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Evaluation of Decontamination Efficacy of Cleaning Solutions on Stainless Steel and Glass Surfaces Contaminated by 10 Antineoplastic Agents

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Objectives: The handling of antineoplastic agents results in chronic surface contamination that must be minimized and eliminated. This study was designed to assess the potential of several chemical solutions to decontaminate two types of work surfaces that were intentionally contaminated with antineoplastic drugs.

Methods: A range of solutions with variable physicochemical properties such as their hydrophilic/hydrophobic balance, oxidizing power, desorption, and solubilization were tested: ultrapure water, isopropyl alcohol, acetone, sodium hypochlorite, and surfactants such as dishwashing liquid (DWL), sodium dodecyl sulfate (SDS), Tween 40, and Span 80. These solutions were tested on 10 antineoplastic drugs: cytarabine, gemcitabine, methotrexate, etoposide phosphate, irinotecan, cyclophosphamide, ifosfamide, doxorubicin, epirubicin, and vincristine. To simulate contaminated surfaces, these molecules (200 ng) were deliberately spread onto two types of work surfaces: stainless steel and glass. Recovered by wiping with a specific aqueous solvent (acetonitrile/HCOOH; 20/0.1%) and an absorbent wipe (Whatman 903®), the residual contamination was quantified using high-performance liquid chromatography (HPLC) coupled to mass spectrometry. To compare all tested cleaning solutions, a performance value of effectiveness was determined from contamination residues of the 10 drugs.

Results: Sodium hypochlorite showed the highest overall effectiveness with 98% contamination removed. Ultrapure water, isopropyl alcohol/water, and acetone were less effective with effectiveness values of 76.8, 80.7, and 40.4%, respectively. Ultrapure water was effective on most hydrophilic molecules (97.1% for cytarabine), while on the other hand, isopropyl alcohol/water (70/30, vol/vol) was effective on the least hydrophilic ones (85.2% for doxorubicin and 87.8% for epirubicin). Acetone had little effect, whatever the type of molecule. Among products containing surfactants, DWL was found effective (91.5%), but its formulation was unknown. Formulations with single surfactant non-ionics (tween 40 and span 80) or anionic (SDS) were also tested. Finally, solutions containing 10^{-2} M anionic surfactants and 20% isopropyl alcohol had the highest global effectiveness at around 90%. More precisely, their efficacy was the highest (94.8%) for the most hydrophilic compounds such as cytarabine and around 80.0% for anthracyclines. Finally, the addition of isopropyl alcohol to surfactant solutions enhanced

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their decontamination efficiency on the least hydrophilic molecules. Measured values from the stainless steel surface were similar to those from the glass one.

Conclusion: This study demonstrates that all decontamination agents reduce antineoplastic contamination on work surfaces, but none removes it totally. Although very effective, sodium hypochlorite cannot be used routinely on stainless steel surfaces. Solutions containing anionic surfactant such as SDS, with a high efficiency/safety ratio, proved most promising in terms of surface decontamination.

Keywords: decontamination/methods; detergents; equipment contamination/prevention and control; hazardous substances/analysis; occupational exposure/analysis

INTRODUCTION

Nowadays, antineoplastic drugs are widely used in cancer therapies. Given their high toxicity, these substances represent a potential risk for professionals at each step of the healthcare process. The National Institute for Occupational Safety and Health has estimated that around 5.5 million healthcare workers are potentially exposed to hazardous drugs in the USA. Despite publications of guidelines describing handling protocols and the use of biology safety cabinets (BSCs) or barrier isolators, surface contamination still exists in hospital pharmacy units (Acampora *et al*., 2005; Crauste-Manciet *et al*., 2005; ISOPP, 2007). Environmental monitoring has indicated that all surfaces could be potentially contaminated (Turci *et al*., 2003; Bussières *et al*., 2006; Heinemann *et al*., 2008; Käslin *et al*., 2010). Biological monitoring has proved that genotoxic effects can be detected by the Ames test or SOS chromo tests (urine mutagenicity, chromosomal aberrations, sister chromatid exchanges, and micronuclei) in the urine of nurses and pharmacy technicians (Poyen *et al*., 1988; Sessink *et al*., 1994; Cavallo *et al*., 2005; Quillardet and Hofnung, 2009). Physical effects such as skin rashes, adverse reproductive effects (abortions, stillbirths, and congenital malformations), leukaemia, or cancers can occur (Skov *et al*., 1990; Connor and McDiarmid, 2006). Traces of contamination have been described in patients' rooms and hospital effluents, among operating theatre personnel, pharmacy technicians, and pharmacists (Mahnik *et al*., 2004, 2006; Sottani *et al*., 2010, 2011). Several papers have reported antineoplastic drug contamination on vials, surfaces, floors, countertops, carts, storage bins, waste containers, tabletops, chairs, and linen and in the atmosphere of pharmacy units (Mason, 2003; Connor and McDiarmid, 2006; Touzin *et al*., 2008). The main exposure routes have been by dermal contact with contaminated surfaces and by inhalation of particles (Kromhout *et al*., 2000; Fransman *et al*., 2005; Connor and McDiarmid, 2006). To confront this challenge, the pharmacist strategy is first to confine contamination in specific pharmacy areas within closed working areas (biosafety cabinets and isolators) and secondly to reduce the risk of contamination on pharmacists and on pharmacy technicians by using specific devices such as containment safety devices rather than needles for example. However, there is still a risk of accumulation over time. Efficient decontamination of surfaces is therefore of the utmost importance.

Several studies are available on the impact of decontamination procedures to reduce chemical contamination by cytotoxic agents. Raghavan *et al.* studied a water rinsing method on cisplatin decontamination using liquid chromatography (Raghavan *et al*., 2000). Chlorine-based agents reduced the mutagenicity of methotrexate (MTX) by inactivating it (Wren *et al*., 1993). Earlier studies described various other solutions for cytotoxic agents on different surfaces. Multiple compounds [carmustine (BCNU), lomustine (CCNU), chlorozotocin, N-[2-chloroethyl]-N'-[2,6-dioxo-3-piperidinyl]-N-nitrosourea (PCNU), 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosoure (Methyl-CCNU), mechlorethamine, melphalan, chlorambucil, cyclophosphamide, ifosfamide, uracil mustard, and spiromustine] were degraded using nickel aluminium in a potassium hydroxyde solution without any toxic degradation (Lunn *et al*., 1989). Barek *et al.* proposed two methods for surface decontamination: the first reported almost total degradation of melphalan based on its oxidation by potassium permanganate in a sodium hydroxide solution, and the second degraded multiple compounds (amsacrine, azathioprine, asparaginase, and thiotepa) using sodium hypochlorite and a Fenton reagent [an oxidizing solution based on hydrogen peroxide oxidized by catalyst ferrous iron (II)] (Barek *et al*., 1987, 1998). Oxidizing agents had already been tested on antineoplastic agents (Hansel *et al*., 1996; Castegnaro *et al*., 1997; Roberts *et al*., 2006) and assessments established on different antineoplastic classes: oxazophosphorine or anthracycline molecules, using

hydrogen peroxide and sodium hypochlorite, which proved to be effective. Vaporized hydrogen peroxide and detergents were also investigated with positive results on 5-Fluorouracil, doxorubicin (DOX), and cyclophosphamide. Despite all previous studies, to date, no clear, effective, and evidence-based cleaning recommendations for daily practice exist. The aim of this paper is to evaluate the surface decontamination efficacy of different cleaning solutions through a step-by-step controlled study, to provide advice for cleaning steps in pharmacy units.

The first part of this experimental work was performed on stainless steel, where aqueous solutions, aqueous alcohol solutions, or organic solutions were first screened. An improvement was then made on selected solutions and finally the optimal volume required for decontamination was determined. The second part was performed on glass to test the effectiveness of selected solutions. The final objective is to provide an effective and clear review of cleaning solutions for the periodic decontamination of work areas.

