PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A study protocol for the assessment of nurses internal contamination by antineoplastic drugs in hospital centres: a cross sectional multicentre descriptive study
AUTHORS	Villa, Antoine; Molimard, Mathieu; Bignon, Emmanuelle; Martinez, Béatrice; Rouyer, Magali; Mathoulin-Pelissier, Simone; Baldi, Isabelle; Verdun-Esquer, Catherine; Canal Raffin, Mireille

VERSION 1 – REVIEW

REVIEWER	Melissa McDiarmid, MD, MPH, DABT University of Maryland School of Medicine Division of Occupational and Environmental Medicine 11 S. Paca Street, Suite 200 Baltimore, MD 21201 USA
REVIEW RETURNED	30-Jul-2019

GENERAL COMMENTS	Characterizing and controlling the occupational exposure of oncology nurses to highly toxic, hazardous antineoplastic drugs (AD) continues to be one of the most urgent issues in occupational health. Indeed, this is one of the only examples in high resource countries where workers are permitted to be exposed to Group 1 IARC carcinogens and documented human abortifacients, with safety measures only weakly practiced or enforced. Thus, this paper is of potential significance to a wide readership from both a clinical medicine, but also a public health viewpoint. There are some significant weaknesses, however, which should be addressed prior to publication. From a general viewpoint, the literature review is quite dated and significant papers either about biomonitoring of exposed populations or methods of measuring exposure intensity are omitted. The complexities assessing this occupational health problem are several, including: 1) there are > than 100 possible drugs in use across the population 2) various work tasks present exposure opportunity and 3) exposure controls (take care measures) are variably applied (containment, use of PPE. These issues have been discussed elsewhere in the literature and could inform the decisions made in this proposed protocol, but do not appear in the literature review. Examples include: Connor, et al., 2010; McDiarmid et al., 2010. Other more recent biomonitoring studies that might inform the protocol, questionnaire or exposure assessment include: Ramphal et al., 2015; Baniasadi et al.,2018. In the Strengths and Limitations section of this paper, the authors discuss the need for analytical methods to be specific and sensitive to avoid misclassification of a result as 'uncontaminated' when drug is actually present. However, the authors seem to be unaware that there are several other aspects of their methods,

	other than the laboratory LODs and LOQs, that limit the likelihood of finding a 'positive' urinary result, even when one is present. To this reviewer, the major issue is with the eligibility criteria that a participant must have EITHER handled at least one of the five AD drugs of interest on the day of study participation OR cared for a patient who received one of those drugs on that day. The work tasks listed in Table 3 that do not involve drug or drug package handling are likely not of equal intensity of those involving drug handling. An important exception here may be handling patient waste, as most of these drugs are eliminated in the urine of treated patients, some in the metabolically active form, for up to 48 hours. However, other tasks that do not present exposure 'opportunity' from drug mixing or administering, are not of equal 'intensity' and thus, may be insufficient to permit exposure. Nurses who do not actually handle drug are not at the same exposure risk as those who do. If the nurses actually prepare drug as well as administer them, then in Table 2, I would also include what type of biologic safety cabinet is used for drug preparation, if any, and if they use closed system transfer devices. One is concerned that study participation/ inclusion criteria requires a window of only one day of work. This is quite narrow in which to find a breach in presumed safety protocols permitting an exposure of sufficient intensity to be measured on the one day you looked for it. The elimination times of most of these drugs suggest that the urine collection schedule would permit measurement of an exposure that took place up to several days prior to sample collection, especially for doxorubicin. Requiring nurses to have worked several days before the study day gives you a 'look back' period for potential exposure that could raise your likelihood of seeing a signal. Related to this, while the work history of the several days before urine collection is documented, a participant just returning to work after a week's vacation will have had a lesser likelihood of exposure, minimizing a possible positive result. Also, the elimination times of the drugs are based on studies where the drugs were administered either orally or intravenously. However, the exposure route of most importance for health workers is DERMAL. This may mean that elimination kinetics are altered, all suggesting more thorough consideration of the working time (exposure opportunity) that you should permit for study participants. In Step 4, Nurses Inclusion, I am curious as to why you are depending on them returning the questionnaire by mail. It seems this permits loss of data. Some parts of the questionnaire (health, exposure, repro history) could be completed prior to the study day and collected on the day of study, limiting possible loss of data. In the public involvement sections, it is concerning that the questionnaire and protocol was developed without input from the exposed nurses. The major objective of the study is to determine the "rate of internal contamination" of nurses handling these drugs. In the introduction it is stated that, "the best approach to measure internal contamination is biomonitoring…". While this is true, it has been discussed extensively in the literature that due to the complexities described above, of multiple different drugs in use by nurses on different days of work and the need to limit the drugs measured to only a few of those handled, the laboratory methods challenges etc, it is presumed that biomonitoring is not the best way to document occupational exposure. In most (but not all countries) environmental surface sampling has been accepted as documentation of worker exposure, in light of the evidence that the

