Original Contribution

Efficacy of Different Hair and Skin Decontamination Strategies with Identification of Associated Hazards to First Responders

Joanne Larner, Adam Durrant, Philip Hughes, Devanya Mahalingam, Samantha Rivers, Hazem Matar, Elliot Thomas, Mark Barrett, Andreia Pinhal, Nevine Amer, Charlotte Hall, Toni Jackson, Valeria Catalani, Robert P Chilcott

University of Hertfordshire, Research Centre for Topical Drug Delivery & Toxicology, Hatfield, UK

Corresponding author:

Robert P. Chilcott

Department of Pharmacy, University of Hertfordshire, Hatfield AL10 9AB, UK.

Phone: +44 (0)7718 696 629

email: tox.publications@herts.ac.uk

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Abstract

Prompt disrobing and minimization of time to casualty decontamination are key to the effective treatment of individuals exposed to toxic chemicals. Established procedures for mass casualty decontamination that involve the deployment of equipment for showering with water (such as the ladder pipe system [LPS] and technical decontamination) necessarily introduce a short, but critical delay. The purpose of this study was to investigate the effectiveness of dry and wet decontamination approaches (individually and in combination) for removing a chemical warfare agent simulant from the hair and skin of human volunteers. A secondary aim was to quantify potential hazards arising from the decontamination processes. Volunteers were exposed to the simulant (mixture of methyl salicylate, fluorophore [curcumin] and mineral oil) as an aerosol within a custom-built dosing chamber. Three decontamination protocols (dry, LPS and technical decontamination) were applied in various sequences. The efficacy of the protocols was evaluated by whole-body fluorescent imaging and measurement of residual simulant recovered from the hair, skin, decontamination materials and air samples using liquid chromatography and thermal desorption gas chromatography. Dry decontamination before LPS or technical decontamination produced significant reductions in methyl salicylate skin contamination. The greatest reductions were seen with the Triple Protocol (dry, then LPS, then technical decontamination). Secondary sources of contamination (e.g. off-gassing of vapor and residue on wash cloths/towels) decreased following dry decontamination. The introduction of dry decontamination prior to wet forms of decontamination offers a simple strategy to initiate treatment at a much earlier opportunity, with a corresponding improvement in clinical outcomes. Our results confirm the value of a “Triple Protocol” response strategy based on the integration of dry and wet decontamination procedures.

Importantly, we highlight how these combined protocols may reduce toxicological risks downstream in the operational process.

Key words: chemical warfare, decontamination, mass casualty, secondary hazard, human volunteer, emergency response

Introduction

The process of decontamination is known to be time-critical for mitigating the adverse health effects from chemical exposures (1-4). Mass casualty decontamination by civilian emergency services has traditionally been based upon showering with water to dilute and remove noxious chemicals from the skin surface (5). One such approach is "gross decontamination" using the ladder pipe system (LPS), where casualties are sprayed with water between two parallel fire engines (6, 7). Purpose-built (tented) showering systems offer a more sophisticated approach and are used for what is commonly termed "technical decontamination" (2, 8), taking advantage of heated water, options for detergent incorporation, use of washing aids and improved casualty comfort and privacy. The importance of physically removing contaminants during wet decontamination through the provision of washcloths and towels has been previously identified (8). However, the arrival, deployment and organization of such wet decontamination techniques takes time.

Dry decontamination (cleansing of the skin with dry absorbent materials) offers the opportunity to reduce the delay between casualty exposure and the onset of decontamination: previous *in vitro* and *in vivo* studies have demonstrated the value of dry decontamination in removing contaminants prior to the arrival and deployment of more formal decontamination measures (9-11). This has consequently been incorporated in the Initial Operational Response to mass casualty chemical exposure in the UK (12) and its operational and clinical value has been evaluated (13) as part of revised US federal guidance for emergency responders (4). However, assessment of individual and combined procedures under controlled conditions is needed. Furthermore, potential secondary contamination from individuals or their clothing is a long-recognized problem (14-20) and extant guidance emphasizes the importance of appropriate personal protective equipment (PPE) and the need to minimize opportunities for

secondary or cross-contamination at the scene of a chemical incident (4, 6, 21). Whilst there is a lack of evidence-based analysis of the chemical contamination hazards facing emergency responders (22), obvious causes include assisting casualties, handling of potentially hazardous materials (e.g. casualty clothing), inadequate decontamination, inadvertent contact during removal of contaminated PPE, and contact with items (e.g. washcloths and/or towels) used in the decontamination processes. Showering enclosures may also present a significant inhalation risk from the accumulation of potentially toxic chemicals in the atmosphere (23). Therefore, a secondary objective of this study was to evaluate the risk of contamination from casualties and materials used in the different decontamination processes, with a view to evaluating how individual and combined procedures may influence such risks on the scene and during downstream processes.