EXPERIMENTAL

Chemicals and reagents

Antineoplastic agents. The study was performed with the following commercially available cytotoxic drugs (Table 1). Reconstitution of etoposide phosphate (Etopophos®), gemcitabine (Gemcitabine Teva®), and ifosfamide (Holoxan®) was obtained with water for injection (Bichsel Laboratories, Interlaken, Switzerland). 5% sterile glucose (Sintetica, Bioren SA, Couvet, Switzerland) was used for the reconstitution of Endoxan®.

Liquid chromatography–mass spectrometry/mass spectrometry. Lichrosolv® HPLC grade acetonitrile (ACN) and ultrapure water were purchased from Merck (Darmstadt, Germany), and formic acid (FA) came from Biosolve (Valkenswaard, the Netherlands).

Wiping and desorption material. Filter paper (Protein Saver TM 903 Card) was from Whatman (Dassel, Germany), and 1.5 ml polyethylene (PE) safe-lock tubes were from Eppendorf AG (Hamburg, Germany). Texwipe 3210 cleaning wipers, used as received as desorption material, were from ITW Texwipe (Kernersville, USA).

Cleaning solutions. Products used in cleaning solution formulations are summarized in Table 2. Simple solutions were tested as decontamination procedures. The choice of these solutions was based on current pharmaceutical practice and on scientific publications. Two kinds of solutions were tested: "elimination-type" solutions whose main action is to dissolve chemical products on the surface and "degradation-type" solutions that react with the chemical structure of compounds, leading to their degradation and the formation of expected non-cytotoxic compounds. Among "elimination-type" solutions, ultrapure

Table 1. Commercially available cytotoxic drugs used in the study.

Molecules (acronym)	Brand name	Hydrophilic (H)/ Hydrophobic (h)	Concentration	Manufacturer	Town, country
Molecules in test					
Irinotecan (IRI)	Campto®	h	20 mg ml^{-1}	Pfizer AG	Zürich, Switzerland
Cytarabine (CYT)	Cytosar®	H	20 mg ml^{-1}		
Gemcitabine (GEM)	Gemcitabin Teva®	H	20 mg ml^{-1}	Teva Pharma AG	Aesch, Switzerland
Vincristine (VI)	Vincristine Teva®	h	1 mg ml^{-1}		
Ifosfamide (IF)	Holoxan®	H	40 mg ml^{-1}	Baxter AG	Volketswil, Switzerland
Cyclophosphamide (CP)	Endoxan®	H	20 mg ml^{-1}		
Methotrexate (MTX)	Methotrexate Farmos®	H	2.5 mg ml^{-1}	Orion Pharma	Zug, Switzerland
Etoposide phosphate (ETO)	Etopophos®	h	20 mg ml^{-1}	Bristol-Myers Squibb SA	Baar, Switzerland
Doxorubicin (DOX)	Doxorubine Ebewe®	h	2 mg ml^{-1}	Ebewe Pharma	Cham, Switzerland
Epirubicin (EPI)	Epirubicin Actavis Solution®	h	2 mg ml^{-1}	Actavis	Regensdorf, Switzerland
Internal standard					
IS	[^{13}C, $^{2}H3$]-MTX	H	—	Alsachim	Illkirch, France

Table 2. Products used in formulations of cleaning solutions tested.

Products (acronym)	International name	Manufacturer	Commentaries	Abbreviation	Experimental phase	Concentrations tested
Acetone	Propane-2-one	Merck (Darmstadt, Germany)	Analysis quality	—	Screening	—
Kleralcohol (IPA)	IPA/water	Ecolab (Farmham, UK)	Guidelines reference	IPA	Screening	70/30% (vol/vol)
DWL	—	Migros (Zurich, Switzerland)	Contains anionic and non-ionic surfactants (<30%)	DWL	Screening, Optimization	5%, 10 and 20% in ultrapure water (vol/vol)
Sodium hypochlorite 5%	Sodium hypochlorite	Tempia (Carouge-Geneve, Switzerland)	oxidative agent	NaClO	Screening, Optimization	Diluted at 0.5% (vol/vol) in ultrapure water
SDS	Sodium lauryl-sulfate	Merck (Hohenbrunn, Germany)	CMC: 0.82×10^{-3}M (Mukerjee and Mysels, 1971)	SDS	Optimization	10^{-4}M, 0.5×10^{-3}M, 10^{-3}M, 10^{-2}M and 10^{-1}M in ultrapure water
Tween 40	Polysorbate 40	Hänseler AG (Herisau, Switzerland)	non-ionic surfactant	—	Optimization	10% in ultrapure water (vol/vol)
Span 80	Oleatesorbitan 80 or sorbitan-(Z)-mono-9-octadecanoate		non-ionic surfactant	—	Optimization	10% in ultrapure water (vol/vol)

water was tested single as a cleaning solution reference and as solvent when mixed with surfactants such as dishwashing liquid (DWL), span 80, tween 40, and sodium dodecyl sulphate (SDS). Isopropyl alcohol (IPA) 70/30 was also studied because of recommendations from guidelines for microbiological decontamination in chemotherapy production units (Le Garlantezec *et al*., 2011). Hydrophobic solvents such as acetone were used to determine its expected efficacy on the more hydrophobic compounds. Finally, among "degradation-type" solutions, a sodium hypochlorite solution, the most currently used solution to wash surfaces today, was also tested.

Preparations of compound stock solutions, calibration standards, and internal standard

All solutions (i.e. drug reconstitutions and sample dilutions) were prepared in appropriate conditions (BSC, individual protection) for handling hazardous compounds such as cytotoxic agents. The preparation of solutions and standards was performed with brand drugs to avoid any direct contact of the operator with cytotoxic powder and to minimize contamination risk during the preparation of solutions. Aliquots of the internal standard (IS; 250 $\mu g.ml^{-1}$) were prepared with a mixture of ACN and water (75/25, vol/vol) and stored at −22°C for 12 months with no sample degradation observed. Stock solutions of IS were diluted daily to 50 ng ml^{-1} in 20% ACN (vol/vol) with 0.1% FA (vol/vol) and were kept stable for at least 2 weeks at 2–8°C. A main stock solution containing the 10 cytotoxic drugs was prepared by diluting at 20 $\mu g.ml^{-1}$ concentration each cytotoxic compound in water. This solution was further diluted to obtain five independent stock solutions at 20, 40, 200, 1 000, and 4 000 ng ml^{-1} in 20% ACN (vol/vol) and 0.1% FA (vol/vol). For calibration standards (CS), stock solutions were diluted with the IS solution at 50 ng ml^{-1} to obtain five CS at 1, 2, 10, 50, and 200 ng ml^{-1}.

Equipment and liquid chromatography–mass spectrometry/mass spectrometry conditions

Analyses were carried out with the Accela liquid chromatography system from Thermo Fisher Scientific Inc. (Waltham, MA, USA) consisting of a quaternary pump equipped with an online degasser, an auto sampler and a solvent platform. The chromatographic system was coupled to a Quantum Discovery MS from Thermo Fisher Scientific Inc. equipped with Ion Max electrospray ionization (ESI) interface and a triple quadrupole. The liquid chromatography–mass spectrometry/mass spectrometry system was monitored with Xcalibur software (Thermo Fisher Scientific). Separations were obtained on a ZORBAX SB-C18 RR column with an inner diameter of 2.1 mm, a length of 10 cm, and a particular diameter of 3.5 µm from Agilent Technologies (Waldbronn, Germany). The liquid chromatography–mass spectrometry/mass spectrometry conditions and method validation have been described in detail elsewhere (Nussbaumer *et al*., 2010).

Decontamination

All tests were performed under a laminar airflow hood. The surface to be investigated (10 × 10 cm) was contaminated with 50 µl of stock solution sprayed on surface (solution containing all 10 cytotoxic agents at 4000 ng ml^{-1}) using an adjustable volume micropipette. This voluntary contamination was repeated 10 times for each cleaning solution. For the drying step, contaminated surfaces were protected from light in a laminar airflow hood for a period of 1 h. After drying, different cleaning solutions were applied. These were prepared extemporaneously and used directly. 300 µl of each cleaning solution was poured onto a 100 cm^2 Texwipe 3210 wipe. A single standard motion from top to bottom was adopted to clean each surface.

