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Studies Supporting the Medical Chemical Defense Program
(Task Order 0020): Evaluation of Decontamination
Solutions for Use on Remains

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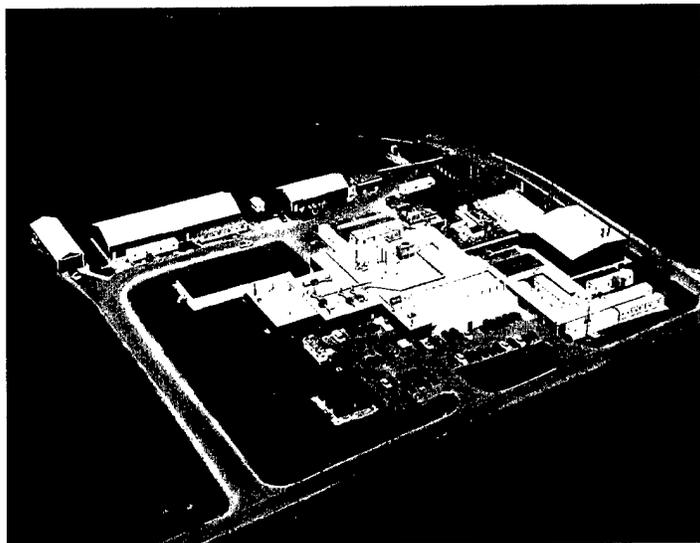
Decontamination Solutions

For Use on Remains

To

U.S. Army Medical Research
Institute of Chemical Defense

March 2004



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Timothy A. Hayes 3-11-04
Program Director Signature Date

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FINAL REPORT

**Contract DAMD17-99-D-0010
A Medical Research and Evaluation Facility (MREF) and Studies
Supporting the Medical Chemical Defense Program**

on

Task Order 0020, Evaluation of Decontamination Solutions for Use on Remains

to

U.S. ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE

March 2004

**Mr. Timothy L. Hayes
Dr. Michael C. Babin
Mr. Thomas H. Snider
Dr. James E. Estep**

**Study Performed by:
Battelle Memorial Institute
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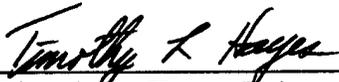
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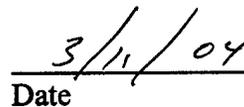
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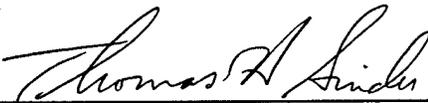
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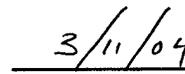
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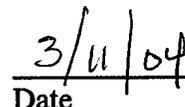
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James E. Estep, D.V.M., Ph.D.
MREF Manager



Date

EXECUTIVE SUMMARY

The objectives of this work were identified by priorities:

- Priority 1: Evaluate the potential decontamination of chemical warfare agents (CA) by three embalming fluids being used by the U.S. military Mortuary Affairs Office. The objective was to determine whether any of the three identified embalming fluids by themselves significantly destroy CA such that they would serve as a decontaminant.
- Priority 2: Use porcine carcass skin to evaluate the decontamination efficacy of (Treatment A) soapy water/2 percent bleach and (Treatment B) DF-200 against topical challenges of HD or VX.
- Priority 3: Evaluate the effect on appearance and integrity of skin with the concurrent use of DF-200 and embalming fluid using porcine skin tissue.

Materials, methods, and results of these studies are detailed in attachments, which indicate that

- Priority 1: Polar Cavity Firminindex[®] 53, Rex 36, and Hexaphene MA64 embalming fluids have limited ability to decontaminate either VX or HD. The materials do increase the destruction slightly, but the decay rate is too slow for them to be considered as adequate decontaminants by themselves.
- Priority 2: Gas chromatography identified no detectable parent compound in any of the 1 inch diameter porcine skin disks exposed to either 30 μ L of VX or 90 μ L of HD and treated with either Treatments A or B.
- Priority 3: DF-200 produced a "bleaching effect" to porcine skin tissue as early as 15 minutes after exposure. The overwhelming majority of DF-200 treated tissues showed moderate tissue differences relative to controls for all three embalming fluids and at all five embalming time points. Two percent bleach resulted in very minor tissue differences for all three embalming fluids at the 24 hour time point. There were no dramatic changes in tissue integrity in all embalmed DF-200 exposed tissues. Both DF-200 and control samples were rubbery in consistency and had the same basic "feel" common to "fixed" tissue.

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Priority 1 Report

Attachment II
Priority 2 Report

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Priority 3 Report

INTRODUCTION

The following task summary report for Task Order 0020 entitled, "Evaluation of Decontamination Solutions for Use on Remains." Is a compilation of reports from the individual work assignments that have been previously reported. The task was initiated to provide information for the evaluation of foam technology as a replacement for the procedures outlined in Joint Publication 4-06, by the US Army Medical Research Institute of Chemical Defense (USAMRICD). The work statements as provided to Battelle by USAMRICD are listed below:

Priority 1 – Embalming Fluid Effects on Chemical Warfare Agent – The objective was to determine if embalming fluids alone would significantly destroy chemical warfare agents (HD and VX) such that it would serve as a decontaminant. This would simplify the decontamination procedure and provide a cost savings to the Army. The study evaluated embalming fluids Rex 36, Hexaphene MA64, and Polar Cavity Firminde[®] 53, as potential decontamination material by assaying the extracts with time.

Priority 2 – Compare DF-200 with Soapy Water/2 Percent Bleach – The objective was to determine if DF-200 was any better than plain soapy water/2 percent bleach as a decontamination treatment. This study tested chemical warfare agents (HD and VX) on pigskin obtained from a slaughterhouse using DF-200 and soapy water/2 percent bleach as decontamination treatments.

Priority 3 – Effect of DF-200 on Skin Integrity under Expected Human Remains Storage Conditions – The objective was to determine if DF-200, in the presence of various embalming fluids, caused cosmetic or structural damage to the skin under the conditions that we would expect human remains to be handled. The concern was that the DF-200 might affect the integrity of the skin resulting in severe damage from normal handling procedures or possible damaging cosmetic effects. The testing procedures were carried out using porcine skin tissue. The skin was exposed to DF-200 and various embalming fluids and stored at room temperature for approximately 1 week. Two concentrations of bleach were also evaluated in the presence of the embalming fluids. No chemical warfare agents were used.

In order to meet the aggressive timeline established by USAMRICD the results of these studies were reported as they were generated in separate reports. Each report has been included in this task summary report as Attachments 1 through 3.

Attachment I
Task 0020 Priority 1

Task 0020 Priority 1

INTRODUCTION

Objective: The purpose of this study was to evaluate the potential decontamination of chemical warfare agents (CA) by three embalming fluids being used by the U.S. military Mortuary Affairs Office. The objective is to determine if any of the embalming fluids by themselves significantly destroy CA such that it would serve as a decontaminant. If so, the decontamination procedure would be simplified. The study was performed as *in vitro* testing with CA placed into small aliquots of embalming fluid and the amount of CA remaining measured over time.

Materials and Methods: The test materials identified as embalming fluids used by the U.S. Army Mortuary Affairs Office are:

Polar (triple base) **Cavity Firminindex[®] 53** manufactured by The Embalmers Supply Co. Stratford, Ct 06615

16 percent Formaldehyde
3 percent Methanol
11.5 percent Phenolic resin
Remainder water

Rex 36 Arterial Index 36 manufactured by Gold Crest Chemical Co. Stratford, Ct 06615
Used diluted 10 oz to 1 gallon per the label.