	major exposure route is dermal. This is important because we do not want to 'exonerate' a contaminated work environment just because on the one day we looked in urine we did not find any of a very limited number of drugs which were assessed. The Authors may think that contaminated urine is likely to occur. While this has been the case in some settings, in locations with reasonable safety measures (biological safety cabinets, work practices and safety PPE, it's uncommon to see drug in urine. Thus, the protocol must be refined to raise the likelihood of finding a positive result by considering the issues raised above. Also, performing surface sampling for the same five drugs at the time of biomonitoring could give you a more complete picture of the extent of contamination in the work environment, even if your biomonitoring results yield few positive results.

REVIEWER	Roberta Bonfiglioli University of Bologna Italy
REVIEW RETURNED	13-Aug-2019

GENERAL COMMENTS	The protocol describes a study designed to primarily evaluate the rate of internal contamination by antineoplastic drugs (AD) among hospital nurses. Another aim of the study is to identify factors associated with AD contamination. It is an ambitious study given the number of subjects to be included and the strong effort for biological monitoring with a conspicuous number of drugs analysed in each urine sample. However there is a matter within the second aim of the study which deserve a comment. As stated by Authors AD occupational exposure is possible through several possible routes: dermal, respiratory and oral. Biological monitoring reveals the burden of contamination but cannot disclose the source of exposure and the route (if dermal or respiratory). Especially when centralized reconstitution units are available, ward nurses are probably more prone to have cutaneous than respiratory exposure. In order to identify source of contamination environmental monitoring is recommended. Poor correlation exists between biological and environmental monitoring in workers exposed to AD, this could be explained by their complementary role (Kibby T. A review of surface wipe sampling compared to biologic monitoring for occupational exposure to antineoplastic drugs. J Occup Environ Hyg. 2017 Mar;14(3):159-174). I suggest Authors to revise the study protocol by including environmental monitoring in order to pinpoint the true sources of exposure and to obtain precious information for prevention (Dal Bello F, Santoro V, Scarpino V, Martano C, Aigotti R, Chiappa A, Davoli E, Medana C. Antineoplastic drugs determination by HPLC-HRMS(n) to monitor occupational exposure. Drug Test Anal. 2016 Jul;8(7):730-7). If this is not possible it should be discussed as a relevant limitation.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Melissa McDiarmid

Characterizing and controlling the occupational exposure of oncology nurses to highly toxic, hazardous antineoplastic drugs (AD) continues to be one of the most urgent issues in occupational health. Indeed, this is one of the only examples in high resource countries where workers are permitted to be exposed to Group 1 IARC carcinogens and documented human abortifacients, with safety measures only weakly practiced or enforced. Thus, this paper is of potential significance to a wide readership from both a clinical medicine, but also a public health viewpoint. There are some significant weaknesses, however, which should be addressed prior to publication.

1.From a general viewpoint, the literature review is quite dated and significant papers either about biomonitoring of exposed populations or methods of measuring exposure intensity are omitted.

We actualised the literature review by adding 31 publications (most of which are recent) on biomonitoring of exposed populations and recent publications on methods of measuring exposure intensity and also contamination work surfaces. Please find below the reference added numbered as in the manuscript (these new references are highlighted in yellow in the main manuscript):