Wiping and analytical procedure

The wiping step can recover the residual contamination present on the surface after the decontamination step. A validated wiping procedure was performed to reclaim remaining cytotoxic compounds (Nussbaumer *et al*., 2012). To do so, a 1-cm^2 blotting paper (Whatman 903®) was soaked with 100 µl of an aqueous desorbing solution [ACN: water, 20/80 (vol/vol) with 0.1% FA]. The contaminated surface was then wiped for 30 s, turning the blotting paper regularly. Blotting papers were placed in PE safe-lock tubes, and 1 ml IS solution at 50 ng ml^{-1} was added. Then samples were ultrasonicated for 20 min and centrifuged at 4000 rd min^{-1} for 5 min. All samples were immediately placed in the LC auto sampler at 15°C and analysed within the day.

Decontamination evaluation

Data extracted from the analytical procedure correspond to residual contamination ($RC_{i,m}$) of each antineoplastic agent. For each molecule, an efficiency index was generated ($Eff_{i,m}$; Equation 1). Then, to be able to compare cleaning solutions with each other, an overall effectiveness index was calculated (Eff_i). It was the average of the 10 efficiency indexes (Equation 2). So, this Eff_i corresponded to the overall effectiveness of a solution on the 10 antineoplastic agents, during a single attempt. To validate the overall effectiveness of a solution, each cleaning procedure was tested 10 times. As a conclusion, in this paper, the median value of those 10 attempts (EP_{value} or Efficiency performance value) was used to compare cleaning solutions (Equation 3). Results were presented as follow: median value [minimum value − maximum value].

$$Eff_{i,m} = 100 - RC_{i,m}(\%) \quad (1)$$

$$Eff_i = \frac{\sum Eff_{i,m}}{n_i} \quad (2)$$

$$EP_{value} = \text{median value } (Eff_i)n \quad (n = 10) \quad (3)$$

Standard deviation (SD) per compound for each cleaning solution was also calculated on 10 attempts. It indicated the reproducibility of the cleaning solution on each compound. Due to the numerous manual steps throughout the procedure, its acceptance threshold was arbitrarily set at 10%.

Sequence of experiments

In the first part of the study, tests were performed on stainless steel. The first "Screening" step involved screening solutions with various physicochemical characteristics. Working on a solubility procedure, tests were carried out with ultrapure water, aqueous-alcoholic solutions and organic solvents such as ACN. An oxidative solution was assessed using an aqueous solution of 0.5% sodium hypochlorite. At last, complex micellar formulations such as DWL diluted in ultrapure water were tested to focus on surfactant molecules. In the "Optimization" step, other detergent solutions were also tested using single anionic and neutral surfactants. SDS was especially focused on to consider the impact of its concentration on decontamination efficacy. Different formulations of aqueous-alcoholic solutions with stable SDS concentration were also tested to reduce surfactant deposit. Up to this point, tests were performed with normalized surfaces and volumes, non-representative of current decontamination activity. So, in the "Practical" step, additional tests were carried out over a 0.2 m^2 surface area with different volumes of optimized solution to simulate current cleaning methods. Finally, the second part involved tests on glass to validate the effectiveness of our solution on the most commonly used materials in closed working areas. All data are summarized in Table 2.

Statistical analysis

Statistical analysis was performed by analysis of variance on ranks following the method of Conover and Iman (Conover and Iman, 1981). This method was used to compare the effectiveness performance value of cleaning solutions. When this analysis revealed a significant P value ($P < 0.05$), contrasts were established with the Tukey–Kramer test to detect significant differences between couples of cleaning solutions with a statistical threshold of 5%. Analysis was performed with XLSTAT® software (Addinsoft).

RESULTS

Screening phase

Considering physicochemical properties of the 10 antineoplastic agents, two groups of molecules can be distinguished: first one corresponding to the most hydrophilic substances with cytarabine (CYT), gemcitabine (GEM), MTX, etoposide phosphate (ETO), cyclophosphamide (CP), and ifosfamide (IF) and second one to more hydrophobic compounds with irinotecan (IRI), DOX, vincristine (VI), and epirubicin (EPI). All data and statistical analyses performed on the stainless steel surface are summarized in Fig. 1 and Table 3.

Ultrapure water, aqueous alcohol, and organic solvents. Ultrapure water effectiveness was considered to be insufficient (Fig. 1). It was effective to remove CYT, GEM, IF, CP, and VI, but for MTX, ETO, IRI, DOX, and EPI, $Eff_{i,m}$ values were between 39 and 73%. Reproducibility was low on hydrophobic molecules (e.g. DOX 15.5%), except for VI. For IPA/water 70/30 (vol/vol), EP_{value} was slightly higher than that of ultrapure water ($P = 0.041$). $Eff_{i,m}$ values for hydrophilic molecules (CYT, GEM, MTX, ETO, IF, and CP) were lower than for ultrapure water and inferior to 90.0%. On the other hand, efficacy on the most hydrophobic molecules (IRI, DOX, and EPI) was superior to that obtained with ultrapure water (Fig. 1). Acetone EP_{value} was significantly lower than ultrapure water and IPA (both with $P < 0.0001$).

Sodium hypochlorite. The 0.5% sodium hypochlorite had the highest EP_{values} (97.5%) and was significantly superior to all other solutions (all $P < 0.0001$). All removal values were superior to 90.0% Table 3. For CYT, GEM, MTX, IRI, and VI, $Eff_{i,m}$ were even superior to 99.0%. For these compounds, SDs were inferior to 5%. The lowest $Eff_{i,m}$ were found for ETO, IF, and CP.

Surfactants. As shown in Fig. 1, 10% DWL obtained a 91.5% EP_{value}. Results are reported in Fig. 2.

Optimization phase: focus on surfactants molecules

Complex surfactants assessments (DWL). During screening phase, 10% DWL reached a promising 91.50% EP_{value}. Two more concentrations (5 and 20%) were also tested in order to observe the potential of DWL concentration on the antineoplastic removal. No significant difference was observed between the three DWL concentrations tested. 20% DWL obtained an EP_{value} (89.7%) significantly

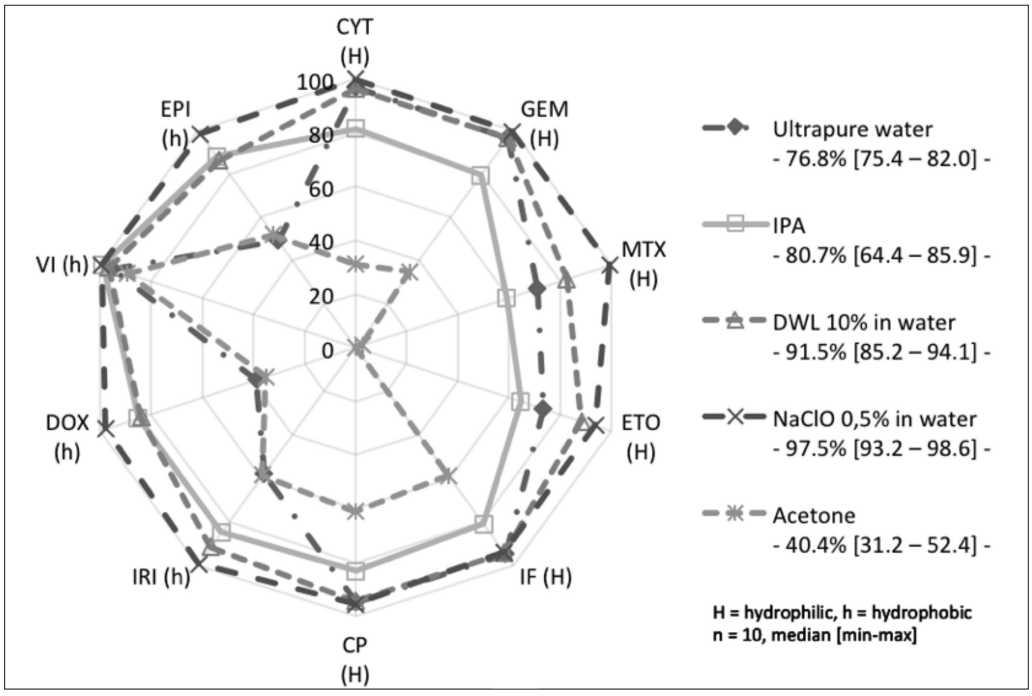

Fig. 1. Efficacy per compound and effectiveness performance of each cleaning solution on ten antineoplastic agents during tests on stainless steel surface

Table 3. Efficacy per compound of surfactant solutions on 10 antineoplastic agents and on stainless steel surface.