36 percent Formaldehyde
8.6 percent Methanol
1.2 percent Phenol
Remainder water

Hexaphene MA64 Cavity embalming fluid manufactured by Gold Crest Chemical Co. Stratford, Ct 06615

18 percent Formaldehyde
8 percent Methanol
1.6 percent Ethylene Glycol
0.6 percent Carbopol 934 (acrylic acid based polymer)
Remainder water

The test was conducted by spiking 5 mL of each material contained in a glass vial with 35 μ L HD or 6 μ L VX. Following the spiking, each vial was vortex mixed for approximately 30 seconds and then allowed to stand at room temperature for either 30 minutes or 4 hours. After the contact time, agent was extracted with 5 mL of methylene chloride. Each sample test matrix was prepared in triplicate. In addition to the embalming fluids, a separate control sample for each agent was prepared by spiking 5 mL methylene chloride with either 35 μ L HD or 6 μ L VX.

In addition, a triplicate set of aqueous recovery controls were prepared by spiking 5 mL HPLC grade water with either 35 µL HD or 6 µL VX and then extracting with methylene chloride.

The spiked samples for each test material, including aqueous controls, were extracted using 5 mL methylene chloride except for Hexaphene, which had to be extracted with hexane since an emulsion was formed with methylene chloride. The methylene chloride/Hexaphene emulsion could not be separated with salting or centrifugation.

Results and Discussion: The extracts from all samples were analyzed by gas chromatography and the results are shown in Table 1.

Table 1 Results of Task 0020, Priority 1

Chemical Agent HD				
Test Article	% Remaining after 30 minutes	%CV	% Remaining after 4 hours	%CV
Rex 36	90%	3%	75%	2%
Polar Cavity Firminindex® 53	52%	8%	36%	4%
Hexaphene MA64	21%	76%	21%	17%
HPLC H ₂ O	94%	N/A	49%	N/A

Chemical Agent VX				
Test Article	% Remaining after 30 minutes	%CV	% Remaining after 4 hours	%CV
Rex 36	54%	4.5%	49%	3%
Polar Cavity Firminindex® 53	51%	11%	48%	6%
Hexaphene MA64	11%	9%	10%	17%
HPLC H ₂ O	78%	N/A	64%	N/A

Conclusions: The three embalming fluids have limited ability to decontaminate the CA tested. The materials do increase the destruction slightly, but the decay rate is too slow for them to be considered as adequate decontaminants by themselves.

Attachment II

**Task 0020 Priority 2:
Evaluation of Soapy Water/2 Percent Bleach and DF-200
As Decontaminants of HD or VX on Porcine Skin**

**Task 0020 Priority 2: Evaluation of Soapy Water/2 Percent Bleach and DF-200
As Decontaminants of HD or VX on Porcine Skin**

Objective: The objective of this study was to use porcine carcass skin to evaluate the decontamination efficacy of soapy water/2 percent bleach (Treatment A, Group 1 in the protocol) and DF-200 (Treatment B, Group 2 in the protocol) against topical challenges with HD or VX.

Materials and Methods: These tests were conducted in accordance with MREF Protocol 284 (Attached) over two consecutive test days, one with HD and one with VX. A section, approximately 12x12 inch square, of full-thickness porcine skin with subcutis attached was obtained from a local butcher and refrigerated overnight in a sealed plastic bag. On the morning before the first test day, the section was clipped of hair, and the subcutis removed using a scalpel. On each test day, 24 - 1 inch diameter disks were cut from the section using a nylon board, arch punch, and hammer. Each disk was placed onto a gauze pad wetted with normal saline in a glass petri dish and covered for storage. For dosing, alternating disks were either placed on a wire basket inverted in a 2 L bucket (Treatment A) or placed in an empty glass jar (Treatment B). For the study involving sulfur mustard (HD) challenges, each disk was dosed with 90 μ L of HD (91.5 percent pure, or 105 mg total dose). This volume was determined from the first replicate disk to be sufficient to completely cover the skin disk. For the study with VX, the volume needed was 30 μ L (90.8 percent pure, or 27.5 mg total dose) per disk. Treatments began after a 2 minute exposure with either Treatment A: a 10 mL rinse from a syringe (no needle) with soapy water and placement into a jar containing 50 mL of two percent bleach (n = 12), or Treatment B: addition of 50 mL of DF-200 to the jar (n = 12). The jars were capped and left undisturbed for 1 hour. Then each disk was removed and placed into a new glass jar containing 25 mL of chloroform. The jars were capped and left undisturbed overnight for agent extraction. On the next day, a sample was transferred from each extraction jar into a gas chromatography (GC) vial for analysis. Standard concentration curves ranged from 0.279 to 4.0 mg/mL for HD and from 0.046 to 0.92 mg/mL for VX.

Results: For each sample, regardless of challenge agent or treatment, gas chromatography identified no detectable parent compound in the chloroform.

Discussion: Both 1 hour treatments were effective in decontaminating a 2 minute maximum exposure to HD and VX such that there was no detectable parent agent remaining on or in the skin. Absence of parent agent, however, does not assure no contact hazard remaining due to oxidation products of HD and VX.

APPENDIX A
MREF Protocol 284

Study performed by

Battelle Memorial Institute
Medical Research and Evaluation Facility (MREF)
505 King Avenue, Building JM-3, Columbus, Ohio 43201-2693

Study Title:

**Evaluation of Soapy Water/2 Percent Bleach and DF-200
As Decontaminants of HD or VX on Porcine Skin**

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I. Nontechnical Synopsis

Scope: The preparation of cadavers resulting from lethal exposure to chemical agents (CA) on the battlefield includes thorough skin decontamination prior to transfer in body bags. There may be more effective alternatives than the current practice of using soapy water and a 2 percent aqueous solution of sodium hypochlorite (bleach). The U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) is investigating the decontamination efficacy of Modec Decon Formulation 200 (DF-200) as an alternative decontaminant against 2,2'-dichlorodiethyl sulfide (sulfur mustard; HD) and O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX). This task uses porcine carcass skin to evaluate rinsing with soapy water followed by soaking in 2 percent bleach versus just soaking in DF-200 for removing any hazard due to contact with HD- or VX-contaminated skin.

II. Background

Study Rationale: Information is needed on how well soapy water/2 percent bleach and DF-200 each remove HD and VX from cadaver skin, and this protocol provides a model for making those assessments.

III. Objective/Hypothesis

The objective of the proposed research is to use porcine carcass skin to evaluate the decontamination efficacy of (1) soapy water/2 percent bleach and (2) DF-200 against topical challenges of HD or VX.

IV. Military Relevance

An important mission of the Military Mortuary Affairs Units is to search for, recover, and prepare for transfer any military members lost as a result of combat. This includes ensuring that the skin surface is free of lingering CA that would pose a threat to handlers, shipment recipients, and funeral participants. These studies are needed to determine whether the use of DF-200 can replace the current method for decontaminating casualties.

V. Materials and Methods

A. Test Materials

1. Test Articles

- a. DF-200 is supplied by USAMRICD or its designee. It is the responsibility of USAMRICD to ensure that appropriate identification (batch number and/or lot number), expiration date (if available), safety data, and storage requirements are supplied for DF-200 received by Battelle. DF-200 is prepared on the day of use according to manufacturer's instructions.
- b. Bleach is prepared at Battelle and verified by chlorine analysis to be between 1.975 and 2.025 percent (w/v) sodium hypochlorite in distilled water.
- c. "Soapy water" is Tincture of Green Soap USP (vegetable soap, lavender oil, glycerin, and ethanol) diluted 1:100 in distilled water.

2. HD and VX are supplied by USAMRICD. CA purity and appropriate identification data (batch number, lot number) are supplied by USAMRICD. Battelle periodically confirms the purity of CA, and these data are archived in the facility records.
3. Surety, security, and safety procedures for the use of CA are thoroughly outlined in facility plans, in personnel requirements for qualification to work with agents, and in agent storage and use SOPs. All safety procedures given in Battelle SOP MREF. I-002, "Standard Operating Procedure (SOP) for the Storage, Dilution, and Transfer of GA, GB, GD, GF, TGD, VX, HD, HL, HN, and L When CA Concentration/Quantity is Greater Than Research Dilute Solution (RSD)", are observed during handling and dosing of CA. References to specific SOPs have been included in this document to ensure the safety of the personnel conducting this experiment.
4. Full-thickness porcine dorsal skin is obtained from a local slaughterhouse and stored in a refrigerator at approximately 4 C prior to use.

