OXFORD

SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 171(1), 2019, 269–279

doi: 10.1093/toxsci/kfz145
Advance Access Publication Date: June 26, 2019
Research Article

Decontamination and Management of Contaminated Hair following a CBRN or HazMat Incident

Hazem Matar, Andreia Pinhal, Nevine Amer, Mark Barrett, Elliot Thomas, Philip Hughes, Joanne Larner, and Robert P. Chilcott[1]

Research Centre for Topical Drug Delivery and Toxicology, University of Hertfordshire, Hatfield AL10 9AB, UK

[1]To whom correspondence should be addressed. E-mail: tox.publications@herts.ac.uk.

ABSTRACT

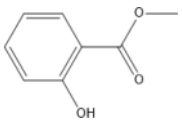
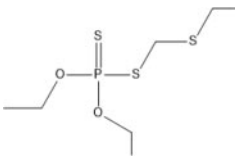
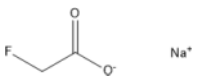
This *in vitro* study evaluated the "triple protocol" of dry decontamination, the ladder pipe system (a method for gross decontamination), and technical decontamination for the decontamination of hair following chemical contamination. First, we assessed the efficacy of the 3 protocols, alone or in combination, on excised porcine skin and human hair contaminated with either methyl salicylate (MS), phorate (PHR), sodium fluoroacetate (SFA), or potassium cyanide (KCN). A second experiment investigated the residual hair contamination following decontamination with the triple protocol at different intervals postexposure. In a third experiment, hair decontaminated after exposure to MS or PHR was evaluated for off-gassing. Though skin decontamination was highly effective, a substantial proportion (20%–40%) of the lipophilic compounds (MS and PHR) remained within the hair. The more water-soluble contaminants (SFA and KCN) tended to form much smaller reservoirs within the hair. Interestingly, substantial off-gassing of MS, a medium volatility chemical, was detectable from triple-decontaminated hair up to 5 days postexposure. Overall, the decontamination strategies investigated were effective for the decontamination of skin, but less so for hair. These findings highlight the importance of contaminated hair serving as a source of potential secondary contamination by contact or inhalation. Therefore, consideration should be given to the removal of contaminated hair following exposure to toxic chemicals.

Key words: hair decontamination; skin decontamination; mass casualty; secondary contamination; decontamination; off-gassing.

The deliberate release of hazardous materials, as exemplified by the Tokyo subway attack (Okumura *et al.*, 1996), and accidental releases of hazardous materials, such as the Seveso and Bhopal incidents (Broughton, 2005; Eskenazi *et al.*, 2018), are acknowledged as worldwide chemical disasters affecting several hundreds, if not thousands of people. In recent years, attacks such as the assassination of Kim Jong-nam with VX and the use of novichok in the UK, had the potential to develop into mass casualty incidents due to the brazen way these chemicals were deployed in public spaces, posing a significant threat to public health.

Mass casualty decontamination within the United States following a hazardous materials (HAZMAT) or chemical, biological, radiological, and nuclear (CBRN) event has traditionally used a gross decontamination method known as the "ladder pipe" decontamination system (LPS) (Chilcott *et al.*, 2018; Lake *et al.*, 2013). This ad hoc procedure involves positioning fire engines in parallel to each other to deliver a high-volume, low-pressure water mist into a corridor through which casualties pass. The intricacies of this response vary across the United States (Power *et al.*, 2016). However, the overall response to mass casualty chemical incidents has been revised to incorporate disrobing (the removal of clothing), an improvised form of dry decontamination (DD) and specialist methods of decontamination (technical decontamination [TD]), similar to the principles being adopted in the United Kingdom (Chilcott *et al.*, 2019a; UK Home Office, 2015).

Table 1. Summary of Chemical Contaminants

	MS	PHR	SFA	KCN
				
Molecular weight (g/mol)	152.149	260.365	100.024	65.116
Log P	2.234	3.56	No data	No data
Vapor pressure (mmHg) at 20°C	0.045	0.0008	0	No data

Data obtained from PubChem open chemistry database, U.S. National Library of Medicine.

Current research focuses mainly on skin, rather than hair decontamination. Mass casualty decontamination guidelines emphasize the use of improvised DD, wet decontamination (including the LPS), and TD (Amlôt *et al.*, 2017; Chan *et al.*, 2013; Kassouf *et al.*, 2017; Matar *et al.*, 2014; Taysse *et al.*, 2007; Thors *et al.*, 2017). Human volunteer trials have demonstrated the effectiveness of such procedures (Chilcott *et al.*, 2019b). Recently, emphasis has been placed on decontamination of scalp hair (Josse *et al.*, 2015; Rolland *et al.*, 2013; Spiandore *et al.*, 2014, 2017, 2018). It is well established that the removal of contaminants from hair is difficult (Duca *et al.*, 2014) and the affinity with which chemicals bind to hair has been exploited for forensic purposes (Blank and Kidwell, 1995).

Hair decontamination studies have found that the efficacy of the decontamination protocols employed for skin decontamination may not apply in the case of hair decontamination, particularly for more lipophilic compounds such as methyl salicylate (MS) and phorate (PHR) (Matar *et al.*, 2018). Thus, there is a need to investigate the potential dangers of hazardous chemicals that are bound or otherwise retained within the hair shaft after decontamination protocols have been completed.

The purpose of this study was to simulate and evaluate the efficacy of DD, LPS, and TD (Chilcott *et al.*, 2019b), alone or in combination, following contamination of skin and hair. The main aim of this study was to investigate the binding affinity of contaminants to hair and whether these pose a secondary hazard over time following decontamination.

MATERIALS AND METHODS

Materials. Methyl salicylate (99%) was purchased from Acros Organics, UK. Potassium cyanide (KCN; > 98%), inhibitor-free diethyl ether (99.9%), and Amberlite XAD-2 (20–60 mesh) were purchased from Sigma Aldrich (St. Louis, Missouri). Phorate (PHR; 95%) and sodium fluoroacetate (SFA; 99%) were custom synthesized by American Radiolabeled Chemicals (St. Louis, Missouri). Acetonitrile (HPLC grade), ethanol (Absolute), and propan-2-ol (HPLC grade) were purchased from Fisher Scientific, Leicestershire, UK. Ultra-pure water (> 18.2 MΩ) for receptor fluid media and sampling was obtained by ultrafiltration of the municipal supply via a MilliQ Integral 3 (Millipore, Massachusetts).

Ring-labeled (^{14}C) MS (70 mCi mMol^{-1}), PHR (50 mCi mMol^{-1}), SFA (50 mCi mMol^{-1}), and KCN (58 mCi mMol^{-1}) were purchased from American Radiolabeled Chemicals (Table 1). Their nonradioactive analogs were added in an appropriate proportion to give a working solution with a nominal activity of 0.5 μCi μl^{-1}. Soluene-350 and Ultima Gold liquid scintillation counting (LSC) fluid were purchased from PerkinElmer, Cambridgeshire.