24. Guichard N, Rudaz S, Bonnabry P, et al. Validation and uncertainty estimation for trace amounts determination of 25 drugs used in hospital chemotherapy compounding units. J Pharm Biomed Anal 2019;172:139-48. doi: 10.1016/j.jpba.2019.04.042 [published Online First: 2019/04/30]
25. Atgé B, Da Silva Cacao O, Ducint D, et al. Tool development for assessing antineoplastic drugs surface contamination in healthcare services and other workplaces. 39th International Congress of the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) 21-24 May 2019, Naples, Italy [abstract]. Clinical Toxicology 2019;57(6):423-602.
26. Colombo M, Jeronimo M, Astrakianakis G, et al. Wipe Sampling Method and Evaluation of Environmental Variables for Assessing Surface Contamination of 10 Antineoplastic Drugs by Liquid Chromatography/Tandem Mass Spectrometry. Ann Work Expo Health 2017;61(8):1003-14. doi: 10.1093/annweh/wxx070 [published Online First: 2017/10/14]
27. Dal Bello F, Santoro V, Scarpino V, et al. Antineoplastic drugs determination by HPLC-HRMS(n) to monitor occupational exposure. Drug Test Anal 2016;8(7):730-7. doi: 10.1002/dta.1827 [published Online First: 2015/06/05]
28. Nussbaumer S, Geiser L, Sadeghipour F, et al. Wipe sampling procedure coupled to LC-MS/MS analysis for the simultaneous determination of 10 cytotoxic drugs on different surfaces. Anal Bioanal Chem 2012;402(8):2499-509. doi: 10.1007/s00216-011-5157-2 [published Online First: 2011/06/28]
29. Turci R, Sottani C, Spagnoli G, et al. Biological and environmental monitoring of hospital personnel exposed to antineoplastic agents: a review of analytical methods. J Chromatogr B Analyt Technol Biomed Life Sci 2003;789(2):169-209. [published Online First: 2003/05/14]
30. Stokvis E, Rosing H, Beijnen JH. Liquid chromatography-mass spectrometry for the quantitative bioanalysis of anticancer drugs. Mass Spectrom Rev 2005;24(6):887-917. doi: 10.1002/mas.20046 [published Online First: 2004/12/16]
31. Nussbaumer S, Bonnabry P, Veuthey JL, et al. Analysis of anticancer drugs: a review. Talanta 2011;85(5):2265-89. doi: 10.1016/j.talanta.2011.08.034 [published Online First: 2011/10/04]
32. Sottani C, Tranfo G, Bettinelli M, et al. Trace determination of anthracyclines in urine: a new high-performance liquid chromatography/tandem mass spectrometry method for assessing exposure of hospital personnel. Rapid Commun Mass Spectrom 2004;18(20):2426-36. doi: 10.1002/rcm.1642 [published Online First: 2004/09/24]
34. Hedmer M, Tinnerberg H, Axmon A, et al. Environmental and biological monitoring of antineoplastic drugs in four workplaces in a Swedish hospital. Int Arch Occup Environ Health 2008;81(7):899-911. doi: 10.1007/s00420-007-0284-y [published Online First: 2007/12/11]