- B. Application of CA to Skin: The application of a CA onto porcine dorsal skin, and the subsequent handling and decontamination of skin samples, is performed according to

Battelle SOP MREF.II-012, "Percutaneous Application of Either Liquid or Vaporous G and V Agents onto Intact or into Dermal Lesions on Rabbit, Hairless Guinea Pig, or Porcine Skin, and Either Liquid or Vaporous HD, L, HL, and HN Agents onto Intact Rabbit, Hairless Guinea Pig, or Porcine Skin to Test Defensive Methods/Materials". During CA application and throughout the exposure period for each test, skin disks are positioned inside chemical fume hoods approved for use with CA.

C. The Porcine Skin Decontamination Model

1. Test Groups and Allocation of Skin Disks: HD and VX are used on consecutive test days. Each test day consists of two treatment groups of 12 disks each. For each CA, the groups are defined in the following table:

Group	Sample Size (Skin disks)	CA Dose Volume	Decontaminant
1	12	To be determined	Soapy water/2% Bleach
2	12	To be determined	DF-200

The CA dose may be different for each CA and is the volume (determined when the first disk is dosed) needed to saturate the surface without runoff.

2. Skin Disk Preparations: On each test day, 24 approximately 2.5-cm diameter disks of skin are cut with a hammer and arc punch, and each disk is placed on a normal saline-soaked 4x4-in gauze pad in a petri dish. All petri dishes remain outside the dosing hood. Just before CA application, the petri dish is uncovered and the disk transferred, using forceps, into the chemical fume hood.
 - a. Group 1: Each disk is placed in turn on an inverted metal basket in a 2-L beaker.
 - b. Group 2: Each disk is placed in a separate glass jar.
3. Topical Application of CA
 - a. Dosing Device: CA may be applied from a gas-tight syringe with a blunt-tipped needle.
 - b. Dosing Procedures: The dose volume is applied at the center of each disk. Droplets of CA that may form during and at the end of extruding the dose

are touched to the disk to prevent dripping. If a droplet of CA remains on the end of the needle, the needle may be brought down close to the skin surface so as to "wick" off the droplet.

- c. Dose Confirmation: Two dose confirmation samples may be prepared for each test day. The volume of CA used is that applied on each skin disk, delivered into each of two volumetric flasks containing hexane. Each volumetric flask is then filled to the quantity-contained line with hexane and capped. After mixing, the volumetric flask is uncapped, and two, approximately 1-mL samples are aliquoted from each flask into separate gas chromatography (GC) vials. These samples are analyzed by GC to confirm the amount of CA dosed.
 - d. Exposure Period: Decontamination is initiated on each disk at approximately 5 min after CA application; however, a different time interval may be used if specified by USAMRICD.
4. Decontamination with Test Articles
- a. Group 1: Approximately 10 mL of soapy water is dispensed from a disposable syringe (no needle) onto the skin surface. The disk is held vertically on the platform using forceps until surface runoff is complete and then transferred to a jar containing approximately 50 mL or other, USAMRICD-specified, volume of 2 percent bleach. The jar is then sealed.
 - b. Group 2: Approximately 50 mL or other, USAMRICD-specified, volume of DF-200 is placed in a jar, which is then sealed.
 - c. Decontamination Period: Each skin disk remains submerged in decontaminant for approximately 1 hr. Then each jar is opened, the disk removed with forceps and transferred to a second jar containing approximately 25 mL of chloroform or other suitable solvent, and the second jar sealed.
5. Assay for CA After Treatment: Skin disks remain in solvent overnight or for another, USAMRICD-specified, period. Solvent samples are collected from each jar and prepared for assay by chromatography. Assay concentrations for each disk are entered into a spreadsheet that calculates both the total mass of CA

remaining and the amount remaining as a proportion of the mass applied on the disk (percent of dose, %D).

6. Disposal of Fluids: All skin disks and fluids from the 2-L beaker and decontamination jars are placed into beakers containing decontamination solution in accordance with SOP MREF. I-002. After assay, all used solvent is disposed of in accordance with SOP MREF. I-002.

D. Data Analysis

1. Parameters: For each CA, statistical contrasts between treatment groups are performed using %D.
2. For each CA, %D is tabulated by treatment group and replicate disk, and descriptive statistics calculated.
3. Analytical Tests: For each CA, an F test is applied to determine whether the %D data exhibit the same variance between treatment groups, and the appropriate Student's t test (equal variance or unequal variances) applied to determine whether DF-200 is superior to soapy water/2% bleach.

VI. Records to be Maintained

- A. Test and control articles inventory.
- B. Dosage preparation and administration of test articles and CA.
- C. Decontamination, monitoring, and disposal records.
- D. Data calculations and statistical analyses.

VII. Final Report: Battelle will prepare a final report within 30 workdays of receiving USAMRICD comments on a draft final report.

VIII. Biohazard Safety: Surety, security, and safety procedures for the use of chemical agents are thoroughly outlined in facility plans, in personnel requirements for qualification to work with CA, and in standard operating procedures for storage and use of CA.

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IX. Assurances: As the primary investigator on this protocol, I provide the following assurances:

- A. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

 11/05/02
Study Director Date

BUSINESS SENSITIVE

II-A-8

Attachment III

Priority 3

**Effect Of DF-200 On Skin Integrity Under Expected
Human Remains Storage Conditions**

EXECUTIVE SUMMARY

The preparation of cadavers resulting from lethal exposure to chemical agents (CA) on the battlefield includes thorough skin decontamination prior to transfer in body bags. There may be more effective alternatives than the current practice of using soapy water and a 2 percent aqueous solution of sodium hypochlorite (bleach). The U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) is investigating the decontamination efficacy of Modec Decon Formulation 200 (DF-200) for 2,2'-dichlorodiethyl sulfide (sulfur mustard; HD) and O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX). This task evaluates the effect on appearance and integrity of skin with the concurrent use of DF-200 and embalming fluid using porcine skin tissue.

Porcine skin was used to test the effects of DF-200 when used in conjunction with three different embalming fluids: Rex 36 Arterial Index 36 embalming fluid, Hexaphene MA64 Cavity embalming fluid and Polar Cavity Firmindex[®] 53. For each embalming fluid, tissue exposed to embalming fluid alone was compared to embalming fluid plus a 2 h tissue exposure to DF-200. In addition, different embalming fluid fixation times for each fluid (3, 6, 12, 24, and 48h) were evaluated.

DF-200 "bleached out" the porcine skin at the 2 hour time period resulting in significant changes in skin appearance. At every embalming time point, there was some cosmetic change in the DF-200 treated tissue with the overwhelming majority of tissues having a VOS score of 3 indicating moderate tissue differences relative to its control tissue.

A time course (15, 30, 45 and 60 min) for porcine skin exposed to DF-200 was performed. At 15 minutes, there was a noticeable "bleaching effect" to the skin tissue. By 30 minutes, the "bleaching effect" was almost maximal with little change seen from the 45 and 60 minute samples.

In a separate 24 hour experiment, water, 0.5 percent bleach and 5 percent bleach was also tested with each embalming fluid (n=1) to evaluate tissue appearance and integrity. The experiment was conducted under the same parameters as the DF-200 experiment only with water and bleach being used as the decon material. Water, as expected, resulted in significant tissue dehydration and shrinkage after 24 hour. The 5 percent bleach defatted and reduced the size of the tissue samples. The 0.5 percent bleach appeared to have very little cosmetic changes relative

to its control under all 3 embalming conditions. Tissue Integrity Evaluation (TIE) score results were "NO" for all treated and control groups.