Full-thickness skin was obtained *postmortem* from female pigs (*Sus scrofa*, large white strain, weight range of 15–25 kg) purchased from a reputable supplier following ethical approval. The skin was close clipped and removed from the dorsal aspect of each animal. The excised skin was then wrapped in aluminum foil and stored flat at −20°C. Prior to the start of each experiment, a skin sample from 1 animal was removed from cold storage and thawed for approximately 24 h. The skin was then dermatomed to a thickness of 1000 μm (Humeca Model D80; Eurosurgical, Surrey, UK). Once dermatomed, the skin samples were mounted on diffusion cells (19.64 cm^2 surface area). Skin diffusion cells and manifold delivery system were custom manufactured by Protosheet Ltd, Kent, UK, as previously described in Matar *et al.* (2014).

A variety of human hair swatches were collected from unisex hair salons within the Hertfordshire and Hampshire areas of the United Kingdom or purchased from Pivot Point Education Ltd (Milton Keynes, UK). Hair curtains (swatches) were assembled using 3 different visual hair types: thin (brown), dyed blonde, and thick (dark brown or black). The hair swatches/curtains were prepared as previously described by Matar *et al.* (2018).

Hair and skin decontamination experiments. Each experiment involved the use of 4 (single studies: control, DD, LPS, or TD) or 5 (combined studies: control, DD+LPS, DD+TD, LPS+TD, or DD+LPS+TD [triple protocol]) diffusion cells (Table 2), with each allocated a specific treatment using a randomized design (so that no treatment was performed in the same diffusion cell position relative to the shower manifold). Each experiment was repeated 6 times to give a total of $n = 6$ replicates per treatment, with each replicate being performed on skin from a separate skin donor and a different hair type.

Diffusion cells containing the hair curtains were connected to a peristaltic pump (Watson-Marlow 520S), through which receptor fluid (50% ethanol/water) was infused at a rate of 0.5 mlmin^{-1}. Each diffusion cell was placed on a silicone heat mat that was controlled via a digital controller (both supplied by Holroyd components, UK). The temperature of each heat mat was set to achieve a skin surface temperature of 32°C (confirmed using an infrared camera; FLIR P620). Diffusion cells were left to equilibrate for 1 h to achieve the desired skin temperature, after which pre-weighed 20 ml glass scintillation vials were positioned at the receptor fluid effluent port to collect a 15-min baseline sample of receptor chamber fluid.

Table 2. Summary of Treatment Groups Used for Combined Skin and Hair Decontamination Studies

Study Group	Treatment Group	Parameters
Single techniques	Control	No decontamination
	DD	Decontamination performed 4 min postexposure using wound dressing
	LPS	Showering performed 8 min postexposure using water with a flow rate of 10 ml min^{-1} cm^{-2} at 10°C for 15 s
	TD	Showering performed 12 min postexposure at a flow rate of 74 ml min^{-1} cm^{-2} at 35°C using a washcloth with gentle rubbing for 90 s
Combined techniques	Control	No decontamination
	DD + LPS	DD performed 4 min postexposure followed by LPS at 8 min
	DD + TD	DD performed 4 min postexposure followed by TD at 12 min
	LPS + TD	LPS performed at 8 min postexposure followed by TD at 12 min
	DD + LPS + TD (Triple Protocol)	DD performed at 4 min, LPS at 8 min and TD 12 min postexposure
Hair extraction and hair off-gassing	Triple Protocol	DD, LPS, and TD performed immediately after each other at either 0, 5, 10, 20, 30, 60, 120, or 240 min postexposure

The experiment was started by the addition of 20 μl of either ^{14}C-MS, ^{14}C-PHR, ^{14}C-SFA, or ^{14}C-KCN to the skin and another 20 μl to the hair (total 40 μl dose per cell). Dry decontamination was performed on the skin/hair surface 4 min postexposure by applying a sterile trauma dressing (McKesson Medical-Surgical), cut into an approximately 38.5 cm^2 disk, to the skin/hair surface and placing a weight (157.12 g; approximately 8 g cm^{-2}) on top. After 5 s the weight was removed, the trauma dressing was turned over, a tinfoil disk (approximately 38.5 cm^2) was placed over the dressing and the weight was positioned on top of the tinfoil disk for a further 5 s. Dry decontamination was terminated by removing the weight and placing both the tinfoil disk and the trauma dressing into a petri dish for imaging via digital autoradiography.

During LPS decontamination, the skin was showered with water at a temperature of 10°C and a flow rate of 10 ml min^{-1} cm^{-2}. Decontamination was conducted 8 min postexposure for a total duration of 15 s. The shower effluent was collected in pre-weighed 1 l glass containers, which were reweighed after showering and stored for subsequent analysis. The diffusion cells were then returned to a horizontal position and the skin and hair were dried using a swatch (11 × 11 cm) of towel, which was stored for image analysis.

Technical decontamination was conducted 12 min postexposure, using a water temperature of 35°C and a flow rate of 74 ml min^{-1} cm^{-2}. The diffusion cells were opened, tilted through 45° and showered for a total of 90 s, during which the skin and hair were gently rubbed using a 3 × 3 cm flannel with 100 μl of Johnson's baby shampoo (JBS) held in forceps. The effluent was collected in pre-weighed 120 ml jars, which were then reweighed and stored for analysis. The diffusion cells were then returned to the horizontal position, the skin and hair were dried using a towel swatch and the cells were closed. The flannel and towel were placed into pre-weighed jars and stored for subsequent image analysis via digital autoradiography.

Air from within the donor chamber was sampled using constant volume pumps (Pocket Pump model 210-1002MTX, SKC Ltd, Dorset, UK) set at a sampling volume of 75 ml min^{-1}. Glass sorbent tubes were purchased from Markes International (Llantrisant, UK). Each tube was filled with 150 ± 5 mg of Tenax TA 35/60 sorbent. Filled Tenax tubes were subjected to conditioning prior to use by purging under nitrogen and a heat cycle, according to the manufacturer's instructions.

The radioactivity within the samples (swabs, Tenax, tubing, receptor chamber fluid, skin, etc.) was quantified using a PerkinElmer Tri-Carb liquid scintillation counter (Model 2810 TR) employing an analysis runtime of 2 min per sample and a preset quench curve specific to the brand of LSC fluid (Ultima Gold, PerkinElmer, UK). The amounts of radioactivity in each sample were converted to quantities of ^{14}C-MS, ^{14}C-PHR, ^{14}C-SFA, or ^{14}C-KCN by comparison with standards (measured simultaneously). The standards were prepared on the day of each experiment by the addition of a known amount of each contaminant to (1) cotton wool swabs in 10 ml propan-2-ol or dH_2O; (2) tin foil, Tenax, tubing, flannel, and towel swatches in 10–40 ml in propan-2-ol or dH_2O; (3) 375 ml of shower fluid and 120 ml of TD fluid; (4) trauma dressing in 25 ml propan-2-ol or dH_2O; and (5) skin tissue dissolved in 50 ml soluene-350. The extraction solvent used for each of the samples was chemical-dependent. Propan-2-ol was employed for ^{14}C-MS and ^{14}C-PHR, whereas deionized water was used for ^{14}C-SFA and ^{14}C-KCN. A standard receptor fluid solution was also prepared by the addition of 10 μl of ^{14}C-MS, ^{14}C-PHR, ^{14}C-SFA, or ^{14}C-KCN to 990 μl of fresh receptor fluid (50% aqueous ethanol), from which a range of triplicate samples (25, 50, 75, and 100 μl) were placed into vials containing 5 ml of LSC fluid to produce a standard (calibration) curve. Aliquots (250 μl) of each sample obtained from the experiment were taken and placed into vials containing 5 ml of liquid scintillation fluid for LSC. For clarity, the sum of the amounts of contaminant detected in the receptor fluid, in the skin and on the skin surface is referred to as the bioavailable fraction.

