleeve gown
Wearing and type of mask	- surgical mask - FFP2 mask - FFP3 mask
Wearing and type of eye protection	- protective eyewear - visor
Wearing and type of gloves	- latex/vinyl/nitrile/PVC - simple pair or double pairs of gloves - short or long sleeve
Performed procedure of hand washing after gloves removal (gloves used after AD handling)	- nothing - hand sanitizer use - wash of hands with water only - wash of hands with water and soap

* PPE list proposed to each nurse for each performed task
** for each item the use frequency is ask (never, sometimes, systematically)

Step 4: Nurses inclusion

Each nurse from the selected healthcare departments will receive a briefing note prior to inclusion and will be invited to participate in an information meeting about CACIES study. At the end of the meeting, a kit containing the polypropylene pots to collect urine samples, the self-questionnaire and the participation consent form will be given to each volunteer. During the meeting, the nurse will be asked to collect their urine samples after several days of work. Therefore, the self-questionnaire plans to collect data on work history the previous seven days before urine samples collection (type of studied AD handling, accidental exposure event). For each nurse, the study participation lasts 24 hours.

Three urine samples will be collected at different times in less than 24 hours (Figure 1): the first one within the 3 hours before the start of the work to document an internal contamination following exposure the previous days before the study; the second within 2 hours following the end of the work, to document an internal contamination following exposure during the first hours of the day working day; the third between 7 to 10 hours after the end of the work, to document an internal contamination following exposure at the end of the work. The time of the 3rd sampling was chosen to take into account a delayed absorption by the cutaneous way as indicated by Hirst et al.[59]

A document gathering the date and times of urine samples will be attached to the samples. Urine samples will be sent to the pharmacology and toxicology laboratory of Bordeaux university hospital within 72h at +4°C. Then samples will be aliquoted and stored at -20°C until analysis. At the same time, nurses will complete a self-questionnaire concerning their professional activity throughout the AD handling day. The self-questionnaire is a paper document with a detachable flap. This part will be sent by mail (return postage paid envelopes) to the Coordinating Centre, which will monitor the completed data and the other part will be kept by the nurse. After urine sample reception by the lab, the latter will immediately informs the Coordinating Centre of this reception. The coordinating centre will contact the nurses within 7 days if the self-questionnaire has not been received yet, limiting possible loss of data. Moreover, in case of missing or discordant data, each subject will be contacted by a member of the coordinating center to complete the self-questionnaire.

Step 5: Urine assays

For each urine sample, four extraction methods followed by a validated analytical method will be performed. Moreover, urine creatinine will be analysed for each urine sample to account for dilution.[60 61] The result will be expressed according to the AD concentration level (ng/L and ng/g of urinary creatinine). Participant will be considered as contaminated when at least one of the five studied AD, is detected in at least one of the three collected urine samples.

Step 6: Data analysis

Statistical analysis will be performed using SAS® software (SAS Institute, v9.3, North Carolina, USA) by a statistician from the Coordinating Centre.

The rate of internal contamination will be calculated by reporting the number of contaminated subjects by at least one of the studied AD to the total number of subjects included and will be expressed as a percentage. This proportion will be estimated globally then detailed by molecule and department. The extent of the concentration levels achieved will also be described for each sampling time and each drug.

The statistical analysis will include a global descriptive analysis of collected data from the self-administered questionnaire. Then factors associated with internal contamination of nurses will be studied using a multivariate logistic regression model. An univariate analysis will be used to select the variables which will be included into the multivariate model at the significance level of 25%. A step-by-step method will be used to select the significant variables at the 5% threshold in the final multivariate model. Interactions and confounders will be sought and tested throughout the modeling.

Endpoints:

The primary endpoint will be the absence or presence of internal AD contamination for each nurse. It will be determined in the light of AD urine assays results. A subject will be considered contaminated if at least one of the five AD is detected in at least one of the three urine samples.