36. Mathias PI, Connor TH, B'Hymer C. A review of high performance liquid chromatographic-mass spectrometric urinary methods for anticancer drug exposure of health care workers. J Chromatogr B Analyt Technol Biomed Life Sci 2017;1060:316-24. doi: 10.1016/j.jchromb.2017.06.028 [published Online First: 2017/06/28]
38. Mathias PI, MacKenzie BA, Toennis CA, et al. Survey of guidelines and current practices for safe handling of antineoplastic and other hazardous drugs used in 24 countries. J Oncol Pharm Pract 2019;25(1):148-62. doi: 10.1177/1078155217726160 [published Online First: 2017/08/26]
41. Chauchat L, Tanguay C, Caron NJ, et al. Surface contamination with ten antineoplastic drugs in 83 Canadian centers. J Oncol Pharm Pract 2019;25(5):1089-98. doi: 10.1177/1078155218773862 [published Online First: 2018/05/05]
42. Koller M, Bohlandt A, Haberl C, et al. Environmental and biological monitoring on an oncology ward during a complete working week. Toxicol Lett 2018;298:158-63. doi: 10.1016/j.toxlet.2018.05.002 [published Online First: 2018/05/09]
43. Dugheri S, Bonari A, Pompilio I, et al. A new approach to assessing occupational exposure to antineoplastic drugs in hospital environments. Arh Hig Rada Toksikol 2018;69(3):226-37. doi: 10.2478/aiht-2018-69-3125 [published Online First: 2018/10/05]
46. Poupeau C, Roland C, Bussieres JF. Surveillance urinaire des professionnels de la santé exposés aux antinéoplasiques dans le cadre de leur travail : revue de la littérature de 2010 à 2015. Can J Hosp Pharm 2016;69(5):376-87. [published Online First: 2016/11/09]
47. Hon CY, Teschke K, Shen H, et al. Antineoplastic drug contamination in the urine of Canadian healthcare workers. Int Arch Occup Environ Health 2015;88(7):933-41. doi: 10.1007/s00420-015-1026-1 [published Online First: 2015/01/30]
48. Friese CR, McArdle C, Zhao T, et al. Antineoplastic drug exposure in an ambulatory setting: a pilot study. Cancer Nurs 2015;38(2):111-7. doi: 10.1097/NCC.0000000000000143 [published Online First: 2014/05/17]
49. Ramphal R, Bains T, Goulet G, et al. Occupational exposure to chemotherapy of pharmacy personnel at a single centre. Can J Hosp Pharm 2015;68(2):104-12. doi: 10.4212/cjhp.v68i2.1435 [published Online First: 2015/05/13]
50. Ramphal R, Bains T, Vaillancourt R, et al. Occupational exposure to cyclophosphamide in nurses at a single center. J Occup Environ Med 2014;56(3):304-12. doi: 10.1097/JOM.0000000000000097 [published Online First: 2014/02/01]
51. Baniasadi S, Alehashem M, Yunesian M, et al. Biological Monitoring of Healthcare Workers Exposed to Antineoplastic Drugs: Urinary Assessment of Cyclophosphamide and Ifosfamide. Iran J Pharm Res 2018;17(4):1458-64. [published Online First: 2018/12/21]
52. Graeve CU, McGovern PM, Alexander B, et al. Occupational Exposure to Antineoplastic Agents. Workplace Health Saf 2017;65(1):9-20. doi: 10.1177/2165079916662660 [published Online First: 2016/10/21]
53. Anonymous. Guideline on bioanalytical method validation: European Medicines Agency, 2011:23.
54. Hon CY, Teschke K, Shen H. Health Care Workers' Knowledge, Perceptions, and Behaviors Regarding Antineoplastic Drugs: Survey From British Columbia, Canada. J Occup Environ Hyg 2015;12(10):669-77. doi: 10.1080/15459624.2015.1029618 [published Online First: 2015/04/22]
55. Steege AL, Boiano JM, Sweeney MH. NIOSH health and safety practices survey of healthcare workers: training and awareness of employer safety procedures. Am J Ind Med 2014;57(6):640-52. doi: 10.1002/ajim.22305 [published Online First: 2014/02/20]
56. Polovich M, Clark PC. Factors influencing oncology nurses' use of hazardous drug safe-handling precautions. Oncol Nurs Forum 2012;39(3):E299-309. doi: 10.1188/12.ONF.E299-E309 [published Online First: 2012/05/01]
57. Connor TH, DeBord DG, Pretty JR, et al. Evaluation of antineoplastic drug exposure of health care workers at three university-based US cancer centers. J Occup Environ Med 2010;52(10):1019-27. doi: 10.1097/JOM.0b013e3181f72b63 [published Online First: 2010/10/01]

58. McDiarmid MA, Oliver MS, Roth TS, et al. Chromosome 5 and 7 abnormalities in oncology personnel handling anticancer drugs. J Occup Environ Med 2010;52(10):1028-34. doi: 10.1097/JOM.0b013e3181f73ae6 [published Online First: 2010/10/01]
59. Hirst M, Tse S, Mills DG, et al. Occupational exposure to cyclophosphamide. Lancet 1984;1(8370):186-8. doi: 10.1016/s0140-6736(84)92111-1 [published Online First: 1984/01/28]
60. Newman MA, Valanis BG, Schoeny RS, et al. Urinary biological monitoring markers of anticancer drug exposure in oncology nurses. Am J Public Health 1994;84(5):852-5. doi: 10.2105/ajph.84.5.852 [published Online First: 1994/05/01]
61. Anonymous. Biological Monitoring of Chemical Exposure in the Workplace Guidelines: World Health Organization, 1996:314.

In the “Introduction” section, this part of the text ”Several international studies conducted between…….of exposed healthcare professionals” has been moved further down in the introduction section.