Modalities	CYT	GEM	MTX	ETO	IF	CP	IRI	DOX	VI	EPI	EP_{value}
Ultrapure water	97,10	96,60	71,27	73,35	94,94	94,49	58,10	38,76	95,40	49,13	76.8
	2,21	2,28	7,89	9,49	2,30	2,74	7,85	15,50	2,27	19,65	—
Acetone	31,04	34,69	2,90	−16,10	58,98	61,02	58,62	34,82	89,10	51,93	40.4
	16,58	15,38	18,07	22,79	12,50	11,18	10,45	21,42	3,64	14,26	—
IPA	81,43	79,40	59,24	64,54	81,08	83,16	85,07	85,21	99,08	87,76	80.7
	19,40	21,81	24,30	27,86	13,81	14,71	12,45	15,19	3,23	11,15	—
NaClO 0.5%	99,78	99,34	99,61	93,88	94,35	95,35	99,38	97,63	99,36	98,00	97.5
	1,15	2,01	0,78	6,04	13,09	8,45	4,06	2,58	0,05	2,35	—
5% DWL	96,88	96,95	88,00	81,77	94,99	94,91	86,71	80,17	94,52	79,59	86.1
	3,18	2,72	3,99	5,84	4,85	4,80	4,81	12,44	2,49	10,40	—
10% DWL	96,50	96,48	82,70	88,45	94,36	94,20	91,66	83,86	96,68	85,97	91.5
	3,93	4,23	7,33	7,92	4,38	4,09	10,51	15,21	1,96	14,36	—
20% DWL	95,54	95,46	87,13	77,86	92,42	92,33	86,27	76,94	93,28	73,84	89.7
	10,54	8,92	10,33	17,32	10,91	10,37	15,83	19,99	7,22	18,12	—
Span 80	82,36	80,33	66,42	65,82	79,13	80,40	45,47	54,82	79,28	55,06	76.8
	16,25	18,67	26,38	42,38	16,19	15,79	28,35	32,19	8,47	29,79	—
Tween 40	92,59	91,48	74,98	66,58	88,67	89,13	84,05	64,68	99,01	63,43	82.7
	8,88	9,27	10,96	17,27	9,25	9,38	5,52	19,20	0,57	7,73	—
10^{-1}M-SDS	96,91	97,33	92,10	89,78	95,02	94,98	100,00	93,28	99,72	95,90	95.4
	5,32	4,33	6,56	9,27	5,02	4,80	0,00	5,96	0,05	4,14	—
10^{-2}M-SDS	96,57	95,91	84,36	87,38	89,45	89,36	86,92	74,73	96,13	78,03	87.8
	4,44	4,13	6,12	6,41	5,06	5,17	6,57	16,93	1,73	7,94	—
0.5 × 10^{-2}M-SDS	94,90	94,63	81,23	81,55	92,41	91,91	82,79	79,20	92,28	81,60	87.5
	4,83	4,78	8,78	12,64	5,18	5,43	8,86	10,68	4,84	9,49	—
10^{-3}M-SDS	94,85	94,13	77,54	73,85	92,16	91,68	75,20	67,92	91,54	70,67	82.6
	6,69	7,10	14,84	19,22	7,19	7,55	14,55	13,06	5,93	15,73	—
10^{-2}M-SDS + 5% IPA	95,83	95,94	90,13	89,66	94,05	93,65	87,77	79,29	96,49	79,42	90.3
	7,08	5,70	6,33	6,56	6,23	6,31	5,87	8,20	2,16	10,26	—
10^{-2}M-SDS+ 20% IPA	95,13	95,47	90,03	92,35	94,32	94,20	89,56	77,77	96,67	80,43	89.6
	3,71	3,19	2,76	4,56	4,23	4,55	5,72	10,67	3,41	10,98	—
10^{-2}M-SDS + 30% IPA	93,24	93,38	89,25	89,18	92,63	92,06	91,60	80,92	98,31	81,59	89.9
	6,53	5,67	7,35	7,28	6,42	6,27	5,95	10,43	1,41	11,36	—

Notes: $n = 10$.
Results expressed in median values and SD in percentage.

higher than ultrapure water (all $P < 0.001$), but not 5% DWL (86.1%, $P = 0.001$). $Eff_{i,m}$ values for CYT, GEM, IF, CP, and VI were superior to 90.0% and SD values close to 10%, whatever the dilutions tested. On the other hand, for IRI, DOX, and EPI, the highest $Eff_{i,m}$ values were obtained using 10% DWL (Table 3).

Single surfactant assessments. Attempts realized on non-ionic surfactants (Tween 40 and Span 80): Fig. 3 reports results obtained with surfactant solutions. Span 80 effectiveness was not significantly different from ultrapure water. It was significantly inferior to 10% DWL ($P = 0.0001$) and to 10^{-2}M SDS ($P > 0.0002$). All of its $Eff_{i,m}$ values were inferior to the 90.0% threshold, whatever the polarity of the molecules. On the other hand, Tween 40 EP_{value} was significantly superior to Span 80 ($P = 0.018$) but not significantly different from ultrapure water ($P > 0.0610$). Its $Eff_{i,m}$ values were superior to 90.0% for CYT, GEM, and VI. Its lowest $Eff_{i,m}$ values were obtained for DOX and EPI. 10^{-2}M-SDS was significantly superior to Span 80 ($P = 0.0002$) but not to Tween 40 ($P = 0.0610$) and to 10% DWL ($P = 0.9276$; Fig. 3). Statistically as effective as 10^{-2}M-SDS, Tween 40 had nevertheless an SD superior to 10^{-2}M-SDS values for CYT, GEM, MTX, ETO, IF, CP, and DOX. As a result, subsequent evaluations were made with SDS on a concentration range of 10^{-4}–10^{-1}M.

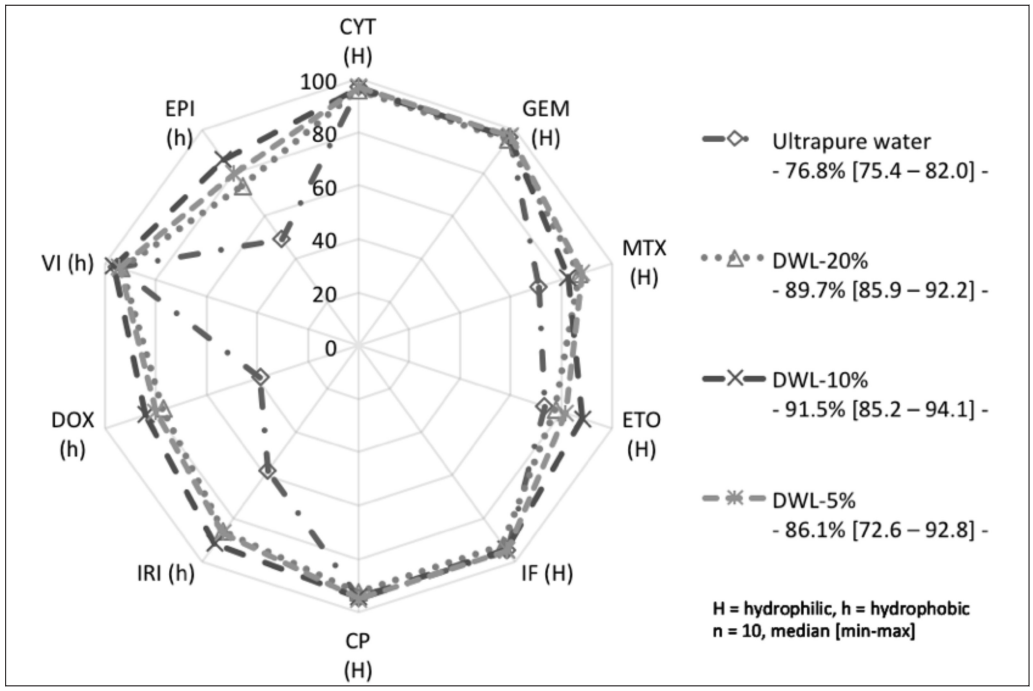

Fig. 2. Efficacy per compound and effectiveness performance of DWL dilutions on ten antineoplastic agents and on stainless steel surface