In the present study, the clinical efficacy of the above three decontamination methods (dry, ladder pipe and technical decontamination), alone and in various sequences, was analyzed following the controlled exposure of human volunteers to an aerosolized, semi-volatile simulant. The simulant, methyl salicylate, was mixed with curcumin, a component of turmeric, for fluorescent traceability and with cosmetic-grade mineral oil to suppress volatility by 50%; this was to ensure consistency with a previously reported large-scale exercise (13). Methyl salicylate is a readily available, semi-volatile chemical that has low toxicity and physicochemical properties similar to those of sulfur mustard. It may thus be used to assess both the dermal exposure of casualties and the potential risks from atmospheric inhalation and secondary exposure. Consequently, it has a long history of use in studies of this type and for the evaluation of protective clothing. (24, 25). The fluorescent property of curcumin enables it to be visualized under near-UV light. Incorporation of such fluorescent tracers into simulants is commonly used for research into washing efficacy and dermal exposure (26, 27).

Methods

Chemicals and Materials

Methyl salicylate (99+%) and curcumin (98%) were sourced from Acros Organics. Johnson's™ Baby Shampoo and Baby Oil were both purchased locally in the UK. Propan-2-ol, methanol, acetic acid, formic acid and acetonitrile (all HPLC grade) were purchased from Fisher Scientific, UK. Deionized water (18.2 MΩcm) was prepared in-house using a MilliQ Integral3 water purifier purchased from Millipore (UK) Ltd. Dosing simulant was prepared by dissolving curcumin in methyl salicylate to produce a 10 mg mL^{-1} solution. This mixture was subsequently diluted 9:1 w:w with Johnson's Baby Oil. The final nominal concentrations of methyl salicylate and curcumin were 1100 and 9 μg mL^{-1}, respectively.

Wound dressings (25 × 75 cm) were purchased from Betty Mills (USA). Disposable cotton towels (80 × 140 cm, Scrummi) were purchased from Fabricmart Ltd. UK, and cotton washcloths (30 × 30 cm, AmazonBasics) from Amazon. Glass jars (125, 250, 500 mL, 1 L and 2 L) were sourced from Fisher Scientific, UK. Glass vials (20 mL, Wheaton) and urine containers (4 L, Urisafe) were sourced from VWR, UK. Cotton pads and cotton buds were purchased locally from Superdrug, UK. Plastic hair swab templates were produced in-house. Syringes (1 mL), syringe filters (PTFE 0.2 μm, Ø 17 mm), autosampler vials (2 mL) and vial inserts (350 μL) were purchased from Fisher Scientific, UK. Crimp caps were sourced from Chromatography Direct, UK. Thermal desorption tubes (Tenax TA 200 mg) were obtained from Markes International Ltd., UK. Video recordings were acquired using multiple cameras (KBA 12005, Kaiser Baas, Australia) on tripods.

Dosing Chamber

A custom-built, enclosed dosing chamber (1.2 m × 1.2 m × 1.8 m, total volume 2.592 m^3) was constructed to expose a seated volunteer to a metered, aerosolized spray of simulant

delivered from a spray gun fitted with a circular nozzle cap (Cobra 1, DeVilbiss UK). The simulant was contained in a kettle pressurized by an air compressor. The stool height was adjusted for each volunteer to ensure consistency of spray delivery from behind and overhead. This manner of administration was designed to reflect the likely scenario of exposure to a spray delivery—e.g. as a result of an explosion. The chamber atmosphere was ventilated for 30 s before the volunteer left the chamber. Before each volunteer was dosed, the simulant delivery per actuation was confirmed gravimetrically.

Ladder Pipe Shower

A custom-built, static showering corridor (7.3 m long) was constructed to deliver water at 50 psi from each of three hoses (2 side, 1 overhead, total rate: 240 L min^{-1}), equivalent to a ladder pipe system using 2 fire engines. Water, maintained at 10°C by a heater chiller (Duratech DURA+19, UK), was pumped from a reservoir tank (16 m^3) via a manifold to the hoses (2.5 inch diameter) and into the corridor using a fire pump (FP750DI-0, Hale, UK). Four-cone spray nozzles (Hy-D, Red Head Brass, LLC, USA) were fitted to each hose.

Technical Decontamination Shower

Technical decontamination consisted of a single-person decontamination tent (SafeFrame3, GRS, UK) fitted with six fixed nozzles (5 side, 1 overhead), each designed to direct a jet of water to the center of the tent. Warmed water (35°C) from a domestic mains supply (approximately 2 bar, flow rate 20 L min^{-1}) was delivered to the tent by a diesel-fired water heater (Porta Heater 75, Hughes Safety Showers UK), powered by a petrol-fueled electrical generator (Honda, Japan).

Fluorescent Photography

A lightproof booth for whole-body photography was constructed. Arrays of near-UV fluorescent LED tubes (T8 Marine Blue, Arcadia Ltd., Surrey, UK) lined one side and the roof of the booth. Digital cameras (Canon 80D, Canon UK) with 18–55 mm and 10–18 mm lenses (EF-S, Canon, UK) fitted with red filters (K&F Concept) were positioned in the center of each array. Cameras were remotely operated from a laptop using Canon software (EOS Utility v3.8.20, Canon, UK). Volunteers stood in the center of the booth for side view and overhead image acquisition, with aperture F5, shutter speed Tv 0.25 and film speed ISO 2000. Spatial calibration images of each volunteer, incorporating a test card, were acquired before the study images.