Hair extraction studies. Human hair swatches were cut to 3 × 1.5 cm and secured to a polystyrene petri dish with duct tape (skin was not used for these studies). For each of the 4 chemicals (^{14}C-MS, PHR, SFA, and KCN), the effects of delayed decontamination of hair were investigated by initiating decontamination at 0, 5, 10, 20, 30, 60, 120, or 240 min postexposure to a 20 μl droplet. At the appropriate time point the triple protocol was conducted. Dry decontamination was performed on the hair as described above. Immediately following the completion of DD, the petri dish containing the hair swatch was transferred to the diffusion cell, where it was subjected to simulated LPS decontamination (described above). The hair was towel dried using a towel swatch (11 × 11 cm) and the petri dish was then immediately transferred to another (clean) showering rig for TD, performed as described above.

Once the triple decontamination procedures had been completed, the hair swatches were placed into pre-weighed 20 ml vials, which were weighed before and after the addition of 20 ml of acetonitrile, ethanol, ether, water, or JBS in water (0.085% v/v). Samples were then stored at 4°C.

The following day, allowing a minimum extraction period of 18 h, hair samples were removed from cold storage. From each vial, a 250 μl aliquot was removed from each sample and placed into a vial containing 5 ml of Ultima Gold liquid scintillation fluid for counting. Samples were replenished with 250 μl of the appropriate solvent and reweighed to maintain a constant volume of 20 ml in all hair samples. Subsampling and replenishment of hair samples were repeated over a total of 5 days. On the fifth day, once all subsampling had been completed, the hair swatches were removed from the solvent, blotted dry with absorbent paper towels (WypAll, Kimberly-Clark) and transferred into fresh pre-weighed jars before the addition of 20 ml of Ultima Gold. The following day, an aliquot of 250 μl was removed from each jar and placed in liquid scintillation vials with 5 ml of Ultima Gold. Sample analysis was performed as described above; standards were prepared on the day of each experiment by the addition of each contaminant to each hair type in their respective solvents.

Hair off-gassing studies. Human hair was obtained as described above and the swatches of hair were prepared and secured to polystyrene petri dishes in the same way. Each of the hair swatches was contaminated with a 20 μl droplet of either ^{14}C-MS or PHR and left for 0, 5, 10, 20, 30, 60, 120, or 240 min before the triple protocol decontamination procedure was performed. Immediately following decontamination, each hair swatch was placed in a small box lined with tin foil (ABS enclosure, 40 × 40 × 20 mm; RS components, UK). Amberlite XAD-2 (approximately 1 g), a passive absorbent was then placed in each box under a perforated metal support to prevent direct contact with the hair. The box was then closed, its lid was screwed tight, and the box was placed on a bespoke silicone heat mat (Holroyd Components Ltd, UK) with a thin metal aluminum sheet to facilitate heat dispersion. The aluminum sheet was set to 31°C to achieve a box temperature of 30°C.

Every 6 h, up to a total period of 120 h, the hair was transferred into a new box with fresh Amberlite and tinfoil, allowing a 4-min delay between each sample. The Amberlite and tinfoil were then removed and placed into their respective preweighed scintillation vials, where each sample was weighed and 20 ml of propan-2-ol was added and left to extract. At 120 h of sample acquisition, the hair was removed from the box and placed into a screwcap vial containing 20 ml of Ultima Gold. All samples were left for a minimum of 24 h to allow for the contaminant to be extracted and each sample was vortexed prior to aliquoting. Each 250 μl aliquot was placed into a 6.5 ml plastic scintillation vial filled with 5 ml of Ultima Gold. Radioactive sample analysis was performed as described above.

Statistics. Statistical analysis was performed using GraphPad Prism 7. Normality tests (Shapiro-Wilk) were performed on all data. Normally distributed data were analyzed using 1-way ANOVA or a 2-tailed t-test. For nonparametric data sets, treatment effects were analyzed using the nonparametric Kruskal-Wallis test. The Mann-Whitney test was performed to verify some of the results, specifically when no significance was shown. The Spearman test was used to find any correlations between groups when data were not normally distributed and the Pearson test for data that were normally distributed.

RESULTS

Skin and Hair Absorption Studies

For the non-decontaminated group (control) the majority of the applied dose was recovered from either the bioavailable fraction (the total amount of receptor fluid, remaining within the skin and on the skin surface) or hair for cells contaminated with ^{14}C-MS, ^{14}C-PHR, ^{14}C-SFA, or ^{14}C-KCN (Figure 1). The bioavailable fraction was found to be significantly smaller ($p < .05$) for all treatment groups when compared with their respective controls. Generally, there were no statistically significant ($p < .05$) differences between the decontamination protocols except between DD and DD+LPS+TD for all the contaminants. Further significant differences were found between DD versus DD+TD and DD versus LPS+TD following contamination with ^{14}C-SFA and ^{14}C-KCN. The amount of contaminant recovered from the hair was greater for hair contaminated with ^{14}C-MS or ^{14}C-PHR than for ^{14}C-SFA and ^{14}C-KCN. Interestingly, all decontamination protocols significantly ($p < .05$) reduced the amount of contaminant in the hair when compared with their respective controls, with the exception of DD for hair contaminated with ^{14}C-MS, PHR, SFA, or KCN, which demonstrated effectiveness, but was not statistically significant. No statistically significant differences were found between the different decontamination methods for hair contaminated with ^{14}C-MS or ^{14}C-PHR. However, a statistical difference was observed between DD versus DD+LPS+TD and DD versus DD+LPS for ^{14}C-SFA and ^{14}C-KCN, respectively. The proportions of unbound (mobile; hair surface) contaminant from hair were generally lower following decontamination when compared with non-decontaminated (control) hair. Furthermore, there were no statistical differences in the amounts of unbound contaminant between all of the decontamination protocols evaluated. Full dose distributions are provided within the Supplementary Data.

Hair Extraction Studies

The triple protocol for hair decontamination was effective for the hydrophilic chemical SFA, as no detectable hair residue could be extracted by the aqueous solvent systems (water or Johnson's shampoo solution) for exposure periods up to 240 min (Figure 2). Furthermore, no residue of KCN could be extracted from hair decontaminated immediately post-exposure, with 5%–25% of the applied dose being recovered after 5 min.

The triple protocol, when performed immediately post-exposure ($t = 0$), reduced the residual amount of MS and PHR extractable from within the hair to approximately 30% of the applied dose (Figure 2). The performance of hair decontamination rapidly declined with time postexposure (after a delay of 5 min or more) as the contaminant residue increased to approximately 65% of the applied dose.