In the “Introduction” section, we added additional sentences:

“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/L32-34 to 0.02ng/L.35 For the others, the LOD value in urine is from 0.05 to 1ng/L.36”

2. The complexities assessing this occupational health problem are several, including: 1) there are > than 100 possible drugs in use across the population 2) various work tasks present exposure opportunity and 3) exposure controls (take care measures) are variably applied (containment, use of PPE). These issues have been discussed elsewhere in the literature and could inform the decisions made in this proposed protocol, but do not appear in the literature review. Examples include: Connor, et al., 2010; McDiarmid et al., 2010. Other more recent biomonitoring studies that might inform the protocol, questionnaire or exposure assessment include: Ramphal et al., 2015; Baniasadi et al.,2018.

In the section “study design” (in step 3), we added seven references used to build our self-questionnaire (references numbered 49,50, 54-58) including those of Ramphal et al., 2015, Connor et al., 2010 and McDiarmid et al., 2010. The text has been modified as follows:
“A self-questionnaire is built, in the light of literature data, concerning work tasks potentially exposing, risk perception.49 50 54-58”

Ramphal et al., 2015 and Baniasadi et al., 2018 references numbered as 49 and 51 were cited in the “introduction” section for recent biomonitoring data on workers contamination.

In the “introduction” section, we added the recent review of Mathias et al, 2019 as a second reference (numbered 38) for recommendations by government agencies for safe handling of antineoplastic drugs. We also added the study of Graeve et al, 2017 as reference (numbered 52) to illustrate that preventive measures (personal protective equipment) are not sufficiently controlled according tasks.

In the “introduction” section, we have added an additional paragraph:
“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”

3. In the Strengths and Limitations section of this paper, the authors discuss the need for analytical methods to be specific and sensitive to avoid misclassification of a result as ‘uncontaminated’ when drug is actually present. However, the authors seem to be unaware that there are several other aspects of their methods, other than the laboratory LODs and LOQs, that limit the likelihood of finding a ‘positive’ urinary result, even when one is present.

For each studied AD, analytical methods are (Dershin et al, 2018 and Canal Raffin et al 2016, under reference number 35 and 33) and will be developed and validated in accordance to the EMEA guideline. (reference numbered 53: European Medicines Agency. Guideline on bioanalytical method validation).
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf (consulted on 05-07-2018).

We have modified the “study design” section (step 1: Development of analytical methods for quantification of AD urine biomarkers) as follows:

“Analytical methods will be developed in the Pharmacology and Toxicology Laboratory of the Bordeaux University Hospital in accordance to the EMEA guideline.53”

Other than the laboratory LODs and LOQs, parameters that limit the likelihood of finding a ‘positive’ urinary result, can exist:
- if an isotopic internal standard is not used to normalize urine matrix effect for each sample (Dershin et al, 2018 and Canal Raffin et al 2016, under reference number 35 and 33)
- if the AD or its metabolite is not stable in the urine during the storage.
- if urine is too diluted, AD may be undetectable.

Then, for each AD, isotopic internal standard will be added in each urine sample to normalize urine matrix effect.
Moreover, during the development of our methods, stability studies of each AD in urine sample have been performed 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.
To assess urine dilution, urine creatinine will be analysed for each urine sample (references numbered 60-61).

We have added this information in the “study design” section (step 1: Development of analytical methods for quantification of AD urine biomarkers) as follows:

“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.”

We have added these informations in “study design” section (step 5: Urine assays):

“Moreover, urine creatinine will be analysed for each urine sample to account for dilution.60 61”

We have modified the two first items in the “Strengths and Limitations” section as follows:

- “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.”

4. To this reviewer, the major issue is with the eligibility criteria that a participant must have EITHER handled at least one of the five AD drugs of interest on the day of study participation OR cared for a patient who received one of those drugs on that day.
The work tasks listed in Table 3 that do not involve drug or drug package handling are likely not of equal intensity of those involving drug handling. An important exception here may be handling patient waste, as most of these drugs are eliminated in the urine of treated patients, some in the metabolically active form, for up to 48 hours. However, other tasks that do not present exposure ‘opportunity’ from drug mixing or administering, are not of equal ‘intensity’ and thus, may be insufficient to permit exposure. Nurses who do not actually handle drug are not at the same exposure risk as those who do.