Open source software (28) was used to analyses images. Each photograph was converted to 8-bit format and the background (un-dosed participant) image data were subtracted before extraction of the red channel data. For overhead images, the individual volunteer's spatial scale was set using calibration results derived from the test card images. For all side-on images, including those of towels and dressings, a fixed scale (26.01 pixels cm^{-1}) calibration was applied, as all objects were photographed at the same distance from the camera. Blanket segmentation threshold limits of 30/255, 20/255 and 120/255 were applied to the side, overhead and dressing/towels image data, respectively. Image noise was reduced by applying a 1-pixel minimum particle size screen. Each image then underwent particle analysis to calculate the total residual contaminated fluorescent area.

Liquid Chromatography UV Analysis

Propan-2-ol extracts of skin/hair and dosing chamber swabs, towels, washcloths and dressings were analyzed by liquid chromatography with diode array detection (LC-DAD) for methyl salicylate. Quantification was performed using a ThermoScientific UltiMate U3000

HPLC system (quaternary pump, autosampler, column oven) and a ThermoScientific U3000 Dionex Diode Array Detector or ThermoScientific Vanquish Diode Array Detector. The column was ThermoScientific HyPurityTM C18 (150 × 2.1 mm, 5 µm internal diameter) with a matching guard column. Instrumentation was controlled via computer running Chromeleon™ version 7.2 software (ThermoScientific). All swab samples were left to extract for 48 hours, after which they were briefly vortex-mixed and syringe-filtered in 1 mL aliquots through 0.2 µm PTFE filters into autosampler vials before being injected (2 µL). The isocratic mobile phase comprised 60% aqueous acetic acid (pH 3) and 40% acetonitrile at a flow rate of 0.4 mL min^{-1}. The column temperature was 30°C and UV detection was at λmax 303 nm.

For each analysis batch, non-matrix calibration standards (0.236–1180 µg mL^{-1}) and quality control samples (0.0474, 3.55 and 829 µg mL^{-1}) were analyzed. Acceptance criteria for accuracy (85–115%) and precision (±15%) were applied. Samples were quantified against a concurrent matrix calibration series diluted from a respective matrix sample spiked with the dosing simulant on the same day of the study and extracted in propan-2-ol (1100 µg mL^{-1}) in the same way as the samples. Unspiked blank matrix samples were also prepared and analyzed concurrently. Amounts of methyl salicylate in propan-2-ol extracts were calculated from predetermined concentrations by adjusting for the volumes of propan-2-ol in each sample.

Thermal Desorption Gas Chromatography-MS

Quantification of air samples taken during technical decontamination was performed using a Markes International TD-100 thermal desorption unit coupled to a ThermoScientific single quadrupole gas chromatography mass spectrometry (GC-MS) system (GC: Trace 1310, MS: ISQ). The GC column was a Durabond-1 MS Ultra Inert column (30 m × 0.25 mm, 0.25 µm

film, Agilent, UK) with helium (99.9999% purity) as carrier gas at a flow rate of 2 mL min^{-1}. All sample tubes were desorbed for 15 minutes at 320°C with a 101:1 split at the thermal desorption focusing trap before injection into the GC column. Samples were recollected into their original desorption tubes to enable repeat analysis. Samples found to be over range underwent reanalysis with a preliminary split (4×) at the tube desorption step prior to the 101:1 trap split. The GC oven temperature was held at 60°C for 1 minute and then ramped to 275°C over 10.75 minutes. Selective ion monitoring for methyl salicylate was by electron ionization for the parent ion (mz 152 ± 0.5) and primary fragment (mz 120 ± 0.5) with ion source temperature 300°C. Maverick™ version 5.2.0 (Markes International Ltd., UK) software was used to control the TD-100, and Xcalibur™ version 3.0.63 (ThermoScientific, UK) software for the GC-MS.

For each analysis batch, standards were prepared and analyzed by spiking freshly conditioned tubes with solutions of methyl salicylate in methanol, producing a nominal standard range of 2.5–1000 ng on tube. Quality control samples were similarly prepared at nominal values of 8, 50, 300 and 800 ng on tube. Two conditioned tubes were run as blanks with each batch. One was spiked with methanol as a solvent blank. Up to 20 samples were run between bracketing QCs to confirm the acceptable accuracy (±15%) and precision (±15%) of the system.

Study Design

This study was conducted in accordance with the principles of the Declaration of Helsinki (2013) and was independently approved by the University of Hertfordshire's Independent Research Ethics Committee. Healthy adult volunteers, male or female, aged between 18 and 60 years were recruited. Prior to their inclusion, all volunteers gave informed consent to their participation in the study, including the taking of photographs. Each volunteer completed a

health questionnaire, which was reviewed by trial staff to exclude those individuals with pre-existing skin conditions, salicylate intolerance, or those who were pregnant or breastfeeding.