Attempts realized on anionic surfactant (SDS): Results obtained for decontamination solutions containing SDS at different concentrations are shown in Fig. 4. The effectiveness of SDS increased proportionally to concentration. Indeed, the lowest EP_{value} was obtained with 10^{-3}M-SDS. These results were significantly lower than 10^{-1}M-SDS ($P < 0.0001$) and 10^{-2}M-SDS ($P = 0.026$) but not significantly different from 0.5×10^{-3}M-SDS ($P = 0.062$). For both concentrations around the critical micellar concentration (CMC) value (10^{-2}M and 0.5×10^{-2}M), no significant difference was found [87.8% (83.9 – 92.3) and 87.5% (83.9 – 92.3); $P = 0.997$]. For both concentrations, all $Eff_{i,m}$ values were close to each other. For CYT, GEM, and VI, they were superior to 90.0% but slightly higher with "10^{-2}M-SDS". However, for DOX and EPI, efficacy was slightly lower with 10^{-2}M-SDS. The highest effectiveness was obtained with 10^{-1}M-SDS. Despite results significantly superior to 10^{-2}M-SDS ($P < 0.0001$), this concentration presented a major drawback. Indeed, a thin surfactant film appeared from time to time on the surface after the cleaning step. Microbiological contamination could appear inside, making it necessary to reduce the risk of formation of the residual film.

Attempts realized with improved anionic surfactant (SDS + IPA): To overcome the problem of surfactant deposit and to increase solution evaporation, the formulation was tested with the addition of IPA. Despite containing as much as 20% IPA, a large deposit of surfactant still remained on the stainless steel surface when "10^{-1}M-SDS + 20% IPA" was spread over it. Therefore, an SDS concentration of 10^{-2}M was selected for further experiments. Results are reported in Fig. 5. IPA ranging from 5 to 30% was diluted in an aqueous solution and mixed with 10^{-2}M-SDS. For all aqueous alcohol mixtures, EP_{values} were significantly higher than those obtained for an ultrapure water solution ($P < 0.0001$ except with "10^{-2}M-SDS + 10%-IPA", $P = 0.031$). However, no significant difference was found between 30%-IPA, 20%-IPA, 5%-IPA (and 10^{-2}M-SDS without IPA; Fig. 5). EP_{values} around 90% were obtained in all cases. Nevertheless, the mixture containing 20%-IPA was the most suitable solution, thanks to SD values inferior to our threshold of 10% and lower than those of other mixtures (Table 3). As already mentioned, IPA improved the decontamination efficacy of the most hydrophobic compounds, while SDS acted on hydrophilic molecules. More precisely, $Eff_{i,m}$ obtained with "10^{-2}M-SDS + 20%-IPA" were superior to SDS alone as far as the most hydrophobic molecules were concerned, but they were slightly lower for the two most hydrophilic molecules, CYT and GEM (Table 3).

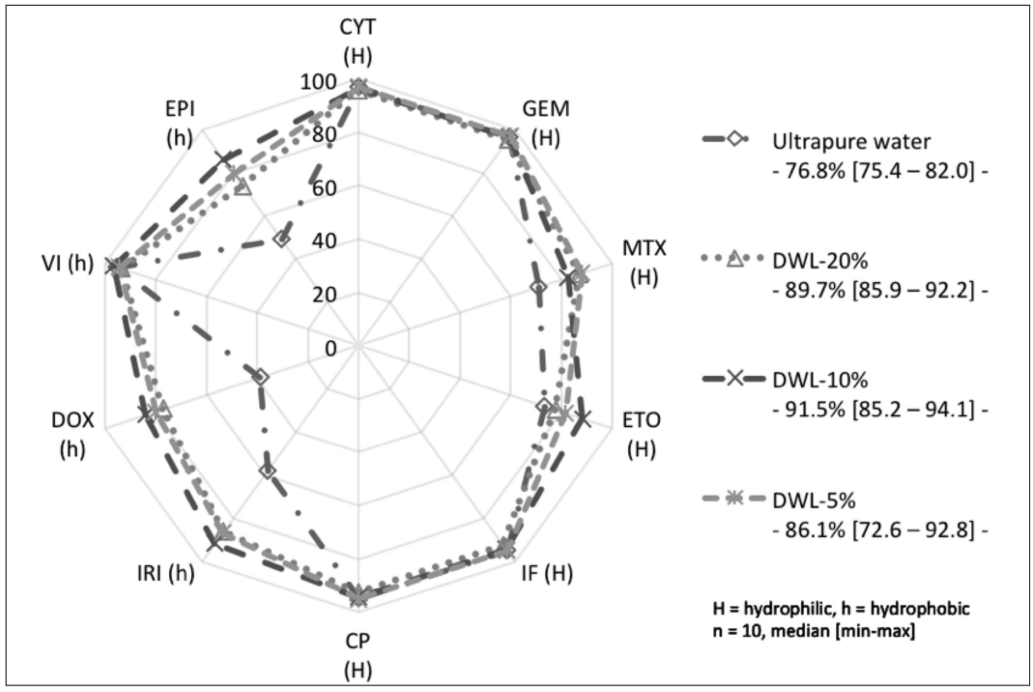

Fig. 3. Efficacy per compound and effectiveness performance of surfactant solutions on ten antineoplastic agents and on stainless steel surface

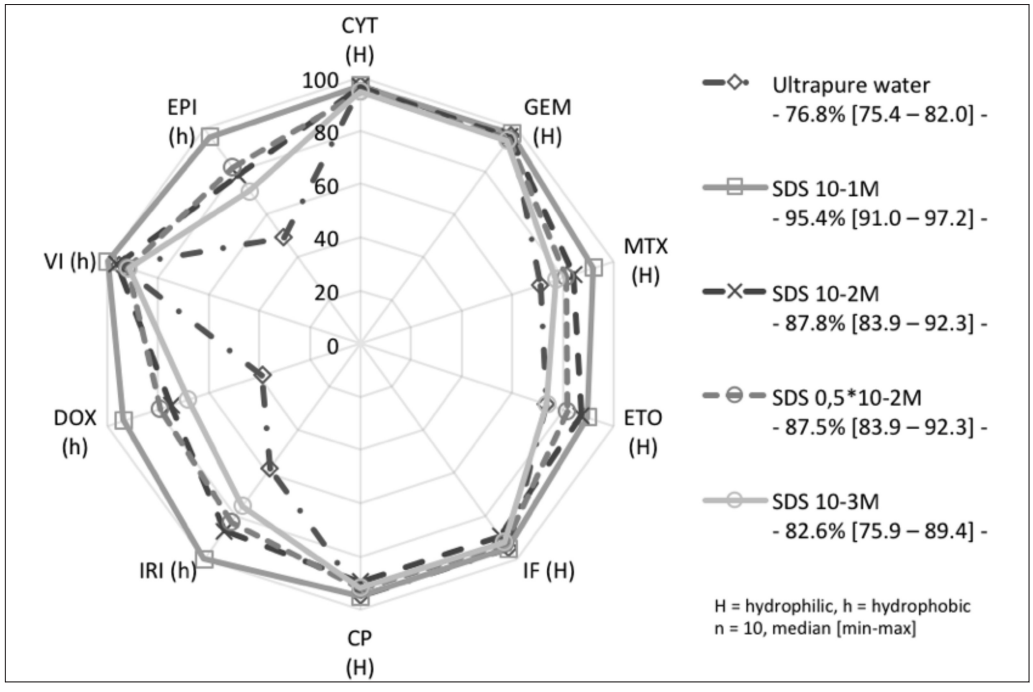

Fig. 4. Efficacy per compound and effectiveness performance of SDS range concentration on ten antineoplastic agents and on stainless steel surface