Differences in the extent to which the different solvent systems extracted the residual hair contamination reflected the known solubility of the contaminants. Water and Johnson's shampoo solution were less effective for extracting the lipophilic contaminants (MS and PHR), whereas the organic solvents were less effective for extracting the hydrophilic contaminants (SFA and KCN; Figure 2).

Hair Off-gassing Studies

Human hair contaminated with ^{14}C-MS was found to off-gas with time (Figure 3). In contrast, minimal but variable amounts of ^{14}C-PHR off-gassed over the 120-h period (Figure 4). With regard to ^{14}C-MS, the amounts off-gassed were generally lower for

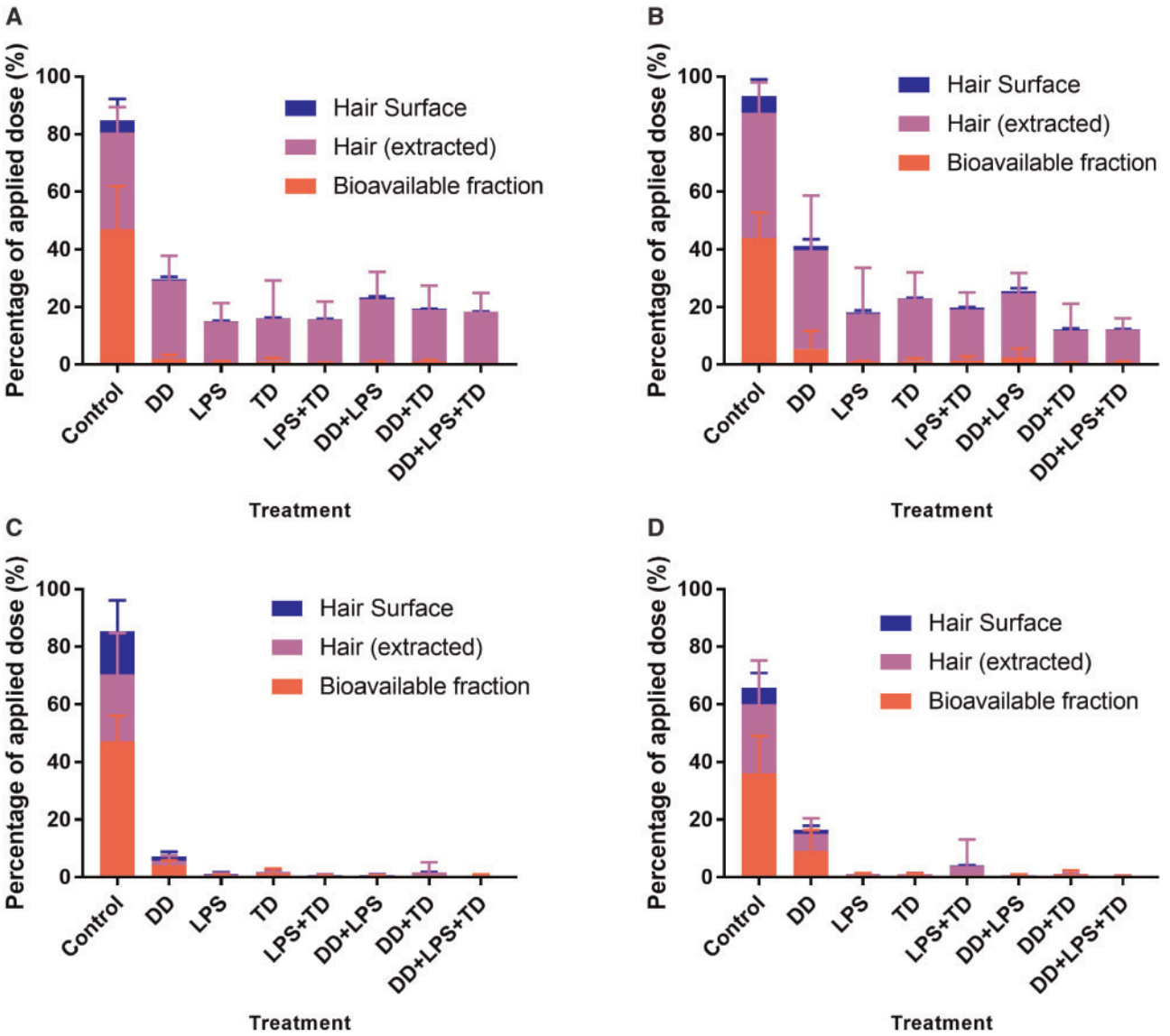


Figure 1. Summary dose distribution of the percentage of applied dose recovered from hair surface, extracted from hair and bioavailable fraction for ^{14}C-methyl salicylate (MS; A), phorate (PHR; B), Sodium fluoroacetate (SFA; C) and potassium cyanide (KCN; D) penetrating untreated (control) or following various decontamination strategies. One 20 μL droplet of 14C-MS, PHR, SFA or KCN was applied to the surface of the hair and one 20 μL droplet directly to the skin surface. A total of eight decontamination strategies were evaluated: untreated (control), ladder pipe system (LPS), dry decontamination (DD), technical decontamination (TD) and various combinations (DD+LPS, DD+TD, DD+LPS+TD and LPS+TD). Dry decontamination (DD) was performed 4 minutes post exposure for a total duration of 10 seconds. Ladder pipe system decontamination was conducted at 8 minutes post exposure using a water flow rate of 10 mL min^{-1} cm^{-2} at a temperature of 10°C for 15 seconds. Technical decontamination was carried out 12 minutes post exposure for a duration of 90 seconds using water at a temperature of 35°C. All points are mean ± standard deviation of up to n=6 diffusion cells, except for controls, which included up to n=12 diffusion cells.

decontaminated than for non-decontaminated (control) groups. Furthermore, the amounts of MS recovered from off-gassing decreased over time for all the exposure times evaluated ($r = -0.87$, $p < .0001$; Figure 3).

The average maximum rate (J_{max}; the maximum rate of vapor loss per treatment group against time) of vapor loss was generally higher within the first 20 min postexposure for non-decontaminated controls (Figure 5). In contrast, the rate of vapor loss was relatively consistent for decontaminated hair. Interestingly, the greater vapor loss within the first 20 min post-exposure appeared to be attributable to the higher amounts of contaminant remaining on the hair surface, and not within the hair, as compared with decontaminated hair.

DISCUSSION

This study utilized a previously characterized skin and hair diffusion cell (Matar *et al.*, 2014, 2018) that models the LPS to investigate and highlight the generic effectiveness of hair and skin decontamination. Further experiments evaluated the consequences of this residual hair contamination.