The study took place over a total of 6 weekends between June and September 2017 at the University of Hertfordshire's toxicology facility in the UK. Participants were permitted to attend more than one study session provided that there was a one-week washout period between visits. Enrolled participants participated in a total of 115 sessions and at each session were allocated to one of 11 treatment groups (Table 1). At least one volunteer from each treatment group was scheduled to be decontaminated according to their protocol on each study day. Allocation to a protocol was not randomized, but no volunteer who attended on more than one occasion underwent the same treatment group protocol more than once.

Volunteers provided a baseline urine sample (10–50 mL) and were directed to remove all their clothes and don only (non-fluorescent) black briefs. Male and female volunteers were dressed identically. Each volunteer underwent their protocol individually, with preservation of modesty paramount: i.e. attending trial staff were kept to a minimum and were of the same gender as the volunteer when requested. Before and after dosing ("baseline" and "post-dose") and after each decontamination procedure ("post-dry", "post-LPS" and "post-technical"), participants underwent fluorescence photography as described above. A final set of photographs were acquired after skin/hair swabbing ("post-swab"). Materials used during decontamination were also photographed. Images were processed as described above.

Each participant was directed to enter the dosing chamber and sit on a stool facing the door with their hands on their knees. Participants were provided with goggles and a nose-clip to prevent inhalation or eye irritation from the simulant. An airline looped from the ceiling with a disposable mouthpiece provided fresh air. At time T0, the participants were dosed from behind and overhead using a metered spray gun delivering 1 g aerosolized simulant per actuation to mimic a more realistic spray exposure scenario. Dosing comprised two

actuations (total dose 2±0.1 g) completed within 5 seconds. The total doses of methyl salicylate and curcumin introduced to the dosing chamber per volunteer were 1.9 and 0.2 µg, respectively. The dosing of each volunteer was video-recorded to ensure consistency of application and volunteer position. Immediately after dosing, the chamber was ventilated by extraction of chamber atmosphere and replacement with fresh air for 30 s, during which time the volunteer remained in position. After chamber ventilation, the volunteer exited the chamber and a staff member removed the participant's goggles, nose-clip and mouthpiece. The walls of the dosing chamber were immediately sprayed with propan-2-ol and wiped with 10 pre-weighed sheets of blue-roll paper towel. The used blue-roll sheets were placed into a 1 L glass jar and the capped jar weighed.

At 4 min post dose (where applicable) the participant underwent dry decontamination using a wound dressing for 1 min. For the first 10 s they were directed to wipe their face, the next 10 s to wipe their hands, the next 30 s to wipe their bodies, and the final 10 s to wipe their hair. The dry decontamination step was video-recorded to retrospectively assess performance and compliance. The dressing was photographed on both sides in the fluorescence imaging booth before being placed in a 2 L glass jar; the capped jar was then weighed.

At 8 min post dose (where applicable), the participant underwent a ladder pipe system (LPS) shower. The protocol involved each volunteer walking the length of the LPS corridor for 15 s, making a single 360° turn at the point where the three sprays converged. One member of the trial staff counted out the 15 s with the aid of a megaphone; another staff member was present in the corridor with the volunteer to ensure they did not become disorientated or leave the corridor early. The LPS step was video-recorded from both ends of the corridor to identify any deviation in shower duration or protocol. On exiting the corridor (where appropriate), the volunteers were given a disposable cotton towel and instructed to dry

themselves all over for 30 s. The towel was then immediately photographed on both sides in the fluorescence imaging booth before being placed in a 2 L capped glass jar and weighed.

At 12 min post dose (where appropriate), the participants underwent technical decontamination. Each participant was handed a cotton washcloth pre-impregnated with 10 mL Johnson's™ Baby Shampoo and was instructed to enter the showering tent, into which warmed water (35°C) was delivered. The participants were instructed to wash themselves all over for 90 s using the washcloth. The technical decontamination step was video-recorded to retrospectively assess performance. On exiting the tent, each volunteer wrung out the wash cloth and placed it into a 1 L glass jar provided by trial staff, which was then recapped and weighed. The volunteers were given a disposable cotton towel and instructed to dry themselves all over for 30 s. The towel was immediately photographed on both sides in the fluorescent booth before being placed in a 2 L capped glass jar and weighed. Air samples from inside the decontamination tent were collected into freshly conditioned thermal desorption tubes from inside the decontamination tent using a constant volume pump set at 100 mL min^{-1} for 5 mins. A baseline air sample was collected immediately prior to the volunteer's shower (time: 6.5 to 11.5 min post-dose), and a second sample was acquired during and after the shower (time: 11.5 to 16.5 min post-dose).

At 18 min post dose, swab samples were taken from 28 sites on each volunteer (15 on front and 11 on rear of body, 1 on scalp, 1 on hair) **(Figure 1).** Prepared sets of 125 mL glass jars containing 3 cotton pads (n=26 skin sites), 20 mL glass vials containing 5 cotton buds (scalp only) or 250 mL glass vial containing 4 pads (hair only) were weighed before use. Triplicate serial swabs of the skin sites were taken using cotton pads. The first and last swabs were performed using dry pads, whereas the second swab was soaked in propan-2-ol prior to use to ensure effective removal of residual simulant on the skin surface. A single-use plastic template with an 8 cm diameter aperture was applied to each hair site, from which triplicate

serial scalp swabs were taken using cotton buds. The first and last swabs were performed using dry cotton buds, whereas the second swab was moistened in propan-2-ol prior to use. To facilitate access for scalp skin samples, a comb was used to part the hair at the swabbing site before the plastic template was applied. The cotton buds were returned to their original glass vial and capped. The procedure for acquiring hair swabs was similar, except that 4 cotton pads were used (swab 1 and 4 were dry, swabs 2 and 3 were moistened in propan-2-ol).