In all embalmed DF-200 exposed tissues, there were no dramatic changes in tissue integrity from corresponding control tissues. Each embalming fluid caused a characteristic coloring of the tissue that remained throughout the experiment. The Polar Cavity Firminindex® 53 produced a "grainy" texture on the surface of the DF-200 treated tissue that was not seen in the controls or in the other embalming fluids.

Tissue integrity was assessed 6 days (Day 7) after exposure to embalming fluid. No tissue embalmed in Rex 36 embalming fluid for any of the 5 time periods resulted in any visible tissue alterations in the TIE scoring. For the Hexaphene MA64 Cavity embalming fluid, 47 percent of the controls and 40 percent of the DF-200 treated samples cracked when the tissue was bent. For the Polar Cavity Firminindex® 53, 27 percent of controls and 13 percent of DF-200 samples cracked when the tissue was bent. No tissue exposed to any of the embalming fluids resulted in any observable further tissue disruption while twisting.

Porcine skin embalmed tissues at all exposure time points for both DF-200 and control samples were rubbery in consistency and had the same basic "feel" common to "fixed" tissue. There was no dramatic skin integrity difference observed in tissue treated with DF-200 relative to its control tissue. There was however a significant "bleaching" of the skin secondary to DF-200 exposure that seems to be related to the time of exposure.

In conclusion, for all embalming fluids, embalming times, and observation days, at least minor differences were observed in VOS between pairs of DF-200 treated and control tissues, with most comparisons scored as 3. Therefore, it can be concluded in general that clinically significant differences were observed between DF-200 treated and control tissues. For Rex 36 Arterial Index 36 embalming fluid, the effects of embalming time ($p=0.0001$) and length of follow-up ($p=0.0158$) and their interaction (0.0020) were statistically significant. These effects were evident for the 24 hour embalming time on days 2 and 3, where less substantial differences were observed between the treatment and control samples. For Hexaphene MA64 Cavity embalming fluid, the model could not be fit, as all scores were identical and equal to 3 for all embalming times and observation times. For Polar Cavity Firminindex® 53, the effects of

embalming time and length of follow-up were not statistically significant at the 0.05 level. There were no significant differences in any of the embalming fluids for any time points in the TIE.

Effect of DF-200 on Skin Integrity under Expected Human Remains Storage Conditions

1.0 INTRODUCTION

The preparation of cadavers resulting from lethal exposure to chemical agents (CA) on the battlefield includes thorough skin decontamination prior to transfer in body bags. There may be more effective alternatives than the current practice of using soapy water and a 2 percent aqueous solution of sodium hypochlorite (bleach). The U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) is investigating the decontamination efficacy of Modec Decon Formulation 200 (DF-200) for 2,2'-dichlorodiethyl sulfide (sulfur mustard; HD) and O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX). This task evaluates the effect on appearance and integrity of skin with the concurrent use of DF-200 and embalming fluid using pig and harvested human skin tissue.

2.0 MATERIALS AND METHODS

2.1 Test Materials (Table 1)

- A. Modec Decon Formulation 200 (DF-200, Modec Inc., Denver, CO) was supplied by USAMRICD or its designee. It was the responsibility of USAMRICD to ensure that appropriate identification (batch number and/or lot number), expiration date (if available), safety data, and storage requirements were supplied. DF-200 was prepared on the day of use according to manufacturer's instructions.
- B. Rex 36 Arterial Index 36 Embalming fluid (Gold Crest Chemical Company, Stratford, CT). Rex 36 was diluted 10 oz to a gallon of water and used according to directions from the sponsor.
- C. Hexaphene MA64 Cavity embalming fluid (Gold Crest Chemical Company, Stratford, CT). Hexaphene was used undiluted according to sponsor specifications.

D. Polar Cavity Firminindex[®] 53 (The Embalmer's Supply Company, Stratford, CT). Polar Cavity Firminindex[®] 53 embalming fluid was used undiluted according to sponsor specifications.

2.2 Experimental Design

Porcine skin was used to test the effects of DF-200 when used in conjunction with three different embalming fluids: Rex 36 Arterial Index 36 embalming fluid, Hexaphene MA64 Cavity embalming fluid and Polar Cavity Firminindex[®] 53. For each embalming fluid, tissue exposed to embalming fluid alone was compared to embalming fluid plus a 2 hour tissue exposure to DF-200. In addition, different embalming fluid fixation times for each fluid (3, 6, 12, 24, and 48h) were evaluated.

2.2.1. Preparation of porcine skin samples

Porcine skin was transported in a cooler from the abattoir and refrigerated overnight at approximately 4C. Ninety, approximately 2.5-cm diameter disks of skin (Appendix A) were cut with an arc punch, and each disk placed in a 2 oz. labeled covered wide-mouth glass jar. Adjacent pieces of tissue were used for control and treatment samples to reduce variation due to sample location. Only light colored porcine skin was available.

2.2.2 Treatment Group

Porcine skin tissue samples were placed into wide mouth 2 oz. glass jars, 20 mL of DF-200 added, the jars covered with lids and the samples left undisturbed for 2 hours (Day 0). The DF-200 was then poured off, the jars recapped and the tissues refrigerated overnight at approximately 4C. The next day (Day 1), skin samples were exposed to 5 different fixation times to each embalming fluid. At the end of each fixation time, embalming fluid was poured from the containers. The containers were re-covered, stored at room temperature and the tissues evaluated for appearance and integrity at the scheduled observation times.

2.2.3 Control Group

For the control group, skin tissue samples were not covered in DF-200 for 2 hours but were left in the hood for a 2 hour period in a covered 2 oz. jar (Day 0). The covered jars were then refrigerated overnight at approximately 4C. The next day (Day 1), skin samples were exposed to 5 different fixation times to each embalming fluid. At the end of each fixation time, embalming fluid was poured from the container. The containers were re-covered, stored at room temperature and the tissues evaluated for appearance and integrity at the scheduled observation times.

2.2.4 Evaluation

Each of the three embalming fluids groups were prepared in the same manner. Pictures of each control and treatment group skin were taken prior to exposure to DF-200 (day 0), and on days 1, 2, 3, 6 and 7. The appearance of skin samples was described daily by a scoring system (Table 1). Comments about the appearance were recorded when the treatment VOS was different than its associated control VOS. On the last day of the experiment (Day 7), each tissue sample was evaluated for integrity (TIE) by bending and twisting (Table 2).

2.2.5 Disposal of Fluids

All skin samples, DF-200 and embalming fluids were placed into waste containers and disposed of in accordance with Battelle SOPs.

Table 1 Test Material Labels

Test Materials	Label
Rex 36 Arterial Embalming fluid	A
Hexaphene MA64Cavity embalming fluid	B
Polar Cavity Firminindex [®] 53	C

Table 2 Visual Observation Score (VOS)

Score	Tissue Observation*
0	No observable tissue differences
1	Very minor tissue differences seen
3	Moderate tissue differences seen
5	Severe tissue differences seen

*VOS scoring was relative to the control tissue at each observation time.