Overall, the decontamination strategies investigated were effective for the decontamination of skin (Chilcott *et al.*, 2019b). However, relatively large proportions of the contaminant remained in the hair even following several decontamination methods. It was apparent that the majority of the applied dose was removed by whichever decontamination protocol was performed first. However, DD was generally not as effective as wet

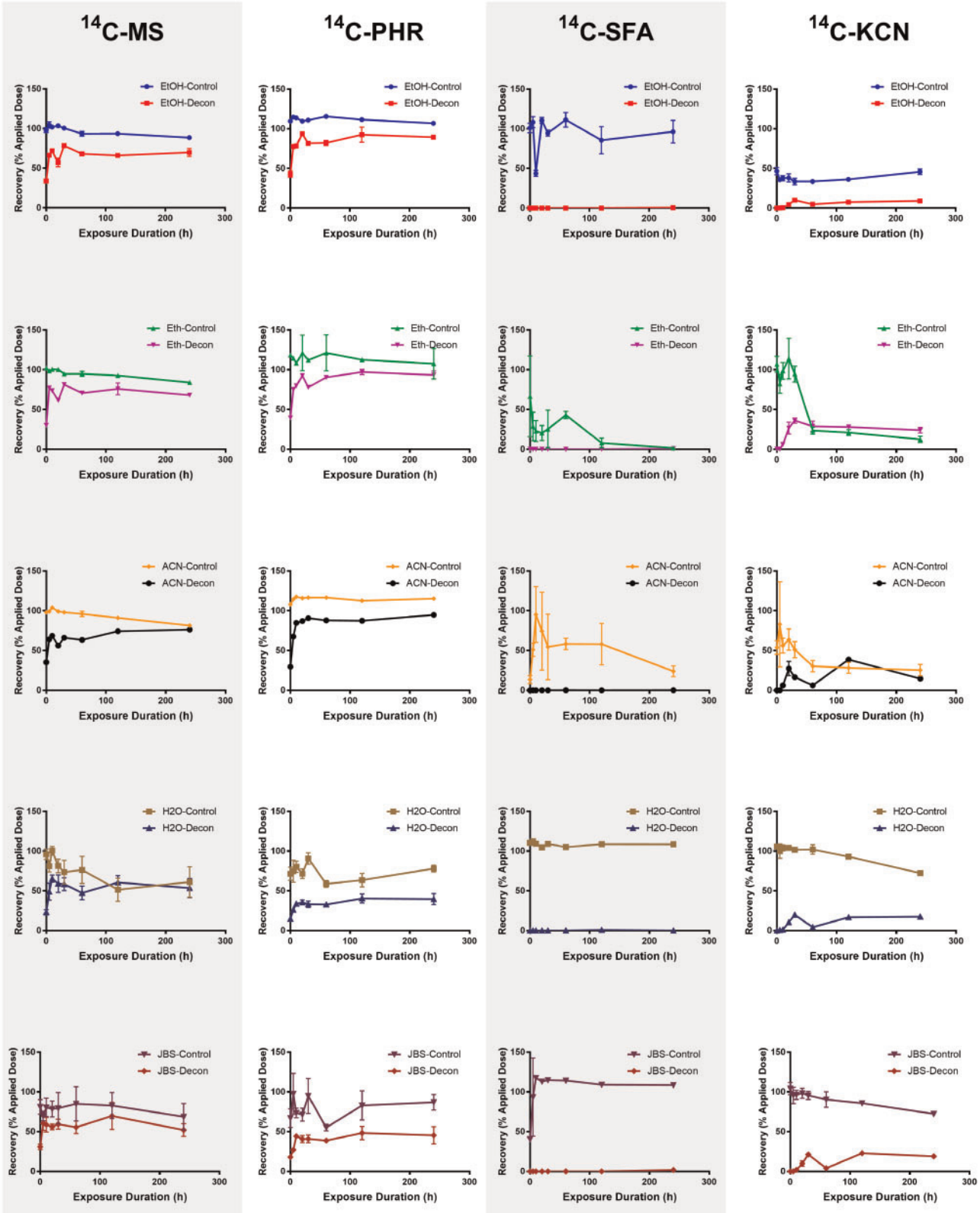


Figure 2. Extraction (expressed as percentage of applied dose) of methyl salicylate, phorate, sodium fluoroacetate, and potassium cyanide from hair curtains originally exposed to a liquid droplet of contaminant for durations of 0–240 min prior to either triple decontamination (dry, LPS, and technical) or no treatment (control). Extractions were performed in ethanol, ether, acetonitrile, water, or a 0.5% aqueous solution of Johnson's Baby Shampoo. Each data point is average ± standard deviation ($n = 5$).