Propan-2-ol was added to each skin swab (100 mL), hair swab (200 mL), scalp swab (10 mL), dosing chamber blue-roll swab (700 mL), cotton washcloth (700 mL), wound dressing (1500 mL) and disposable cotton towel (1500 mL). All jars were reweighed after the addition of solvent (to confirm the volume of solvent used) before storage in light-proof boxes at ambient temperature prior to analysis.

All samples were left to extract for 48 hours before LC-DAD analysis for methyl salicylate. Quantification was determined against spiked calibration standards, as described above. Air samples captured on Tenax TA thermal desorption tubes from within the technical decontamination tent were analyzed for methyl salicylate content by thermal desorption GC-MS. Quantification was determined against spiked calibration standards, as described above.

Finally, participants were provided with instructions for collecting all urine into a pre-weighed 4 L urine container for the following 24 hours. The volunteers were instructed not to shower for at least 4 hours post dose. The 24 h urine samples were returned by each volunteer the following day and processed the same day for storage in compliance with the requirements of the UK Human Tissue Act (29). Participants were asked at time of return to declare whether they had ingested any medication since dosing and whether samples had been missed from the collection. Baseline and 24 h urine samples were analyzed by LC-MS

for salicyluric acid, the primary metabolite of methyl salicylate. The protocol for each treatment group is summarized in Table 2.

Statistics

Statistical analysis was performed using Prism® v6.07 (Graphpad Software Inc., USA). A normality test (Kolmogorov–Smirnov) was performed on all data and descriptive statistics generated. Where data were not found to be normally distributed, treatment effects were subsequently analyzed using the non-parametric equivalent of a one-way t-test (Mann–Whitney) or one-way ANOVA (analysis of variance; Kruskal–Wallis) followed by Dunn's multiple comparisons post-test. Correlation testing was by the non-parametric Spearman's correlation test.

Results

A total of 48 volunteers (20 male, 28 female, age range 20–60 years) enrolled and participated in the trial. Several volunteers attended more than one session (no more than 6, each 1 week apart), resulting in a total of 115 participant sessions (51 male, 64 female) in total (**Table 1**). All volunteers except one were pale-skinned (Fitzpatrick skin type 1–3) (30).

A one-way ANOVA demonstrated that there was no significant difference in the delivery of simulant into the dosing chamber across the treatment groups. All actuations fell within the dosing acceptance criteria for accurate delivery of 2 g ± 5%. The amount of simulant dispensed was 2.03 ± 0.05 g (mean ± SD), comprising 1.87 g methyl salicylate (range 1.75–1.97 g) and 0.02 g curcumin (range 0.02–0.02 g).

Analysis of skin and hair swabs collected from volunteers for methyl salicylate content showed a consistent pattern of recovery related to their position during dosing (hair > back > arms > scalp > legs > face > chest) with only the back and hair/scalp sites providing quantifiable swab data for most volunteers. Image analysis of side and overhead photographs

of volunteers to determine the total area of fluorescent contamination confirmed the swab analysis data for areas predominantly contaminated with methyl salicylate (**Figure 2**). In general, there was no significant difference in the total area of volunteer contamination across the treatment groups at each photographed stage of activity.

Median recoveries of methyl salicylate from the back, hair and scalp were lower than those from the untreated control group for each of the individual decontamination protocols, although no statistical significance was detectable (**Figure 3**). Dry decontamination before technical decontamination or before LPS (+towel) produced significant reductions in skin contamination. However, the greatest reductions in methyl salicylate contamination were seen with the Triple Protocol. The mean amount of methyl salicylate recovered in back swab samples from volunteers who had undergone dry, LPS (+towel) and technical decontamination was 28.6 µg, compared with 445 µg in controls, indicating a 16-fold reduction in contamination. Mean recovery of methyl salicylate from the hair in control volunteers was 1531 µg compared to 92.8 µg from the underlying scalp (Group 1).

The amounts of methyl salicylate extracted from wound dressings used during dry decontamination (reflecting removal of simulant from the volunteers during the procedure) varied from 2.6 to 73.7 mg. The mean (±SD) amount extracted was 17.7 ± 15.0 mg: no statistically significant difference was observed between the six treatment groups that incorporated the initial dry decontamination, nor were there any significant differences in the total contaminated area on the wound dressings detected by fluorescence photography.

Quantifiable amounts of methyl salicylate were extracted from all towels used after LPS showering (reflecting mechanical removal of simulant from the volunteers by the action of actively drying after a 15 s shower). However, significantly smaller quantities of methyl salicylate were extracted from LPS towels of volunteers who had undergone prior dry decontamination (**Figure 4a**). Fluorescence photography detected no significant differences

between the total areas of contamination on the LPS towels used in LPS and technical decontamination. However, those areas were smaller than those on the wound dressings used for dry decontamination (**Figure 6**).