Table 3 Tissue Integrity Evaluation (TIE)

Score	Tissue Bending	Tissue Twisting
Yes	Loss of tissue integrity (cracking/ breaking etc) while bending.	Loss of tissue integrity (cracking/ breaking etc) while twisting.
No		

2.2.6 Statistical Analysis

Study Design: Three pairs of porcine skin samples were tested at each of five embalming times (3, 6, 12, 24, and 48 hours), for each of 3 embalming fluids. Within each pair, one skin sample was treated with DF-200 decontamination fluid for 2 hours and the other control sample was not treated. Both samples were refrigerated overnight, then treated with embalming fluid for the specified time interval. Visual observation scores (VOS, Table 2) that compared the DF-200 treated sample to the matched control sample were recorded on day 0 prior to DF-200 treatment, day 1 prior to embalming fluid treatment, and days 2, 3, 6, and 7. The 48 hour embalming groups were not observed on day 2, as the embalming treatment was not complete. On day 7, the samples were evaluated for tissue integrity when bent or twisted. VOS were defined as follows: 0= No observable difference seen; 1= Very minor observable difference seen; 3= Moderate to serious observable difference seen; 5= Severe tissue destruction. Tissue integrity was evaluated as yes/no for loss of tissue integrity (cracking/breaking, etc) while bending or twisting.

Statistical Methods: A nonparametric analysis of variance (ANOVA) technique was used to evaluate the effects of embalming time and length

of follow-up on visual observation scores. The nonparametric method entailed fitting a standard parametric model to the ranks of the VOS data. A separate model was fitted to the data for each embalming fluid. McNemar's test was used to assess for an association in tissue integrity evaluations between pairs of skin samples. Because the sample size was small, the data for all embalming times was pooled for the tissue integrity evaluation. All statistical analyses were conducted using the RANK, GLM, and FREQ procedures in Version 8.2 of SAS.

2.2.7 Missing Value Handling

All tissues used in this study were individually identified and accounted for at the conclusion of the study.

2.2.8 Additional unplanned experiments

Because of the unexpected "bleaching effect" in the treated tissue, two unplanned experiments were conducted with the remaining unused porcine skin. A time course (15, 30, 45 and 60 minutes) for porcine skin exposed to DF-200 was performed. Briefly, 1 mL of DF-200 was placed on the dorsal surface of approximately 2 square inch pieces of porcine skin (n=1). The DF-200 was removed at time intervals of 15, 30, 45 and 60 min. Pictures were taken after the last time point.

In a separate 24 hour experiment, water, 0.5 percent bleach and 5 percent bleach was also tested with each embalming fluid (n=1) to evaluate tissue appearance and integrity. The experiment was conducted under the same parameters as the DF-200 experiment with water and bleach being used as the decon materials (n=1).

3.0 RESULTS

3.1 Statistical Analysis (APPENDIX G)

For all embalming fluids, embalming times, and observation days, at least minor differences were observed between pairs of DF-200 treated and control tissues, with most comparisons scored as 3. Therefore, it can be concluded in general that clinically significant differences were observed between DF-200 treated and control tissues (APPENDIX G).

For embalming fluid A, the effects of embalming time ($p=0.0001$) and length of follow-up ($p=0.0158$) and their interaction (0.0020) were statistically significant. These effects were evident for the 24 hour embalming time on days 2 and 3, where less substantial differences were observed between the treatment and control samples. For embalming fluid B, the model could not be fit, as all scores were identical and equal to 3 for all embalming times and observation times. For embalming fluid C, the effects of embalming time and length of follow-up were not statistically significant at the 0.05 level. There were no significant differences in any of the embalming fluids for any time points in the Tissue Integrity Evaluation (TIE).

3.2 Data Summation and Observations

A summation of VOS and TIE scores are presented in Tables 4, 5 and 6.

Table 4 Visual Observation Score (VOS) Summary For Porcine Skin Tissues Exposed To 3 Different Embalming Solutions. VOS Scores Represent An Observed Difference In The DF-200 Treated Tissues Relative To The Untreated Control Tissues.

Observation Day	Rex 36		Hexaphene MA64		Polar Cavity Firminex [®] 53	
	VOS	% of total samples	VOS	% of total samples	VOS	% of total samples
0	0	100	0	100	0	100
1	3	100	3	100	3	100
2	1	17	3	100	3	100
	3	83				
3	1	13	3	100	1	13
	3	87			3	87
6	3	100	3	100	1	13
					3	87
7	3	100	3	100	1	7
					3	93

Table 5 Observation Day 7 Tissue Integrity Score (TIE) Summary For Porcine Skin Tissues Exposed To 3 Different Embalming Solutions. A "Yes" Score Indicates That The Tissue "Cracked" Or Was "Compromised" In Some Manner As A Result Of The Applied Test Procedure.

		Tissue Integrity Score					
		Rex 36		Hexaphene MA64		Polar Cavity Firminex [®] 53	
Test	Sample	Score	No. of samples tested	Score	No. of samples tested	Score	No. of samples tested
Bending	Control	No	100	Yes	47	Yes	27
	DF-200 Treatment	No	100	Yes	40	Yes	13
Twisting	Control	No	100	No	100	No	100
	DF-200 Treatment	No	100	No	100	No	100

Table 6A Visual Observation Score (VOS) Summary For Porcine Skin Tissues Exposed To 3 Different Embalming Solutions after Decontamination with Water or Bleach. VOS Scores Represent An Observed Difference In The Decontaminated Tissues (Water Or Bleach) Relative To The Untreated Control Tissues.

		Visual Observation Score					
		Rex 36		Hexaphene MA64		Polar Cavity Firminindex® 53	
Time Point	Sample	Score	No. of samples tested	Score	No. of samples tested	Score	No. of samples tested
24 hours	Control	0	1	0	1	0	1
	Water	1	1	1	1	1	1
	0.5% bleach	1	1	1	1	1	1
	5.0 % bleach	3	1	3	1	3	1

Table 6B Day 7 Tissue Integrity Score (TIE) Summary For Porcine Skin Tissues Exposed To 3 Different Embalming Solutions And The Indicated Decon (Water, Bleach). A Yes Or NO Score Indicates That The Tissue Did Or Did Not "Crack or Break" As A Result Of The Applied Test Procedure.

		Tissue Integrity Score					
		Rex 36		Hexaphene MA64		Polar Cavity Firminindex® 53	
Test	Sample	Score	No. of samples tested	Score	No. of samples tested	Score	No. of samples tested
Bending	Control	NO	1	NO	1	NO	1
	Water	NO	1	NO	1	NO	1
	0.5% bleach	NO	1	NO	1	NO	1
	5.0% bleach	NO	1	NO	1	NO	1
Twisting	Control	NO	1	NO	1	NO	1
	Water	NO	1	NO	1	NO	1
	0.5% bleach	NO	1	NO	1	NO	1
	5.0% bleach	NO	1	NO	1	NO	1

Unexpectedly, the DF-200 "bleached out" the porcine skin at the 2 hour time period resulting in significant changes in skin appearance (Table 4). At every embalming time point, there was some cosmetic change in the tissue with the overwhelming majority of tissues having a VOS score of 3 (moderate tissue differences seen). There were only a few tissues that had a VOS score of 1 (very minor tissue differences seen). There were no tissues with a VOS score of 0 (no changes seen between control and DF-200 treated tissue).

A time course (15, 30, 45 and 60 minutes) for porcine skin exposed to DF-200 was conducted to determine a more appropriate exposure time to the decon. Even at 15 minutes, there was a noticeable "bleaching effect" to the skin tissue. By 30 minutes, this "bleaching effect" was almost maximal with little change seen from the 45 and 60 minute samples (Appendix A).

In an additional 24 hr experiment, water, 0.5 percent bleach and 5 percent bleach was also compared to DF-200 but using only 1 porcine skin sample for each decon. The experiment was conducted under similar parameters as the DF-200 experiment only with water and bleach being used as the decon material. Water, as expected, resulted in significant tissue dehydration and shrinkage after 24 hour. The 5 percent bleach defatted and reduced the size of the tissue samples. The 0.5 percent bleach appeared to have very little cosmetic changes relative to its control under all 3 embalming conditions (Table 6A and APPENDIX E). TIE score results were "NO" for all treated and control groups (Table 6B).