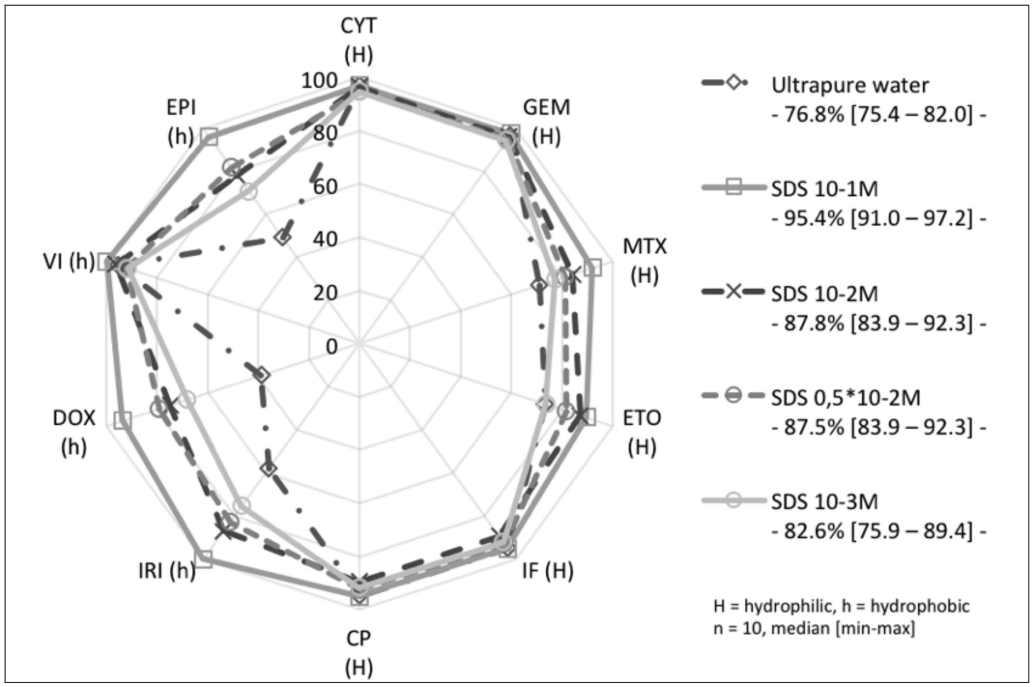

Fig. 5. Efficacy per compound and effectiveness performance of concentration range of optimised solutions, on ten antineoplastic agents and on stainless steel surface

Practical phase: volume sprayed and decontamination effectiveness

In this test, the solution was sprayed directly on the contaminated surface (0.2 m²), and a new whole Texwipe 3210 was used for each trial. Results are presented in Table 4. Whatever the volume of mixture used ("10^{-2}M-SDS + 20 %-IPA"), EP_{value} was superior to 90% and even to 93%. Effectiveness with "6 ml" was significantly higher than with "3 ml" [97.3% (96.5 – 98.1) versus 96.12% (92.77 – 97.27); $P = 0.008$] or with "1 ml" [97.3% (96.5 – 98.1) versus 93.9% (78.4 – 94.4); $P < 0.0001$]. Moreover, SD was inferior to the 10% threshold (Table 4).

Decontamination procedure glass versus stainless steel surface

Results are presented in Fig. 6, and all data are compiled in Table 5. Similar to results found on stainless steel, IPA was the least effective on glass. With $Eff_{i,m}$ values superior to the 90% threshold except for IF and CP, sodium hypochlorite was the most effective and was significantly different from IPA ($P < 0.0001$) on glass. Nevertheless, it was not statistically different from "10^{-2}M-SDS", unlike its use on stainless steel. With an EP_{value} of 88.3%, "10^{-2}M-SDS" had the same effectiveness on glass as on stainless steel. The dispersion of its values was less important than those of sodium hypochlorite, which may explain the non-statistical difference between the two modalities.

DISCUSSION

As the most widely used solvent in cleaning solutions, ultrapure water had to be evaluated. Its performance highlighted the minimal performance required for all other aqueous cleaning solutions. According to our results, its effectiveness is not sufficient and optimization was required, especially for hydrophobic compounds. IPA/water 70/30 (vol/vol) was expected to improve decontamination of the most hydrophobic compounds. In practice, an improvement was found on IRI and anthracyclin compounds, but at the same time, deterioration was measured on the most hydrophilic ones. Acetone was no more suitable at improving the decontamination process. Despite a lower polarity than IPA, hydrophobic molecules were not more effectively removed than with IPA. Furthermore, as far as hydrophilic molecules are concerned, acetone was the least effective solution tested. As a result, evaluating the solubility of single solvents did not seem to be the proper solution for decontamination procedure. Two other

Table 4. Efficacy per compound of "10^{-2}M-SDS + 20% IPA" solutions on 0.2 m² stainless steel surface.

Modalities (ml)	CYT	GEM	MTX	ETO	IF	CP	IRI	DOX	VI	EPI	EP_{value}
1	94,68	94,82	94,57	90,52	93,79	93,58	94,47	91,51	96,64	91,76	93.9
3	97,47	97,77	97,35	93,72	97,35	97,19	96,44	93,83	97,11	93,96	96.1
6	97,46	97,65	97,91	95,96	97,69	97,47	99,00	95,22	98,36	95,23	97.3

Notes: $n = 2$. Results expressed in median values.

hypotheses were considered: oxidative action and modification of solvents' solubility by adjunction of surfactants. For sodium hypochlorite, results are in accordance with those obtained by Hansel *et al*. who reported degradation efficacy for CP and an IF superior to 98.0% (Hansel *et al*. 1996). Nevertheless, despite its high decontamination potential, the use of sodium hypochlorite solutions has major drawbacks. First of all, the possibility of cytotoxic agents to be degraded in mutagenic residues has already been mentioned (Barek *et al*., 1987, 1998). To avoid this phenomenon, a time gap after cleaning (minimum 1 h) should be respected, but this delay is not feasible in everyday pharmacy routine (Castegnaro *et al*., 1997). It is necessary to clean the surface after use with a soaked wipe, otherwise corrosion phenomena appear on metals such as stainless steel. Nowadays, most barrier isolators and BSCs are made of stainless steel and manufacturers do not recommend the use of sodium hypochlorite. Finally, according to United States Pharmacopeia (USP) (797, Table 2), sodium hypochlorite can cause side effects such as skin, eye, and respiratory irritations or systemic toxicity. To overcome these inconveniences, another decontamination method as surfactant should be considered. Already available on the food market, DWL could be convenient. The poorer efficacy of 5% DWL can be explained by an insufficient concentration of surfactants to remove hydrophobic compounds properly because of the lack of micelle structures. With 20% DWL, a residual film was observed on the stainless steel surface, which persisted after the wiping step. This was probably the reason for the higher residual contamination observed (Table 3). This film can be removed with a large volume of water spilled over the surface, but this solution is not suitable within BSCs. None of these limits were found with the intermediate dilution 10% DWL. Even if its results were less effective than those obtained with 0.5% sodium hypochlorite, the main advantage of 10% DWL was undoubtedly its safety not only for humans but also for work surfaces. These results confirmed a previous work that studied cyclophosphamide chemical contamination on a glass vial surface (Touzin *et al*., 2008). Nevertheless, as already mentioned, the exact composition of DWL was unknown and depending on the supplier tested. DWL formulations are usually based on mixtures of anionic and non-ionic surfactants. For a better understanding of DWL action and to simplify formulation of cleaning solutions, subsequent experiments were focused on a single surfactant. Span 80 did not appear to be efficient, so attempts were not pursued further. Tween 40 and SDS were both effective on stainless steel surface. Nevertheless, after a brief literature review, SDS appeared to be the most widely employed surfactant on decontamination products. An additional benefit of SDS is that it is commercially available in certified laboratory quality powdered form. The use of a standardized formulation would allow users to guarantee the quality of the cleaning agent. Despite its high effectiveness, SDS 10^{-1}M was not selected because a residual film was noticed after each decontamination procedure. This residual film was similar to the one observed with 20% DWL. CMC is the main characteristic to take into account when using surfactants. This is found in our results. With a concentration 10 times inferior to CMC (SDS 10^{-3}M), the effectiveness of the cleaning solution decreased. The highest ratio "effectiveness/residual film on surface" was found for concentrations around CMC (10^{-2}M and 0.5×10^{-2}M). To promote the formation of micelles, concentration has to be superior to CMC. So, SDS 10^{-2}M was selected as the cleaning solution for further experiments. To further minimize the risk of residual film in everyday use, adjunction of IPA in SDS formulation was tested. Deposit of surfactants was less serious on a stainless steel surface, and its removal by evaporation was found to be especially fast with the 20%-IPA concentration. Moreover, no decrease of effectiveness (compared with SDS 10^{-2}M) was noticed using the "10^{-2}M-SDS + 20%-IPA" mixture. Finally, the "10^{-2}M-SDS + 20%-IPA" mixture presented the best balance between decontamination profile and reduced deposit and so was selected for further trials.