Methyl salicylate was detected in all propan-2-ol washcloth and towel extracts used during technical decontamination (reflecting mechanical removal of simulant from the volunteers by the action of washing during the shower and actively drying afterwards. Significantly lower quantities of methyl salicylate were detected in washcloths from all the treatment groups who underwent multiple decontamination procedures compared to those who underwent technical decontamination alone (**Figure 5**). A similar trend was observed for recoveries of methyl salicylate from the towels (**Figure 4b**). The amounts recovered were generally lower than those from the LPS towels and the variability in results was also less than with the LPS towels. A qualitatively smaller total fluorescent contaminated area was observable on the technical decontamination towels from those treatment groups who underwent more than one decontamination activity, although this effect was not statistically significant (**Figure 6**).

Analysis of air samples from within the technical decontamination tent before and after showering confirmed a significant reduction in atmospheric methyl salicylate in the tent if the contaminated volunteers had undertaken prior decontamination (either dry or LPS) (**Figure 7**).

Video reviews of dry decontamination, LPS and technical decontamination confirmed that all volunteers were compliant with the study protocol. Dry and technical decontamination scores for percentage body area wiped and intensity of effort for facial, body and hair regions confirmed that areas of the body that were more difficult to reach (i.e. upper back, backs of legs, feet) could be neglected, but concurrent prompting by staff during dry decontamination improved levels of volunteer activity compared to technical

decontamination, where instructions were provided immediately before volunteers entered the showering tent (**Figure 8**).

Analysis of the baseline and 24 h urine collections for salicyluric acid detected the presence of the methyl salicylate metabolite in all samples. However, no significant differences across the treatment groups were detected.

Discussion

This study demonstrates the benefit of combining dry and wet decontamination procedures to limit the chemical exposure of casualties. It also illustrates the importance of the correct handling of materials used in the decontamination process in order to avoid the risk of secondary contamination.

In the present study, individual dry, LPS and technical decontamination methods and various combinations thereof were successfully tested under carefully controlled conditions in order to allow objective evaluation and comparison of their relative efficacy and to limit possible confounding factors. Dosing was demonstrated to be tightly controlled on all study days, with a spray delivery precision of <5%. Individual adjustment of the seat level ensured that the orientation of the spray from behind and overhead was consistent for all volunteers, independently of any height differences. Dosing was therefore deemed considered reflective of a spray exposure and comparisons could subsequently be made between different decontamination protocols. Dry decontamination, LPS and technical decontamination, following current US emergency personnel guidance (4, 31), were then compared to the proposed improved procedures derived from *in vitro* hair and skin decontamination studies (10, 32) and other human volunteer trials incorporating an initial dry decontamination step (12, 13).

All three individual methods achieved qualitative reductions in the median methyl salicylate skin and hair and scalp contaminations (**Figure 3**). However, a combination of two or, ideally, all three methods (the "Triple Protocol") was significantly superior to any single method alone. This was shown not only directly, by the much smaller quantities of methyl salicylate measured in back swabs, but also indirectly, by the significantly smaller amounts of contaminant recovered from the washcloths and towels used in the technical decontamination phase and the lower vapor concentrations of MS in the decontamination unit when protocols were combined. Moreover, the higher (16-fold) relative recoveries of methyl salicylate from hair in relation to scalp skin of control (untreated) volunteers were in good agreement with previous studies, confirming that hair protects the underlying scalp skin (13, 32).

In this study, we used wound dressings for dry decontamination. Our results showed no difference in methyl salicylate recovery from dressings between treatment groups, but this is understandable given that all dry decontamination occurred at the same time post dose and prior to any other treatment. Wound dressings are commonly found in ambulances and are thus likely to be rapidly and readily available at the scene of an incident. Alternative materials, such as incontinence pads and "blue-roll" tissue paper (also normally available in ambulances), have also been found effective for this purpose (10). The importance of active washing with a washcloth introduced with the ORCHIDS protocol (8) was confirmed as a key aspect of technical decontamination.

These findings further document the importance of a combined decontamination strategy utilizing an initial dry decontamination step prior to the deployment and application of wet decontamination procedures. Our findings showed that initial dry decontamination, performed promptly after exposure and in conjunction with subsequent wet decontamination, had a significant and positive effect on the overall success of the decontamination process. This confirms the findings from a large-scale exercise, "Operation Downpour", which used

both ambulant and non-ambulant volunteers to evaluate the operational and clinical effect of incorporating dry decontamination into the US emergency response (13). Areas of fluorescent contamination on towels used for technical decontamination were smaller when individuals had already undergone other decontamination procedures, suggesting that opportunities for earlier decontamination are important to reduce casualty exposure.

With respect to wet decontamination, it is normal practice for LPS to use unheated water supplied directly from a local hydrant. In contrast, technical decontamination utilizes heated water commonly delivered at more comfortable temperatures (~35–40°C). Previous *in vitro* studies on the effect of water temperature on brief showering have indicated that warmer temperatures may be more effective (33), suggesting that the inclusion of technical decontamination is essential, particularly under cold weather conditions where the use of unheated water would be contraindicated because of the risk of hypothermia.