In all embalmed DF-200 exposed tissues, there were no dramatic changes in tissue integrity from corresponding control tissues. Each embalming fluid caused a characteristic coloring of the tissue that remained throughout the experiment. The Polar Cavity Firminde[®] 53 (brownish in color) resulted in the most dramatic color changes. The Polar Cavity Firminde[®] 53 also produced a "grainy" texture on the surface of the DF-200 treated tissue that was not seen in the controls or in the other embalming fluids. This effect seemed to be limited to the tissue surface (pathology not performed) and did not seem to affect the integrity of the tissue.

Tissue integrity was assessed 6 days (Day 7) after exposure to embalming fluid. Each piece of tissue was "bent over" as far as possible using the thumb and forefinger. The tissue was then rotated 90 degrees and "bent over" a second time. A "Y" or yes was recorded if the tissue cracked upon bending. The extent to which the tissue would bend was limited by the thickness of the tissue. No tissue embalmed in Rex 36 embalming

fluid for any of the 5 time periods resulted in any visible tissue alterations in the TIE scoring. For the Hexaphene MA64 Cavity embalming fluid, 47 percent of the controls and 40 percent of the DF-200 treated samples cracked when the tissue was bent. For the Polar Cavity Firminindex[®] 53, 27 percent of controls and 13 percent of DF-200 samples cracked when the tissue was bent.

Each piece of tissue was then subjected to a twisting motion as it was held between the thumb and forefinger of both hands. A "Y" or yes was recorded if any further tissue destruction occurred. No tissue exposed to any of the embalming fluids resulted in any observable further tissue disruption while twisting.

Porcine skin embalmed tissues at all exposure time points for both DF-200 and control samples were rubbery in consistency and had the same basic "feel" common to "fixed" tissue. There was no dramatic skin integrity difference observed in tissue treated with DF-200 relative to its control tissue. There was however a significant "bleaching " of the skin secondary to DF-200 exposure that seems to be related to the time of exposure.

4.0 DISCUSSION

This experiment was planned to mimic the circumstances surrounding the decontamination of the body of a soldier in the field. It was anticipated that the remains would be exposed to DF-200 for about 2 hours then refrigerated overnight before being embalmed the following day.

Results from porcine skin seem to indicate that DF-200 does not interfere with the integrity of the skin and therefore should not preclude the ability of the body to be embalmed. There may however, be some cosmetic changes from the "bleaching effect" of the decon.

A very limited water and bleach decon study showed that 5 percent bleach has a rather dramatic disruptive effect on the tissue. The 0.5 percent bleach however, did not seem to

have any significant cosmetic or integrity effects. More extensive experiments might need to be conducted to verify this data.

These experiments were conducted with porcine skin obtained from an abattoir. It is unknown if results from these experiments can be extrapolated to human skin. Studies using human skin samples should be conducted to verify these results.

5.0 ARCHIVES

Records pertaining to the conduct of this study are contained in Battelle laboratory record books that are specific to this study. These records and the final report will be archived at Battelle. No samples will be archived at Battelle.

6.0 ACKNOWLEDGEMENTS

The names, titles and degrees or certification of the principal contributors to this study are listed below:

NAME	TITLE	DEGREE
Ms. Michelle Tussing	Lead Technician	A.S., LATG
Ms. Nancy A. Niemuth	Statistician	M.A.

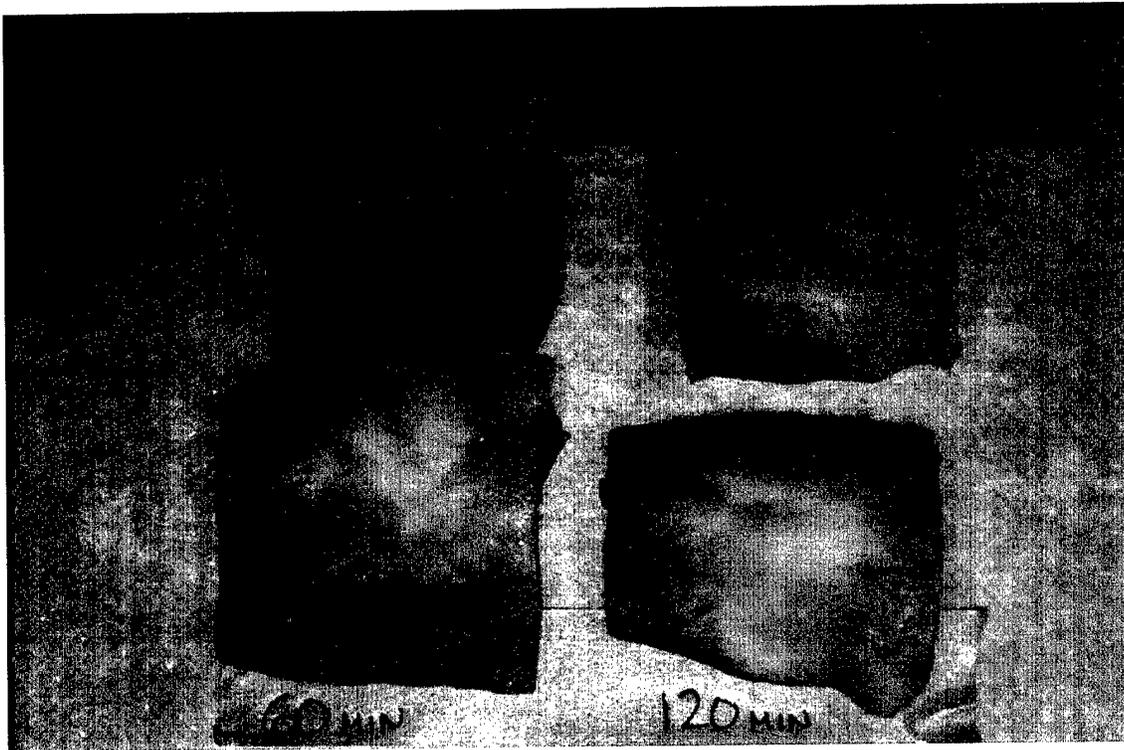
APPENDIX A

DF-200 Time Course

**Effect of DF-200 on Skin Integrity under Expected Human Remains Storage
Conditions**

APPENDIX A

A time course (15, 30, 45 and 60 minutes) for porcine skin exposed to DF-200 was performed to determine a more appropriate exposure time to the decon. Even at 15 minutes, there was a noticeable "bleaching effect" to the skin tissue. By 30 minutes, this "bleaching effect" was almost maximal with little change seen from the 45 and 60 minute samples.