Others endpoints will be studied:

- AD internal contamination stratified by drug and by sampling times (S1, S2, S3).
- Descriptions of the studied population from the self-questionnaire data: (I) socio-demographic data; (II) occupational data; (III) AD handling data (IV) take care modalities of

treated patients by studied AD. This description will be stratified by centre and by department (stratification conditioned by the number of participants)
Following these descriptions, the factors, described above, associated with internal contamination of nurses will be studied.

Calculation of the number of participants:
The main objective is to estimate the rate of nurse internal AD contamination in two hospitals. Thus, no sample size calculation will be made for the main criterion since it will be estimated from the total eligible population. Given the total number of nurses working in the 11 selected care departments to participate in the study, 300 nurses are potentially eligible.
Since this protocol is not very constraining for participants, with only one day of inclusion and only three noninvasive urinary samples, we expect a participation rate around 75% for the nursing staff. With this participation rate, the number of recruited subjects expected for this study will be about 225 subjects.

Impact of the study:
The impact of this study will be: (1) the assessment of the rate of nurses internal contamination in care departments, (2) awareness of nurses about their contamination, (3) implementation of corrective actions, (4) improvement of AD handling and transport safety, (5) improvement of nurse professional practices and particularly the use of protection equipement, (6) powerful (highly sensitive) analytical tools set up in the laboratory, adapted to the follow-up of professionals exposed to "dangerous handling drugs", and available for occupational physicians.

Patient and public involvement:
The research question and the protocol have been developed by a multidisciplinary team and an analysis of the workplace. As indicated in step 3 of the study protocol, a pilot study was previously conducted, in a healthcare unit of Bordeaux university hospital during which a draft version of a self-questionnaire was developed and pre-tested on a small group of nurses and modify according to their feedback.
Representative workers of hospital personnel, managers of the two hospitals, health managers will be informed of the study. Each nurse from the selected care departments will receive a briefing note prior to inclusion and will be invited to participate in an information meeting about CACIES study.

Ethics and dissemination:
The study protocol has been approved by the French Advisory Committee on the Treatment of Information in Health Research (CCTIRS) and by the French Data Protection Authority (CNIL).
Collected data will be subject to a computerized treatment in the Coordinating Centre of this study (Research Platform in Pharmacoepidemiology, BPE, CIC Bordeaux CIC1401) in compliance with law n° 78-17 (January 6, 1978) relating to data processing, files and freedoms modified by the French law 2004-801 (August 6, 2004). Collected data will be kept during five years.
The results from this study will be submitted to peer-reviewed journals and reported at suitable national and international conferences or workshops.

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Contributors
MCR, MM designed the initial study concept,
CVE, AV, IB, SMP, BM, contributed to the design of the study and development of the protocol,
MCR, CVE, EB, AV, MR, participate to develop the self-questionnaire,
AV, MCR wrote the manuscript with the contributions of others authors for each work packages.
All authors have taken part in the academic discussions of the manuscript's content, and in revising the article.
All authors read and approved the final manuscript.
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Competing interests
The authors declare that they have no competing interests.

Patient consent for publication
Not required

Ethics approval
Following the opinion of the French Regional Ethic Committee for the Protection of Persons (CPP n° 2014/87), this study is considered as outside the scope of the provisions governing biomedical research and routine care. Indeed, CACIES is an observational study which does not modify working methods and/or the use of protective equipment. The study protocol has been approved by the French Advisory Committee on the Treatment of Information in Health Research (CCTIRS) and by the French Data Protection Authority (CNIL).
This study is registered on the ClinicalTrials.gov website, under the number NCT03137641.

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Figure legend/caption:

Figure 1: urine samples collection time. D: day of participation of nurses in the CACIES study; swt: start work time; ewt: end work time; S1: urine sample collected within 3 hours before the start of the work; S2: urine sample collected within 2 hours following the end of the work; S3: urine sample collected between 7 to 10 hours following the end of the work