Video scoring suggested better performance intensity and an increase in the total area of body wiped during dry decontamination when compared to technical decontamination. This may reflect the fact that volunteers were prompted to address specific body areas during the 60 s protocol. In contrast, volunteers only received instructions prior to entering the technical decontamination shower. The video evidence demonstrated that hard-to-reach areas were commonly overlooked, i.e. feet, upper back, backs of legs. This underlines the importance of clear and detailed instructions if decontamination is to be performed both effectively and compliantly. Our results therefore support previous studies regarding the need for clear communication and potential additional staffing assistance, particularly when directing “at risk” populations (e.g. those with physical, cognitive or language difficulties), throughout the decontamination process (13).

Whilst analysis of 24 h urine collections confirmed exposure to methyl salicylate, the lack of any significant differences between groups indicated no treatment differences in

systemic exposure. This is most likely attributable to uncontrolled, exogenous sources of salicylates in the volunteers' diet (34).

We consider the prompt initiation of dry decontamination to be applicable to the majority of chemical contamination situations, including those involving caustic or toxic industrial chemicals. The principle of removing as much as possible as soon as possible to mitigate dermal and respiratory exposure and subsequent systemic exposure holds for all chemicals. To date, the approach for caustic exposures has been the prompt flushing of the skin with water (35), but in instances where water is not immediately available, disrobing and blotting of the skin surface should remove excess liquid that could otherwise permeate clothing or cause further skin damage. However, further work is required to confirm the effectiveness of dry decontamination for caustic substances.

From a secondary contamination perspective, our data demonstrate that materials used in the decontamination process constitute a chemical hazard. This applies especially to materials used for dry decontamination, which make the "first contact" with the contaminant. However, if initial disrobe and dry decontamination are performed, then the extent of the hazard from towels and washcloths used in subsequent wet decontamination procedures is substantially reduced. The performance of early dry decontamination does, therefore, reduce the risk of downstream contamination and will reduce the risk of "clean" areas becoming "dirty" during the operational response. However, it would still seem prudent to consider all used towels and washcloths as hazardous materials, even when prior dry decontamination has taken place: all discarded clothing and used decontamination aids should be collected and placed into approved waste containers by response personnel wearing appropriate PPE, prior to disposal according to local rules for hazardous waste.

Importantly, the performance of preliminary dry decontamination and/or LPS was shown to markedly reduce the subsequent volatilization of contaminants from casualties in the

technical decontamination shower. In practice, this will minimize the accumulation of contaminant vapors within the decontamination units and thus mitigate the exposure of casualties, responders and equipment. However, the detectable presence of vapor within the showering unit emphasizes the need to ensure adequate ventilation of such structures during the incident response.

Limitations

The method used for simulant delivery into the dosing chamber precluded an accurate determination of dosage to each volunteer. However, the aerosolized delivery into the chamber was well controlled and preliminary validation (data not shown) confirmed that dosages would be within safe limits (1 g). Furthermore, since the positioning of volunteers and their time spent in the chamber was consistent, any differences in dosing would be minimal and reflective only of volunteer size.

The decontamination protocols were implemented at 4 (dry), 8 (LPS) and 12 mins (technical). We acknowledge that, with the possible exception of dry decontamination, these are not likely to be realistic response times from first responders or emergency services. However, these timings were selected to optimize objective measurements of contamination in order to identify statistically significant differences between treatment groups. In this regard, the data obtained from skin swabs and secondary hazardous materials succeeded in achieving this objective. Previous work by our group has addressed the temporal importance of decontamination (1, 32). Moreover, this present study is in agreement with previous work performed under more realistic environmental conditions and response times (13).

A few volunteers (<10) did not comply with urine collection instructions; either they missed a collection or did not collect over the full 24 h period, as instructed. The urine data from these volunteers were omitted from subsequent statistical analysis.

Conclusions

The results of this study confirm the value of the "Triple Protocol" response for optimizing casualty treatment. Initial dry decontamination was associated with significantly lower levels of contaminant recovered from volunteers during subsequent stages of the response process. Furthermore, prompt initiation of dry decontamination is of clinical benefit in making more effective use of the time delay associated with the operational deployment of wet decontamination assets. Dry decontamination could feasibly be repeated during this period to further improve efficacy and provide a focus for casualties. It is also important to note that the materials used for dry decontamination, as well as discarded clothing, washcloths and towels used in the later phases of decontamination, all represent potential secondary hazards and require careful handling to reduce toxicological risks downstream in the operational process. Incorporation of the dry decontamination procedure, however, would remove a large proportion of contamination before contact with specialist equipment.

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Author Contributions

RPC and JL designed the study. JL, AD, DM, SR and PH conducted the study and performed image and chromatographic sample analysis. HM, MB, ET, AP, NA, CH, VC and TJ assisted

with volunteer enrolment or sample processing on study days. JL reviewed the data, undertook statistical analysis and prepared the manuscript. RPC edited the final version.