In our research so far, effectiveness has been tested on 100 cm² surfaces with 300 µl of decontamination solution, which is not representative of current decontamination in an isolator or a laminar airflow hood. Simulations of practical decontamination on larger surfaces with different volumes were

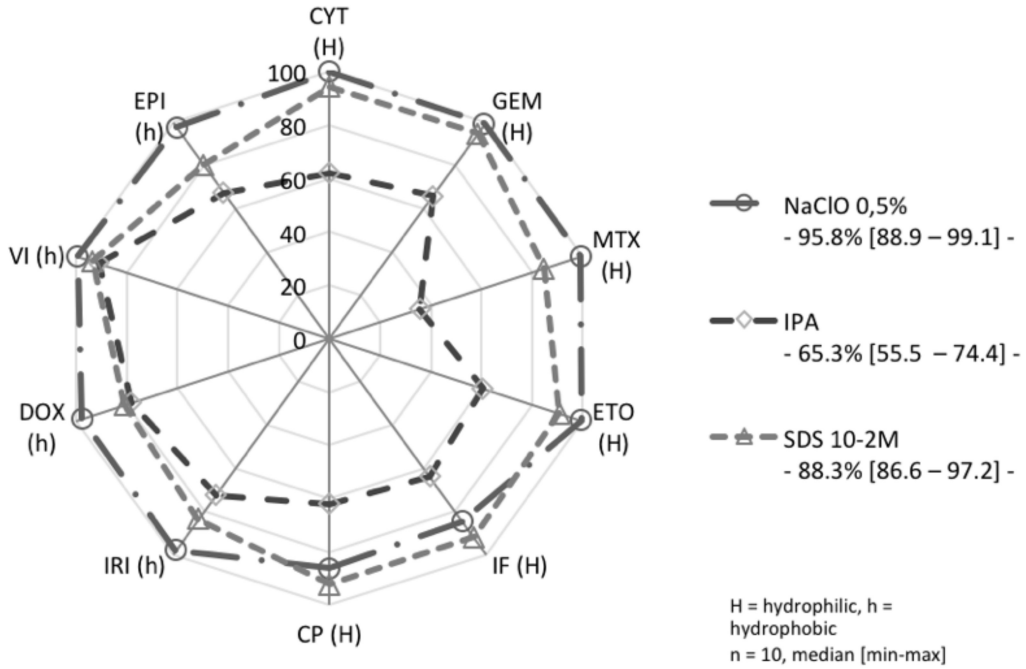

Fig. 6. Efficacy per compound and effectiveness performance of hypochlorite, IPA and SDS 10-2M on glass surface

needed to validate both the effectiveness of the solution and to quantify the volume needed to clean a large surface properly. The best results were obtained when the largest volume was used. Even when using "6 ml", no deposition of surfactant film was found after the wiping procedure. Consequently, a calculation by proportionality between the standardized surface (0.2m²) and a theoretical surface of 1 m² can be evaluated. As a result, ratio of cleaning agent to surface area between 20 and 30 ml m^{-2} should be recommended as an informal rule to clean a stainless steel surface properly.

The most frequently encountered materials in BSCs and isolators are stainless steel and glass. Consequently, further experiments were performed on a glass surface with only three selected aqueous mixtures. IPA/water 70/30 (vol/vol) is recommended by guidelines and has microbiological decontamination effectiveness. During our study, 0.5% sodium hypochlorite, a "degradation-type" solution, presented the best overall EP_{value}, and finally, "10^{-2}M-SDS", developed and tested by ourselves, presented the best ratio between performance and safety. IPA had the lowest EP_{value} (65.3%) and presented no more advantage on glass than on stainless steel. NaOCl effectiveness (95.8%) was equivalent on both stainless steel and glass surfaces. The variations observed between the two surfaces can be accounted for by their physicochemical characteristics. Glass as a more hydrophilic surface has high wettability, and stainless steel as metal has higher hydrophobicity and lower wettability. Finally, 10^{-2}M-SDS with an

Table 5. Efficacy per compound of cleaning solutions on glass surface.

Modalities	CYT	GEM	MTX	ETO	IF	CP	IRI	DOX	VI	EPI	EP_{value}
0.5% NaClO	99,74	99,02	99,23	99,53	84,91	86,34	98,22	97,58	99,42	97,35	95.8
	8,33	8,41	5,83	6,66	19,08	17,61	7,81	8,60	0,68	6,88	—
10^{-2}M-SDS	94,11	94,69	84,58	90,84	92,36	92,21	83,76	80,68	93,46	80,14	88.3
	5,74	4,82	10,12	6,51	7,33	7,71	10,49	16,07	5,62	18,58	—
IPA	62,11	65,85	35,70	60,67	64,09	62,08	72,52	78,11	91,15	67,36	65.3
	16,44	18,08	23,97	15,91	19,02	27,45	11,38	10,36	0,96	9,47	—

Notes: $n = 6$.
Results expressed in median values and SD in percentage.

88.3 EP_{value} was as effective on glass as on stainless steel surfaces.

CONCLUSION

The "degradation-type" solution represented by sodium hypochlorite was very attractive because of oxidation. However, stainless steel as a building material in isolators and BSC prohibits its use. Moreover, this recommendation conveyed by manufacturers themselves is reinforced by the risk of producing agents with unknown chemical structures and cytotoxic potential. The "elimination-type" solutions demonstrated promising results. The use of surfactants such as DWL proves to be efficient and reproducible. Previous studies had already proved its efficiency on glass surface, but those studies were performed with a single antineoplastic agent, the cyclophosphamide or the carboplatin (Lê *et al*., 2012). In our study, the DWL efficiency was again found on 10 antineoplastic agents. Approved on both hydrophilic and hydrophobic agents, DWL had nevertheless a major drawback. Indeed, many manufacturers are on the household cleaning market with their own unknown formulation. Nevertheless, DWLs were very convenient and practical products, and further tests will be performed to evaluate the relevance of their use in daily practical conditions. During our study, surfactants used alone have proved to be effective especially the SDS. Moreover, they have the advantage of being available with the pharmaceutical certification, which eases their use and approval in pharmacy units. SDS allows the desorption of antineoplastic agents and reinforces their solubility. Their effectiveness was successfully proved on both stainless steel and glass surfaces. However, the appearance of residual film at high concentrations can be a potential source of cross and bio contamination. To overcome this problem, IPA was added into the formulation. With a quantity of 20–30 ml m^{-2}, the "10^{-2}M-SDS + 20% IPA" formulation was efficient when sprayed on both stainless steel and glass surfaces with an efficacy superior to 97% on a single run. This effectiveness confirms its suitable use in current practice. Nevertheless, this study was performed on standardized surfaces that cannot be substituted for production units used daily by healthcare workers, where additional parameters have to be taken into account. The best rated decontamination solvents will have to be tested further in real environments, as well as on other materials (transparent thermoplastic such as poly-methylmethacrylate and polycarbonate) and on molecules (platine derivatives and 5-fluorouracil) for which no data is available. The decontamination procedure could also be tested on various supports such as cytotoxic packaging which is known to be contaminated in its industrial area.