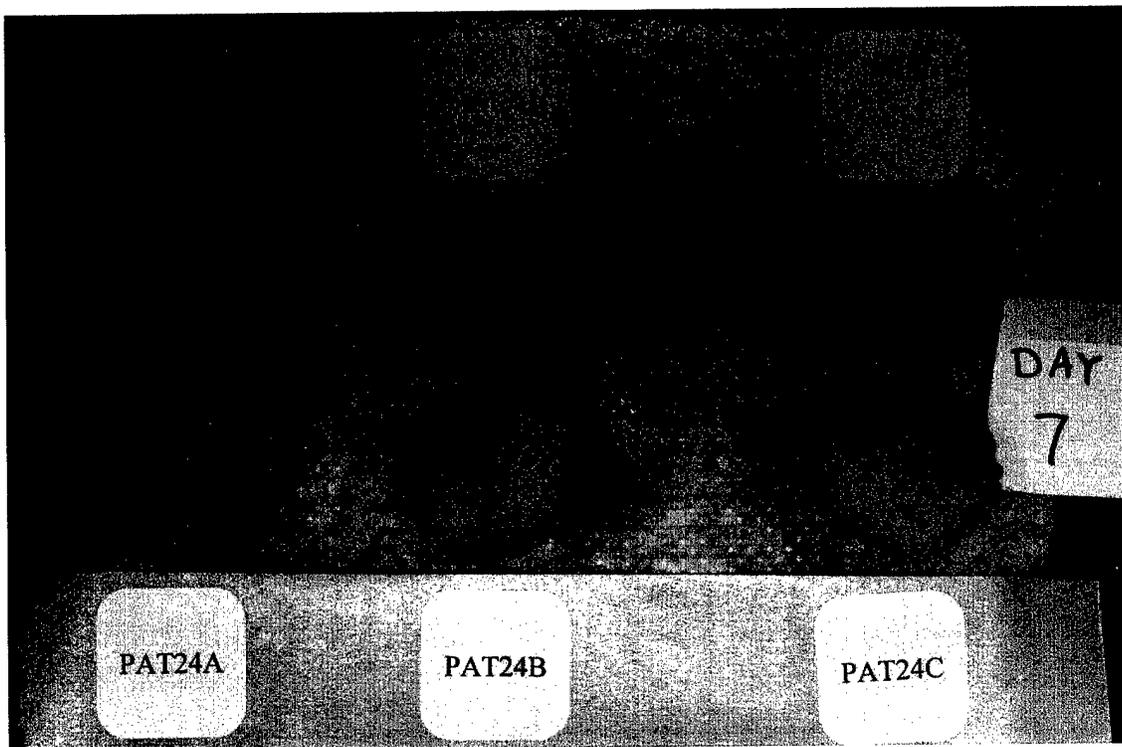


APPENDIX B

DF-200 Treated Tissue Embalmed with Rex 36 Arterial Index

APPENDIX B

Control and treatment group porcine skin tissues were placed into 2 ounce wide mouth glass jars. 20 mL of DF-200 was added to the treatment group tissues which were then incubated in covered jars for 2 hours at room temperature. Control tissues received no DF-200 but were also incubated in covered jars for 2 hours at room temperature. After 2 hours, the DF-200 was poured off and all tissues were refrigerated overnight (~ 4°C). The following day, all tissues were embalmed with 20 mL of Rex 36 Arterial Index 36 embalming fluid. The embalming fluid was poured off after 24 hours, at which time the pictures were taken. The top row are control tissues (no DF-200 exposure) and the bottom row are DF-200 treated tissues. These treated tissues receive a VOS score of 3 (treated relative to its corresponding control) indicating "moderate tissue differences seen". The "bleaching effect" of the DF-200 was very evident in the treated tissues.

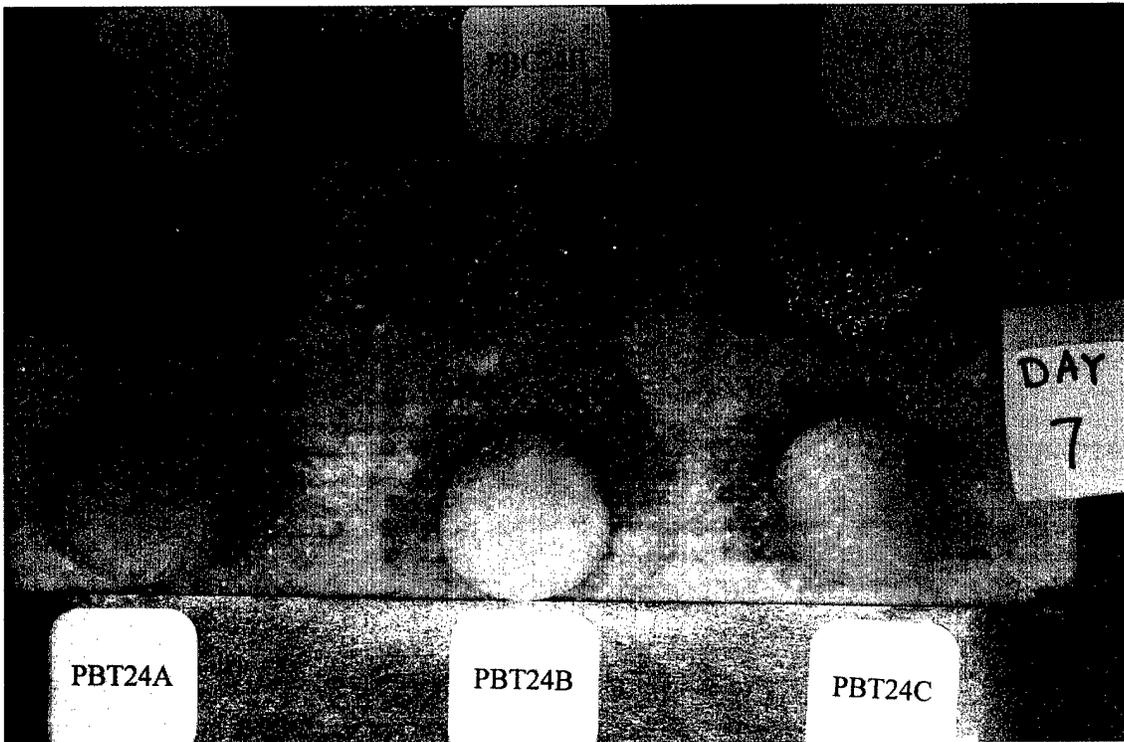


APPENDIX C

DF-200 Treated Tissue Embalmed with Hexaphene MA64

APPENDIX C

Control and treatment group porcine skin tissues were placed into 2 ounce wide mouth glass jars. 20 mL of DF-200 was added to the treatment group tissues which were then incubated in covered jars for 2 hours at room temperature. Control tissues received no DF-200 but were also incubated in covered jars for 2 hours at room temperature. After 2 hours, the DF-200 was poured off and all tissues were refrigerated overnight (~ 4°C). The following day, all tissues were embalmed with 20 mL of Hexaphene MA64 Cavity embalming fluid. The embalming fluid was poured off after 24 hours, at which time the pictures were taken. The top row are control tissues (no DF-200 exposure) and the bottom row are DF-200 treated tissues. These treated tissues receive a VOS score of 3 (treated relative to its corresponding control) indicating "moderate tissue differences seen". The "bleaching effect" of the DF-200 was very evident in the treated tissues.