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Table 1. Treatment groups for the human volunteer study. Volunteers were randomly allocated to a group, each group having a different decontamination protocol.

	Protocol	**N**	**Sex**
1	Control	10	3 ♂, 7♀
2	Dry decontamination (DD)	11	5♂, 6♀
3	Ladder pipe system (LPS) (no towel)	10	5♂, 5♀
4	Technical decontamination (TD)	10	5♂, 5♀
5	DD & LPS (no towel)	12	7♂, 5♀
6	LPS (no towel) & TD	10	3♂, 7♀
7	DD & TD	12	3♂, 9♀
8	LPS (+towel)	10	4♂, 6♀
9	DD & LPS (+towel)	10	5♂, 5♀
10	DD & LPS (no towel) & TD	10	6♂, 4♀
11	DD & LPS (+ towel) & TD	10	5♂, 5♀

Table 2: Activity timings for treatment groups

		Time (min)														Time (h)
		-30	0	2	4	6	6.5	8	10	11.5	12	14	16.5	18	20	24
	Activity	*BL Urine*	*Dose*	*Image*	*DD*	*Image*		*LPS*	*Image*		*TD*	*Image*		*Swab*	*Image*	*24 h Urine collection*
								BL TD air sample			*Shower TD air sample*					
			Video		*Video*			*Video*			*Video*					
								No towel + towel								
Treatment Group																
1	Control															
2	DD				√	√										
3	LPS (no towel)							√	√							
4	TD						√			√	√	√				
5	DD & LPS (no towel)				√	√		√	√							
6	LPS (no towel) & TD	√	√	√			√	√	√	√	√	√		√	√	√
7	DD & TD				√	√	√			√	√	√				
8	LPS (+ towel)							√	√							
9	DD & LPS (+ towel)				√	√										
10	DD + LPS (no towel) & TD				√	√	√	√	√	√	√	√				
11	DD + LPS (+ towel) & TD				√	√	√	√	√	√	√	√				

Figure legends

Figure 1. Swab samples were taken from 28 sites on each volunteer, 15 on the front and 13 on the rear (including 1 hair and 1 scalp swab).

Figure 2. Example of fluorescent photography from front and back of dosed volunteer. Dark areas indicate contaminated skin areas. Hair contamination is not visible because of the small size of the aerosol particles and their variable depth of deposition upon the hair.

Figure 3. Box and whisker comparison of methyl salicylate recovery from a) back b) hair & c) scalp swabs of volunteers across all treatment groups. Error bars are treatment group min/max, upper and lower box limits reflect 25% and 75% of the response, horizontal line inside box reflects treatment group median, • symbol indicates the treatment group mean value. Asterisks denote significant differences from the respective individual treatment group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Figure 4. Box and whisker plot of methyl salicylate recovered from towels from volunteers who underwent active drying after a) 15 s LPS shower or b) 90 s technical decontamination. Error bars are treatment group min/max, upper and lower box limits reflect 25% and 75% of the response, horizontal line inside box reflects treatment group median, + symbol indicates the treatment group mean value. Asterisks denote significant differences from the respective individual treatment group, *$p < 0.05$, ***$p < 0.001$.

Figure 5. Box and whisker plot of methyl salicylate recovered from washcloths from volunteers who underwent technical decontamination (TD). Error bars are treatment group min/max, upper and lower box limits reflect 25% and 75% of the response, horizontal line inside box reflects treatment group median, + symbol indicates the treatment group mean

value. Asterisks denote a significant difference from the TD only treatment group, ***$p <$ 0.001.

Figure 6. Box and whisker plot of total fluorescent contaminated area on wound dressings used during dry decontamination for 1 minute and towels used by volunteers for drying themselves for 30 s after LPS or technical decontamination. Error bars are treatment group min/max, upper and lower box limits reflect 25% and 75% of the response, horizontal line inside box reflects treatment group median, + symbol indicates the treatment group mean value. Derived values based on the sum of contaminated areas from images of both sides of the materials. Dimensions: dressings 25 × 75 cm, towels 80 × 140 cm. Image segmentation threshold was identical for dressings and towels.

Figure 7. Box and whisker plot of amounts of off-gassed methyl salicylate recovered from volunteers who underwent technical decontamination (TD). Air samples from inside the technical decontamination tent were drawn through Tenax TA desorption tubes at a flow rate of 100 mL min^{-1} for 5 min. Error bars are treatment group min/max, upper and lower box limits reflect 25% and 75% of the response, horizontal line inside box reflects treatment group median, + symbol indicates the treatment group mean value. Asterisks denote significant differences from the TD only treatment group, *$p < 0.05$, ***$p < 0.001$, ****$p <$ 0.0001.

Figure 8. Histograms of averaged video scoring data from dry decontamination (DD, n=50/65) or technical decontamination (TD, n=32/47). Videos of volunteer performance were assessed by three independent reviewers and scored from 1 to 5 for % total body area wiped and intensity of effort at facial, body and head hair sites. Lower scores reflect less effort or

smaller % area of body wiped; high scores reflect more rigorous effort or greater % area of body wiped.