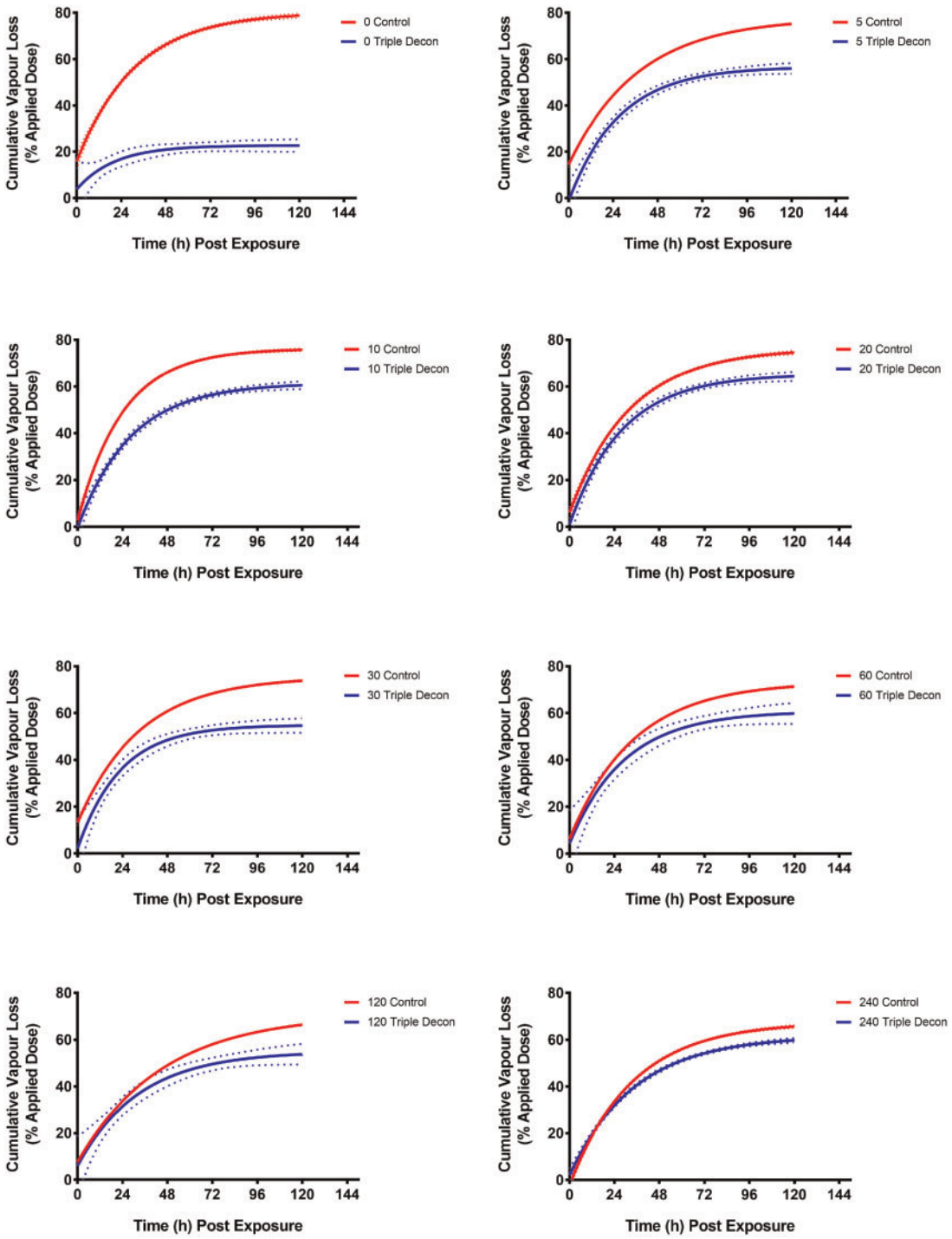


Figure 3. Percentage of applied dose of ^{14}C-methyl salicylate (MS) expressed as cumulative vapor loss from human hair over 120 h (6 hourly intervals). Each swatch of human hair was exposed to a 20 μl droplet of ^{14}C-MS and subjected to either no decontamination (control) or combined dry, ladder pipe system, and technical decontamination (triple protocol) at 0, 5, 10, 20, 30, 60, 120, and 240 min postexposure. Each point represents mean ± 95% confidence interval of up to $n = 6$.

decontamination in decontaminating hair. This is probably attributable to the fact that the area of exposure of the wound dressing pad was limited to the uppermost hair strands, limiting the amount of contaminant available for decontamination. This contrasts with other studies, in which the military decontaminants fuller's earth and RSDL were found to be effective products for decontaminating VX from hair (Rolland *et al.*, 2013). It should be noted that both these substances have the ability to coat or surround contaminated hair strands.

It is widely accepted that hair is a difficult matrix to decontaminate, even after several washes (Duca *et al.*, 2014). This innate ability of hair to retain chemicals has been exploited mainly in the field of forensics and drug analysis (Blank and Kidwell, 1995). It should be noted that certain solvent mixtures

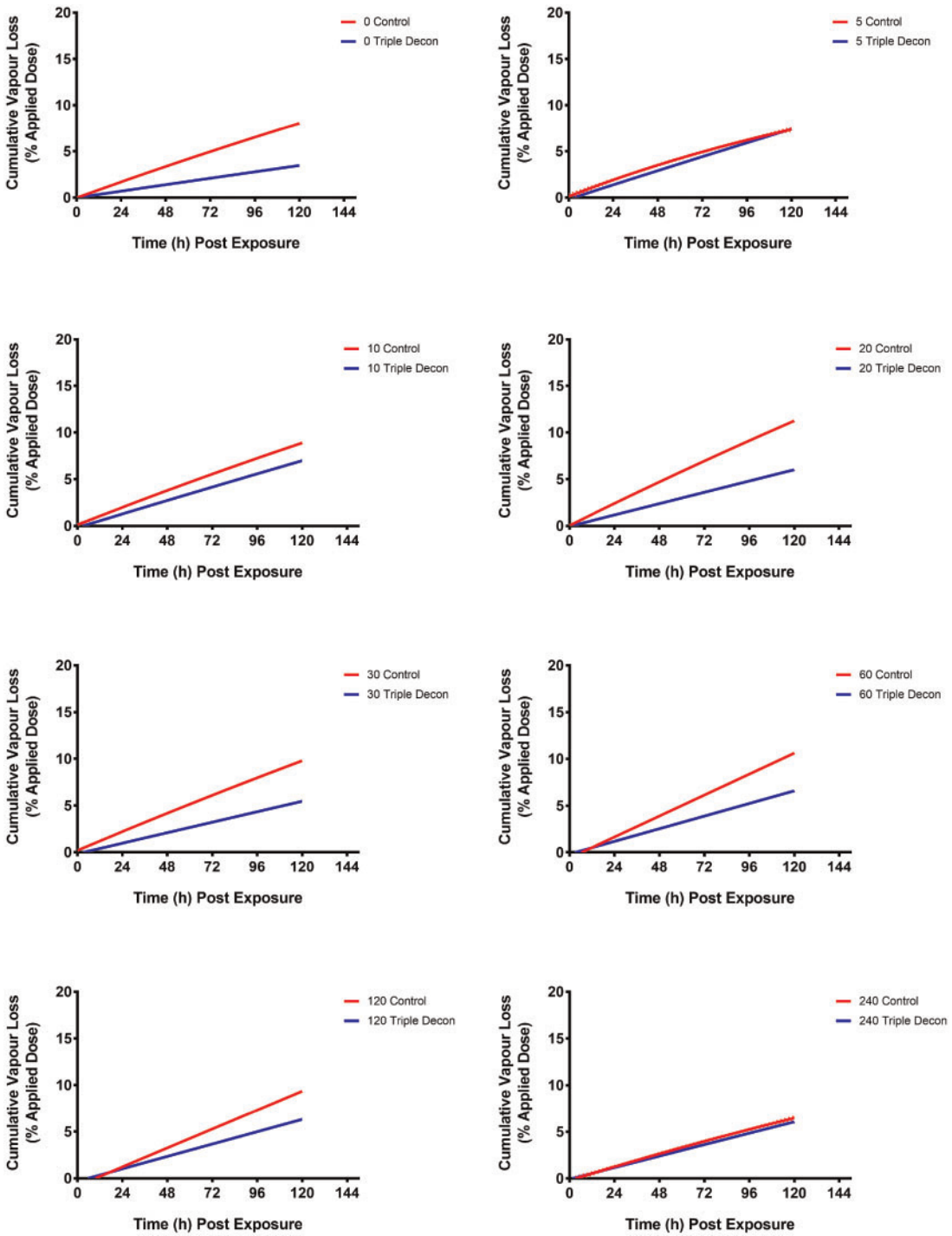


Figure 4. Percentage of applied dose of ^{14}C-phorate expressed as cumulative vapor loss from human hair over 120 h (6 hourly intervals). Each swatch of human hair was exposed to a 20 μl droplet of ^{14}C-PHR and subjected to either no decontamination (control) or decontamination using combined dry, ladder pipe system, and technical decontamination (triple protocol) at 0, 5, 10, 20, 30, 60, 120, and 240 min postexposure.

could be employed to remove chemicals from hair (Duca *et al.*, 2014). However, there is a paucity of data relating to the persistence of chemical contaminants following standard decontamination techniques. For the purposes of managing casualties contaminated as the result of a HAZMAT or CBRN incident, it is important to assess the binding affinity of contaminants to hair. If chemicals are found to leach with time, this may result in secondary vapor exposures affecting the casualty, first responders, and/or medical personnel, as reported with contaminated clothing (Feldman, 2010).