The main objective of this study is to evaluate the rate of internal contamination by AD in nurses. We agree that some work tasks (Table 1) expose workers more than others (Table 2) in term of level of AD concentrations (AD preparation, patient’s urine, washing water after the patient had been washed and cleaning water after a patient toilet had been cleaned, …) (Fransman et al, 2005, reference numbered 37). Level of internal contamination depends on the level of exposure but also on the wearing of protective personal equipment more or less respected according to the tasks (Graeve et al, 2017, reference numbered 52), the respect of the industrial sanitary rules (smoking, onychophagia, washing hands...) and the perception of the risk according to the tasks.
As a result, some less exposing tasks may cause higher internal contamination level than more exposing tasks. Indeed, Fransman et al, 2005 (reference numbered 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 listed 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. This criteria also allows to include all nurse in a health care department such as them who are working during the night and are not likely to AD administer.

In the « eligibility criteria » section, we added the following sentences:
“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.”

5. If the nurses actually prepare drug as well as administer them, then in Table 2, I would also include what type of biologic safety cabinet is used for drug preparation, if any, and if they use closed system transfer devices.

We have previously consulted the centralized units for the preparation of antineoplastic drugs in the pharmacy of the two French hospitals. The latter confirmed us that none of the health care service included in the CACIES study are preparing these drugs. Thus, in Table 1 (ex table 2), we did not include items concerning drug preparation and type of biologic safety cabinet. However, we included the item of “closed system transfer devices” in table 1 (ex table 2).

6. One is concerned that study participation/ inclusion criteria requires a window of only one day of work. This is quite narrow in which to find a breach in presumed safety protocols permitting an exposure of sufficient intensity to be measured on the one day you looked for it. The elimination times of most of these drugs suggest that the urine collection schedule would permit measurement of an exposure that took place up to several days prior to sample collection, especially for doxorubicin. Requiring nurses to have worked several days before the study day gives you a ‘look back’ period for potential exposure that could raise your likelihood of seeing a signal. Related to this, while the work history of the several days before urine collection is documented, a participant just returning to work after a week’s vacation will have had a lesser likelihood of exposure, minimizing a possible positive result. Also, the elimination times of the drugs are based on studies where the drugs were administered either orally or intravenously. However, the exposure route of most importance for health workers is DERMAL. This may mean that elimination kinetics are altered, all suggesting more thorough consideration of the working time (exposure opportunity) that you should permit for study participants.

During the information meeting, nurses will be asked to collect their urine samples after several days of work. The seven days work history information preceding the collection urine samples will be collected in the self-questionnaire (type of studied AD handling, accidental exposure event). In Table 1, the legend (* and **) has been amending accordingly.
The manuscript is modified in the section “study design in step 4: nurses inclusion” as follows:
“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).”

As the reviewer 1 rightly indicates, pharmacokinetic studies of the drugs have been done in patients treated after oral or intravenous administration. However, the most common route of exposure for healthcare workers is the dermal route, which may delay the peak of absorption in blood and therefore the appearance of AD in the urine. Indeed, Hirst et al, in 1984 (reference number 59), highlighted that after cyclophosphamide dermal application in five volunteers: “in most cases, the drug was evident only in urine samples given more than 6h after application”. Therefore, in our study protocol, a third urine sample will be collected between 7 to 10 hours after the end of the work to take into account a delayed absorption by the dermal route.

Clarifications on times for urine samples collection have been added in the section “study design, Step 4: nurses inclusion ” as follows:
“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"

7. In Step 4, Nurses Inclusion, I am curious as to why you are depending on them returning the questionnaire by mail. It seems this permits loss of data. Some parts of the questionnaire (health, exposure, repro history) could be completed prior to the study day and collected on the day of study, limiting possible loss of data.

At the end of the nurse information 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. We will therefore ask nurses to complete some parts of the self-questionnaire (general collected data cited in Table 3) prior to the study day. The data listed in Tables 1 and 2 will be completed the day of study.
At the end of the study day, nurses will send the polypropylene pots containing urine to the Pharmacology and Toxicology laboratory of Bordeaux university hospital through the classic pathway of hospitalised patients samples using a pneumatic pipe system. This system is not compatible with the sending of large paper documents, such as our self-questionnaire. As the questionnaires will be anonymous, it is agreed to return them by mail using a postage paid envelopes, to maintain this anonymity.
Moreover, the Coordinating Centre of this study (Research Platform in Pharmacoepidemiology based in University of Bordeaux) is not based in hospital. This centre has in charge of receiving the self-questionnaire, of checking for missing and discordant data and of contacting nurses individually to complete self-questionnaire if necessary.
We decided that when the laboratory will receive urine samples, it will immediately inform the Coordinating Centre of the receipt of this sample. The Coordinating Centre will contact the nurses within 7 days if the self-questionnaire has not been yet received yet, thus limiting the possible loss of data.