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REFERENCES

Acampora A, Castiglia L, Miraglia N *et al*. (2005) A case study: surface contamination of cyclophosphamide due to working practices and cleaning procedures in two Italian hospitals. Ann Occup Hyg; 49: 611–8.

Barek J, Castegnaro M, Malaveille C *et al*. (1987). A method for the efficient degradation of melphalan into nonmutagenic products. Microchem J; 36: 192–7.

Barek J, Cvacka J, de Méo M *et al*. (1998) Chemical degradation of wastes of antineoplastic agents amsacrine, azathioprine, asparaginase and thiotepa. Ann Occup Hyg; 42: 259–66.

Bussières J-F, Sessink PJM, Prot-Labarthe S *et al*. (2006). Évaluation de l'exposition professionnelle aux antinéoplasiques dans une unité de pharmacie hospitalière. Archives Des Maladies Professionnelles Et De l'Environnement; 67: 880–8.

Castegnaro M, De Méo M, Laget M *et al*. (1997) Chemical degradation of wastes of antineoplastic agents. 2: six anthracyclines: idarubicin, doxorubicin, epirubicin, pirarubicin, aclarubicin, and daunorubicin. Int Arch Occup Environ Health; 70: 378–84.

Cavallo D, Ursini CL, Perniconi B *et al*. (2005) Evaluation of genotoxic effects induced by exposure to antineoplastic drugs in lymphocytes and exfoliated buccal cells of oncology nurses and pharmacy employees. Mutat Res; 587: 45–51.

Connor TH, McDiarmid MA. (2006) Preventing occupational exposures to antineoplastic drugs in health care settings. CA Cancer J Clin; 56: 354–65.

Conover WJ, Iman RL. (1981). Rank transformations as a bridge between parametric and nonparametric statistics. Am Stat; 35: 124–9.

Crauste-Manciet S, Sessink PJ, Ferrari S *et al*. (2005) Environmental contamination with cytotoxic drugs in healthcare using positive air pressure isolators. Ann Occup Hyg; 49: 619–28.

Fransman W, Vermeulen R, Kromhout H. (2005) Dermal exposure to cyclophosphamide in hospitals during preparation, nursing and cleaning activities. Int Arch Occup Environ Health; 78: 403–12.

Le Garlantezec P, Rizzo-Padoin N, Lamand V *et al*. (2011). Manipulation des médicaments anticancéreux à l'hôpital : le point sur l'exposition et sur les mesures de

prévention. Archives Des Maladies Professionnelles Et De l'Environnement; 72: 24–35.

Hansel S, Castegnaro M, Sportouch MH *et al*. (1996) Chemical degradation of wastes of antineoplastic agents: cyclophosphamide, ifosfamide and melphalan. Int Arch Occup Environ Health; 69: 109–14.

Heinemann A, Kiffmeyer T, Stüter H *et al.* (2008). Monitoring Effekt-Studie für Wischproben in Apotheken. Hambourg: Berufsgenossenschaft für Gesundheitsdienst und Wohlfahrtspflege (BGW).

ISOPP. (2007). Section 13 cleaning procedures. J Oncol Pharm Pract; 13: 55–61.

Käslin E, Merz B, Rüegger M *et al*. (2010). Contamination de surface lors de la manipulation de médicaments cytostatiques dans les établissements de santé. Suva Med; 2010: 59–72.

Kromhout H, Hoek F, Uitterhoeve R *et al*. (2000) Postulating a dermal pathway for exposure to anti-neoplastic drugs among hospital workers. Ann Occup Hyg; 44: 551–60.

Lê LM, Jolivot PA, Sadou Yaye H *et al*. (2012) Effectiveness of cleaning of workplace cytotoxic surface. Int Arch Occup Environ Health, in press.

Lunn G, Sansone EB, Andrews AW *et al*. (1989) Degradation and disposal of some antineoplastic drugs. J Pharm Sci; 78: 652–9.

Mahnik SN, Rizovski B, Fuerhacker M *et al*. (2004) Determination of 5-fluorouracil in hospital effluents. Anal Bioanal Chem; 380: 31–5.

Mahnik SN, Rizovski B, Fuerhacker M *et al*. (2006) Development of an analytical method for the determination of anthracyclines in hospital effluents. Chemosphere; 65: 1419–25.

Mason HJ. (2003) Cytotoxic drug contamination on the outside of vials delivered to a hospital pharmacy. Ann Occup Hyg; 47: 681–5.

Mukerjee P, Mysels KJ. (1971) Critical Micelle Concentrations of Aqueous Surfactant Systems. Washington, DC: US Department of Commerce, US Government Printing Office.

Nussbaumer S, Fleury-Souverain S, Antinori P *et al*. (2010) Simultaneous quantification of ten cytotoxic drugs by a validated LC-ESI-MS/MS method. Anal Bioanal Chem; 398: 3033–42.

Nussbaumer S, Geiser L, Sadeghipour F *et al*. (2012) Wipe sampling procedure coupled to LC-MS/MS analysis for the simultaneous determination of 10 cytotoxic drugs on different surfaces. Anal Bioanal Chem; 402: 2499–509.

Poyen D, De Méo MP, Botta A *et al*. (1988) Handling of cytostatic drugs and urine mutagenesis. Int Arch Occup Environ Health; 61: 183–8.

Quillardet P, Hofnung M. (2009). Le SOS chromotest : des cellules bactériennes pour détecter et caractériser produits et radiations génotoxiques. Radioprotection; 29: 539–56.

Raghavan R, Burchett M, Loffredo D *et al*. (2000) Low-level (PPB) determination of cisplatin in cleaning validation (rinse water) samples. II. A high-performance liquid chromatographic method. Drug Dev Ind Pharm; 26: 429–40.

Roberts S, Khammo N, McDonnell G *et al*. (2006) Studies on the decontamination of surfaces exposed to cytotoxic drugs in chemotherapy workstations. J Oncol Pharm Pract; 12: 95–104.

Sessink PJ, Cerná M, Rössner P *et al*. (1994) Urinary cyclophosphamide excretion and chromosomal aberrations in peripheral blood lymphocytes after occupational exposure to antineoplastic agents. Mutat Res; 309: 193–9.

Skov T, Lynge E, Maarup B *et al*. (1990) Risks for physicians handling antineoplastic drugs. Lancet; 336: 1446.

Sottani C, Porro B, Comelli M *et al*. (2010) An analysis to study trends in occupational exposure to antineoplastic drugs among health care workers. J Chromatogr B Analyt Technol Biomed Life Sci; 878: 2593–605.

Sottani C, Porro B, Imbriani M *et al*. (2011) Occupational exposure to antineoplastic drugs in four Italian health care settings. Toxicol Lett; 213:107–15

Touzin K, Bussières JF, Langlois E *et al*. (2008) Cyclophosphamide contamination observed on the external surfaces of drug vials and the efficacy of cleaning on vial contamination. Ann Occup Hyg; 52: 765–71.

Turci R, Sottani C, Spagnoli G *et al*. (2003) Biological and environmental monitoring of hospital personnel exposed to antineoplastic agents: a review of analytical methods. J Chromatogr B Analyt Technol Biomed Life Sci; 789: 169–209.

Wren AE, Melia CD, Garner ST *et al*. (1993) Decontamination methods for cytotoxic drugs. 1. Use of a bioluminescent technique to monitor the inactivation of methotrexate with chlorine-based agents. J Clin Pharm Ther; 18: 133–7.