Differences in extraction efficacy were observed between the different contaminants and solvents used. Generally, ^{14}C-MS and PHR were readily (fully) extracted using ethanol, ether, or acetonitrile within the first 24 h of extraction, whereas for water and shampoo solution the extraction was gradual overtime. This is probably attributable to the solubility of the

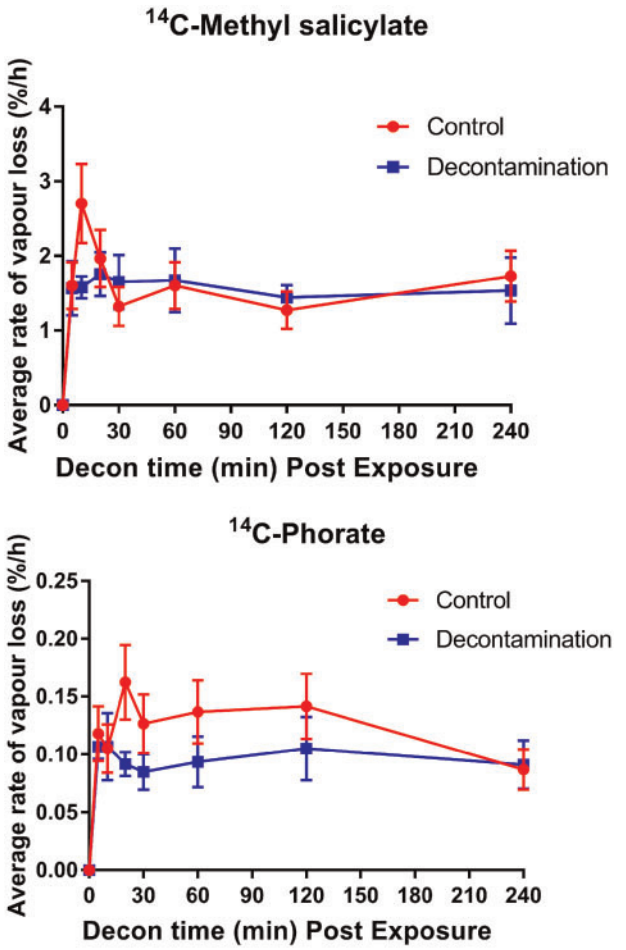


Figure 5. Average rate of vapor loss from hair expressed as percentage per hour. Each swatch of human hair was exposed to a 20 μl droplet of ^{14}C-MS or PHR and subjected to either no decontamination (control) or decontamination using combined dry, ladder pipe system and technical decontamination at 0, 5, 10, 20, 30, 60, 120, and 240 min postexposure.

contaminants in these solutions. Conversely, ^{14}C-SFA and KCN were not as readily extracted in ethanol, ether, or acetonitrile when compared with H_2O and shampoo solution. However, both ^{14}C-SFA and KCN were effectively removed following decontamination, unlike ^{14}C-MS and PHR. It is worth noting that this part of the study was designed to evaluate the affinity with which the contaminants are bound to hair and not the suitability of these solvents as hair decontaminants (given the long, 5-day submersion).

The underlying mechanism behind the affinity of these chemicals for human hair is unclear (Duca *et al.*, 2014), but it is likely to be influenced by the lipophilicity of the contaminant. The structure of human hair, from the inner layer to the outer layer, consists of the medulla, the cortex, and the cuticle. The outermost layer of the cuticle (epicuticle) is a lipoprotein membrane that is estimated to be 10–14 nm thick (Swift and Smith, 2001) and may be coated with sebum (Eberhardt, 1976). Therefore, a strong physicochemical attraction between the lipophilic chemicals and the lipid-rich hair could explain why decontamination of these chemicals from the hair proved to be more problematic. The implications of this association depend on the affinity with which a given contaminant bonds with the hair: if the bond is irreversible, there will be no toxicological hazard (as the contaminant will not transfer, or become mobile for inhalation or dermal exposure); if it is not, then the contaminant may be transferred to either hand or scalp, or absorbed via the follicular pathway, and will thus still pose a threat (Knorr *et al.*, 2009).

An important aspect of this study was the investigation of chemical off-gassing from contaminated human hair. Our findings highlight the significance of contaminant off-gassing, even from hair that has undergone decontamination procedures. An interesting result was that large proportions of the applied dose off-gassed within the first 24 h. This means that contaminated casualties may continue to pose a risk to themselves, emergency responders, and/or hospital staff following decontamination. Conversely, nonvolatile organic chemicals, such as PHR, were found not to off-gas with time. Consequently, large proportions of the contaminant remained within the hair. Upon further analysis, the rate of off-gassing (Figure 5) can provide some evidence that the residual contaminants were absorbed into the hair rather than adhering to the hair surface. The difference between control and decontaminated hair is that most surface contamination would be removed by decontamination. Thus, the higher initial rate of vapor loss from controls implies that hair surface contamination is the predominant factor for early vapor loss. A previous study (Matar *et al.*, 2018) demonstrated that PHR and MS partition rapidly into the hair; thus, there would be less surface contamination to contribute to the initial vapor loss phase. The similarity in rate of vapor loss between control and decontaminated hair over the second, longer phase of vapor loss probably reflects evaporation from within the hair. Raman microscopy was used to assess molecular interactions of the contaminants with hair with no significant differences observed in the spectra (data not shown).

Limitations

The experiments that made up this study had a number of limitations. First, the hair and skin model used did not take into account the potential for chemical absorption via the hair and hair follicle, which might reduce the amount on or within hair but would increase the bioavailable fraction. In addition, the use of ^{14}C-radiolabeled chemicals is unable to distinguish (without further analysis) whether the contaminant is intact, rather than a breakdown product or metabolite bound to hair. However, because this model was designed to represent a conservative approach to hair exposure and decontamination, the measurements assumed the worst-case scenario of no chemical degradation.

The hair and skin decontamination study was performed unrealistically soon after contamination (4, 8, and 12 min for DD, LPS, and TD, respectively) and thus deviates from the longer anticipated response times. The rationale for this was to employ effective decontamination soon after exposure and thus assess whether these contaminants could be removed from hair. Furthermore, the fact that decontamination was performed so rapidly postexposure highlights the speed with which these contaminants adhere to hair. Nevertheless, it is possible that allowing a longer exposure time before the start of decontamination would reveal differences between the decontamination strategies and their combinations that were not apparent within the short time scale of this study.

In the off-gassing study, the placement of contaminated hair within sealed boxes to assess chemical off-gassing is not indicative of a realistic situation, where airflow through the hair would affect chemical evaporation and concentration. However, as this initial model was based on a conservative approach, it nevertheless effectively highlights the fact that some

contaminants represent a significant hazard from off-gassing over surprisingly long durations.

CONCLUSIONS

The use of the triple decontamination protocol (DD + LPS + TD) has been shown to be effective for removing contaminants from skin (Chilcott *et al*., 2019b). In contrast, the use of DD was generally not as effective as wet protocols for decontaminating hair. Furthermore, human hair has shown a capacity to retain certain chemicals and, depending on their physicochemical properties, these may off-gas with time. Therefore, to counteract this risk, it may be prudent to close-clip and remove contaminated hair from individuals. Decontaminating hair using solvents in mass casualty scenarios may not be practical in some cases or may need to be delayed. However, when dealing with a small number of casualties it may be more feasible to decontaminate hair with appropriate solvents in a controlled manner.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ACKNOWLEDGMENTS

The authors would like to thank Marios Konstantinidis for his assistance with the solvent extraction studies from hair.