This information has been added to the "study protocol, step 4: nurses inclusion" section as follows:
"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."

8. In the public involvement sections, it is concerning that the questionnaire and protocol was developed without input from the exposed nurses.

We previously conducted a pilot study (unpublished data) in a healthcare department of Bordeaux university hospital that enabled us to carry out a study of the complete organization around AD and around excreta of treated patients and to collect tasks performed, type and wearing of PPE. During this pilot study, a draft version of a self-questionnaire was developed and pre-tested on a small group of nurses. When it was necessary, questions where changed according to the feedback of the nurses. A final version was elaborated and will be used in the CACIES study.

These informations are added in the "study protocol, step 3: development of a self-questionnaire" section as follows:

“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 patient and public involvement sections was modify as follows:
“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.”

9. The major objective of the study is to determine the “rate of internal contamination” of nurses handling these drugs. In the introduction it is stated that, “the best approach to measure internal contamination is biomonitoring…”. While this is true, it has been discussed extensively in the literature that due to the complexities described above, of multiple different drugs in use by nurses on different days of work and the need to limit the drugs measured to only a few of those handled, the laboratory methods challenges etc, it is presumed that biomonitoring is not the best way to document occupational exposure. In most (but not all countries) environmental surface sampling has been accepted as documentation of worker exposure, in light of the evidence that the major exposure route is dermal. This is important because we do not want to ‘exonerate’ a contaminated work environment just because on the one day we looked in urine we did not find any of a very limited number of drugs which were assessed. The Authors may think that contaminated urine is likely to occur. While this has been the case in some settings, in locations with reasonable safety measures (biological safety cabinets, work practices and safety PPE, it’s uncommon to see drug in urine. Thus, the protocol must be refined to raise the likelihood of finding a positive result by considering the issues raised above.

Biological Monitoring of Occupational Exposure (BMOE) is the only tool for identifying internal contamination of exposed workers. Moreover, for each individual, this allows to take into account (1) all the exposure routes (respiratory, dermal, oral), (2) all the sources of exposure, (3) the effectiveness and/or wearing of protective personal equipment, (4) gesture and professional practices and (5) personal hygiene. It also allows identification of groups at risk and documents events of accidental exposure to AD.
In addition, French recommendations for good practice in the field of Biological Monitoring of Occupational Exposure (BMOE) were elaborated in 2016 by three French scientific societies and three French governmental authorities (INRS, ANSES, SPF) and are referenced as:
Nisse C, Barbeau D, Brunet D, El Yamani M, Fontaine B, Goujon Y, et al. Recommandations de bonne pratique pour la surveillance biologique de l’exposition professionnelle aux agents chimiques (SBEP): recommandations de la Société française de médecine du travail, associée à la Société française de toxicologie analytique et à la Société de toxicologie clinique. ToxAc. 2017;29:351-376.

These recommendations for BMOE have been adapted to occupational exposure to antineoplastic drugs and are referenced as:
Lepage N, Canal-Raffin M, Villa A. Proposals for the practical implementation of the Biological Monitoring of Occupational Exposure for antineoplastic drugs. ToxAc. 2018;29:387-417.

We have not included in the main document these two references because they are in french.

A French study conducted in more than 12 hospitals in 2010 and 2016 reported an internal contamination rate among nurses over 50% (french reference, not included in the main document: Ndaw S, Robert A, Ricolfi C, Denis F, Marsan P. Healthcare workers and cytotoxic drugs. the place of biometrology in risk management over time. Bulletin épidémiologique hébdomadaire 2018; 12-13: 252-257.

In addition, our laboratory assayed in routine, several urine samples for Biological Monitoring of Occupational Exposure of seven antineoplastic drugs prescribed by occupational physicians. According to our database, the current internal contamination rate among nurses is around 30% with current analytical methods. For the CACIES study, using a new highly sensitive equipment (5500 QTrap, sciex®), we aim to increase the sensitivity of current methods by a factor of 10, which could allow a better detection of drug in the urine (Dhersin et al, 2018 reference numbered 35).
The reviewer 1 indicates that “it’s uncommon to see drug in urine”. In the light of the elements mentioned above, a significant number of contaminated nurses is expected for the CACIES study.