FUNDING

This project was funded by Federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advance Research, and Development Authority (HHS0100201500016C).

REFERENCES

Amlôt, R., Carter, H., Riddle, L., Larner, J., and Chilcott, R. P. (2017). Volunteer trials of a novel improvised dry decontamination protocol for use during mass casualty incidents as part of the UK's Initial Operational Response (IOR). *PLoS One* **12**, e0179309.

Blank, D. L., and Kidwell, D. A. (1995). Decontamination procedures for drugs of abuse in hair: Are they sufficient? *Forensic Sci. Int.* **70**, 13–38.

Broughton, E. (2005). The Bhopal disaster and its aftermath: a review. *Environmental Health* **4**, 6.

Chan, H. P., Zhai, H., Hui, X., and Maibach, H. I. (2013). Skin decontamination: Principles and perspectives. *Toxicol. Ind. Health* **29**, 955–968.

Chilcott, R. P., Larner, J., and Matar, H. (2018). Primary Response Incident Scene Management; PRISM Guidance, Vol. 1, 2nd edn. Office of the Assistant Secretary for Preparedness and Response, Biomedical Development Authority, Washington, DC.

Chilcott, R. P., Larner, J., and Matar, H. (2019a). The United Kingdom's initial operational response and specialist operational response to CBRN and HazMat incidents: A primer on decontamination protocols for healthcare professionals. *Emerg. Med. J.* **36**, 117–123.

Chilcott, R. P., Larner, J., Durrant, A., Hughes, P., Mahalingam, D., Rivers, S., Thomas, E., Amer, N., Barrett, M., and Matar, H. (2019b). Evaluation of US federal guidelines (primary response incident scene management [PRISM]) for mass decontamination of casualties during the initial operational response to a chemical incident. *Ann. Emerg. Med.* **73**, 671–684.

Duca, R. C., Hardy, E., Salquèbre, G., and Appenzeller, B. M. (2014). Hair decontamination procedure prior to multi-class pesticide analysis. *Drug Test. Anal.* **6(Suppl. 1)**, 55–66.

Eberhardt, H. (1976). Recoating of human hair by sebum. *J. Soc. Cosmet. Chem.* **27**, 235–239.

Eskenazi, B., Warner, M., Brambilla, P., Signorini, S., Ames, J., and Mocarelli, P. (2018). The Seveso accident: A look at 40 years of health research and beyond. *Environ. Int.* **121**, 71–84.

Feldman, R. J. (2010). Chemical agent simulant release from clothing following vapor exposure. *Acad. Emerg. Med.* **17**, 221–224.

Josse, D., Wartelle, J., and Cruz, C. (2015). Showering effectiveness for human hair decontamination of the nerve agent VX. *Chem. Biol. Interact.* **232**, 94–100.

Kassouf, N., Syed, S., Larner, J., Amlôt, R., and Chilcott, R. P. (2017). Evaluation of absorbent materials for use as ad hoc dry decontaminants during mass casualty incidents as part of the UK's Initial Operational Response (IOR). *PLoS One* **12**, e0170966.

Knorr, F., Lademann, J., Patzelt, A., Sterry, W., Blume-Peytavi, U., and Vogt, A. (2009). Follicular transport route—research progress and future perspectives. *Eur. J. Pharm. Biopharm.* **71**, 173–180.

Lake, W., Schulze, P., and Gougelet, R. (2013). ECBC-SP-036 Guidelines for Mass Casualty Decontamination During a HAZMAT/Weapon of Mass Destruction Incident Volumes I and II. https://www.hsdl.org/?view&did=745138.

Matar, H., Amer, N., Kansagra, S., Pinhal, A., Thomas, E., Townend, S., Larner, J., and Chilcott, R. P. (2018). Hybrid *in vitro* diffusion cell for simultaneous evaluation of hair and skin decontamination: Temporal distribution of chemical contaminants. *Sci. Rep.* **8**, 16906.

Matar, H., Larner, J., Kansagra, S., Atkinson, K. L., Skamarauskas, J. T., Amlot, R., and Chilcott, R. P. (2014). Design and characterisation of a novel *in vitro* skin diffusion cell system for assessing mass casualty decontamination systems. *Toxicol. In Vitro* **28**, 492–501.

Okumura, T., Takasu, N., Ishimatsu, S., Miyanoki, S., Mitsuhashi, A., Kumada, K., Tanaka, K., and Hinohara, S. (1996). Report on 640 victims of the Tokyo subway sarin attack. *Ann. Emerg. Med.* **28**, 129–135.

Power, S., Symons, C., Carter, H., Jones, E., Amlôt, R., Larner, J., Matar, H., and Chilcott, R. P. (2016). Mass casualty decontamination in the United States: An online survey of current practice. *Health Secur.* **14**, 226–236.

Rolland, P., Bolzinger, M. A., Cruz, C., Josse, D., and Briançon, S. (2013). Hairy skin exposure to VX *in vitro*: Effectiveness of delayed decontamination. *Toxicol. In Vitro* **27**, 358–366.

Spiandore, M., Piram, A., Lacoste, A., Josse, D., and Doumenq, P. (2014). Hair analysis as a useful procedure for detection of vapour exposure to chemical warfare agents: Simulation of sulphur mustard with methyl salicylate. *Drug Test. Anal.* **6(Suppl. 1)**, 67–73.

Spiandore, M., Piram, A., Lacoste, A., Prevost, P., Maloni, P., Torre, F., Asia, L., Josse, D., and Doumenq, P. (2017).

Efficacy of scalp hair decontamination following exposure to vapours of sulphur mustard simulants 2-chloroethyl ethyl sulphide and methyl salicylate. *Chem. Biol. Interact.* **267**, 74–79.

Spiandore, M., Souilah-Edib, M., Piram, A., Lacoste, A., Josse, D., and Doumenq, P. (2018). Desorption of sulphur mustard simulants methyl salicylate and 2-chloroethyl ethyl sulphide from contaminated scalp hair after vapour exposure. *Chemosphere* **191**, 721–728.

Swift, J. A., and Smith, J. R. (2001). Microscopical investigations on the epicuticle of mammalian keratin fibres. *J. Microsc.* **204**, 203–211.

Taysse, L., Daulon, S., Delamanche, S., Bellier, B., and Breton, P. (2007). Skin decontamination of mustards and organophosphates: Comparative efficiency of RSDL and Fuller's earth in domestic swine. *Hum. Exp. Toxicol.* **26**, 135–141.

Thors, L., Koch, M., Wigenstam, E., Koch, B., Hägglund, L., and Bucht, A. (2017). Comparison of skin decontamination efficacy of commercial decontamination products following exposure to VX on human skin. *Chem. Biol. Interact.* **273**, 82–89.

UK Home Office. (2015). Initial Operational Response to a CBRN Incident (C. Unit, Ed.). https://www.jesip.org.uk/uploads/media/pdf/CBRN%20JOPs/IOR_Guidance_V2_July_2015.pdf.