10. Also, performing surface sampling for the same five drugs at the time of biomonitoring could give you a more complete picture of the extent of contamination in the work environment, even if your biomonitoring results yield few positive results.

We agree with the two reviewers on the importance of surface sampling in addition to the CACIES study. 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.

We have added this information in the “introduction” section as follows:
“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”.

We have recently developed and validated a multi-residue analytical method for the routine AD monitoring of working surfaces: 15 antineoplastic drugs can be analyzed simultaneously (cyclophosphamide, ifosfamide, doxorubicin, daunorubicin, epirubicin, 5-FU, dacarbazin, etoposide, pemetrexed, vincristine, cytarabine, methothrexate, paclitaxel, gemcitabine, mitomycin C). This analytical method (referenced in the manuscript under number 25) has been optimized and adapted to achieve high sensitivity with very low limits of quantification (25 to 5 000ng/L or 2.5 to 500pg/wipe or 0.025 to 5pg/cm2), equivalent or lower that previously published data, for 13 out of 15 antineoplastic drugs.

Reference numbered 25. Atgé B, Da Silva Cacao O, Ducint D, et al. Tool development for assessing antineoplastic drugs surface contamination in healthcare services and other workplaces. 39th International Congress of the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) 21-24 May 2019, Naples, Italy [abstract]. Clinical Toxicology 2019;57(6):423-602.

Considering the limited budget allocated to the CACIES study (funded by a grant from the French Ministry of Health), we are going to search a complementary grant to support a monitoring of AD environmental contamination in each healthcare department included in the CACIES study. This will allow the identification of environmental exposure sources, the implementation of corrective measures and the verification of the effectiveness of surface decontamination.
In addition, as Kibby et al., (2017, reference under number 40) concluded in a review: “there is no statistically significant correlation between wipe sampling and urine monitoring”. Given these

elements, there is no disadvantage in conducting both studies (biomonitoring and surface monitoring) separately.

In the “strengths and limitations of the study” section, we have modified the last sentence as follows:
• “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.”

We have added in the “introduction section”, the following sentences:
“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.”

The reviewer 2 cited Dal Bello et al, 2016 as published antineoplastic drugs multi-residue method for workplace environmental monitoring. In the « introduction section », we have added five recent references (under numbers 24-28) of AD multi-residue analytical methods for workplace environmental monitoring as follows:
“Several analytical methods have been published for surface metrology of AD 24-28 and for AD urine biomonitoring.29-32”

Reviewer 2: Roberta Bonfiglioli

The protocol describes a study designed to primarily evaluate the rate of internal contamination by antineoplastic drugs (AD) among hospital nurses. Another aim of the study is to identify factors associated with AD contamination. It is an ambitious study given the number of subjects to be included and the strong effort for biological monitoring with a conspicuous number of drugs analysed in each urine sample.

However there is a matter within the second aim of the study which deserve a comment. As stated by Authors AD occupational exposure is possible through several possible routes: dermal, respiratory and oral. Biological monitoring reveals the burden of contamination but cannot disclose the source of exposure and the route (if dermal or respiratory). Especially when centralized reconstitution units are available, ward nurses are probably more prone to have cutaneous than respiratory exposure. In order to identify source of contamination environmental monitoring is recommended. Poor correlation exists between biological and environmental monitoring in workers exposed to AD, this could be explained by their complementary role (Kibby T. A review of surface wipe sampling compared to biologic monitoring for occupational exposure to antineoplastic drugs. J Occup Environ Hyg. 2017 Mar;14(3):159-174).

I suggest Authors to revise the study protocol by including environmental monitoring in order to pinpoint the true sources of exposure and to obtain precious information for prevention (Dal Bello F, Santoro V, Scarpino V, Martano C, Aigotti R, Chiappa A, Davoli E, Medana C. Antineoplastic drugs determination by HPLC-HRMS(n) to monitor occupational exposure. Drug Test Anal. 2016 Jul;8(7):730-7). If this is not possible it should be discussed as a relevant limitation.

We agree with the two reviewers on the importance of surface sampling in addition to the CACIES study. 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.

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