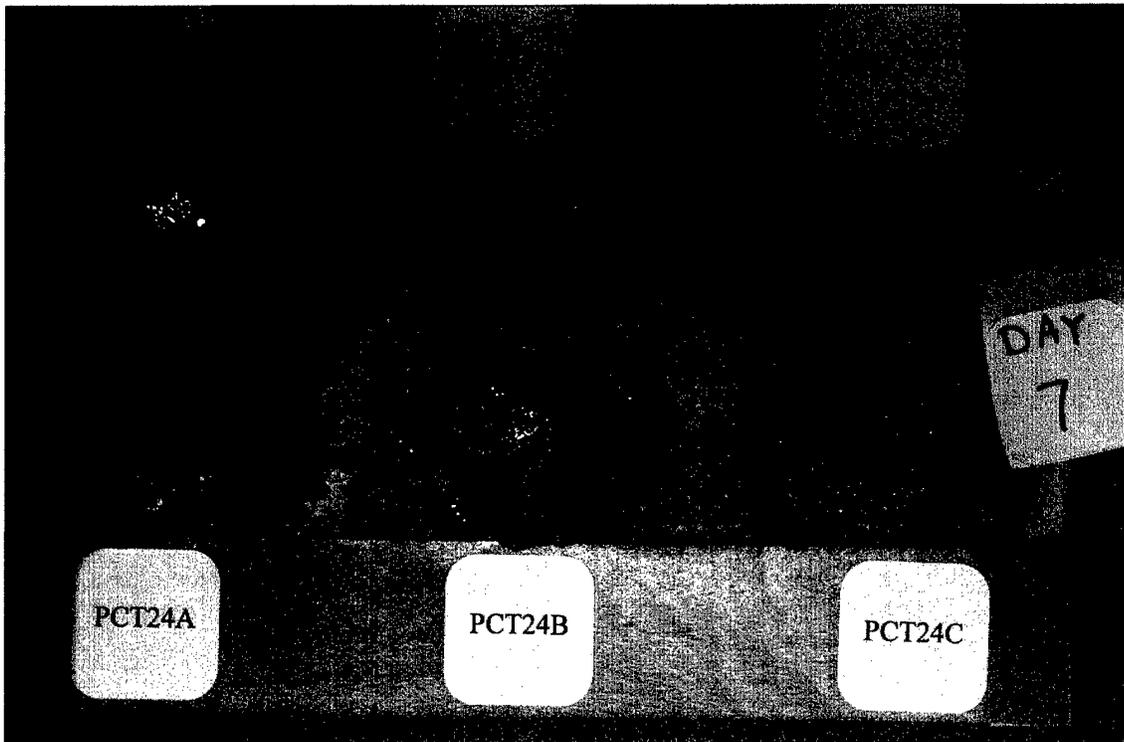


APPENDIX D

DF-200 Treated Tissue Embalmed with Polar Cavity Firmindex® 53

APPENDIX D

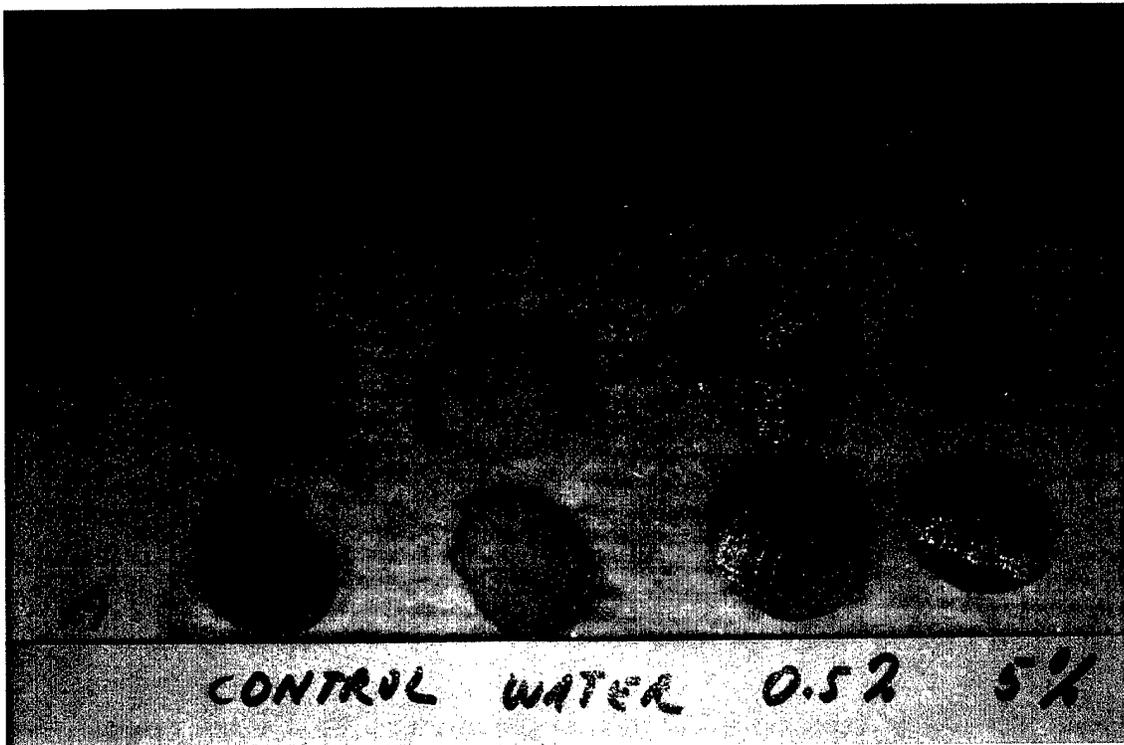
Control and treatment group porcine skin tissues were placed into 2 ounce wide mouth glass jars. 20 mL of DF-200 was added to the treatment group tissues which were then incubated in covered jars for 2 hours at room temperature. Control tissues received no DF-200 but were also incubated in covered jars for 2 hours at room temperature. After 2 hours, the DF-200 was poured off and all tissues were refrigerated overnight (~ 4°C). The following day, all tissues were embalmed with 20 mL of Polar Cavity Firminindex® 53 embalming fluid. The embalming fluid was poured off after 24 hours, at which time the pictures were taken. The top row are control tissues (no DF-200 exposure) and the bottom row are DF-200 treated tissues. These treated tissues receive a VOS score of 3 (treated relative to its corresponding control) indicating "moderate tissue differences seen". The "bleaching effect" of the DF-200 was very evident in the treated tissues.



APPENDIX E
Bleach and Water Comparison

APPENDIX E

In a 24 hour experiment, water, 0.5 percent bleach and 5 percent bleach was also compared to DF-200 but using only one porcine skin sample for each embalming solution. Water, as expected, resulted in significant tissue dehydration and shrinkage after 24 hours. The 5 percent bleach defatted and reduced the size of the tissue samples. The 0.5 percent bleach appeared to have very little cosmetic changes relative to its control under all three (Table 1) embalming conditions (Table 6A and APPENDIX E). TIE score results were "NO" for all treated and control groups (Table 6B).



APPENDIX F
MREF Protocol 293

MREF Protocol 293
293-G472520
Medical Research and Evaluation Facility
October 23, 2002
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Effect of DF-200 on Skin Integrity under Expected Human Remains Storage Conditions

Study performed by

**Battelle Memorial Institute
Medical Research and Evaluation Facility (MREF)
505 King Avenue, Building JM-3, Columbus, Ohio 43201-2693**

STUDY DIRECTOR: Michael C. Babin, D.V.M., Ph.D.

MREF MANAGER: James E. Estep, D.V.M., Ph.D.

**SPONSOR: United States Army Medical Research Institute of Chemical Defense
(USAMRICD)**

PROGRAM MONITOR: LTC Brian Lukey

Approval Signatures



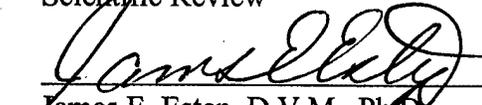
Michael C. Babin, D.V.M.; Ph.D.
Study Director

11/15/02
Date



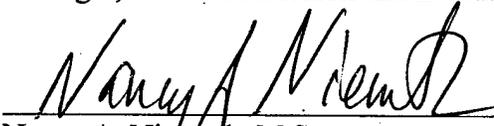
Carl T. Olson, D.V.M., Ph.D.
Scientific Review

11/18/02
Date



James E. Estep, D.V.M., Ph.D.
Manager, Medical Research and Evaluation Facility

11-15-02
Date



Nancy A. Niemuth, M.S.
Statistician

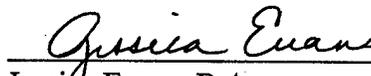
11/15/02
Date

See Attachment, Page 2A, For Sponsor Approval

LTC Brian Lukey
Sponsor Representative
USAMRICD

Date

Reviewed and Registered by:



Jessica Evans, B.A.
Quality Assurance Auditor

11/19/02
Date

MREF Protocol 293
293-G472520
Medical Research and Evaluation Facility
October 23, 2002
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Approval Signatures

Michael C. Babin, D.V.M.; Ph.D.
Study Director

Date

Carl T. Olson, D.V.M., Ph.D.
Scientific Review

Date

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Date

Nancy A. Niemuth, M.S.
Statistician

Date

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LTC Brian Lukey
Sponsor Representative
USAMRICD

15 Nov 02

Date

Reviewed and Registered by:

Jessica Evans, B.A.
Quality Assurance Auditor

Date

Effect of DF-200 on Skin Integrity under Expected Human Remains Storage Conditions

I. Nontechnical Synopsis

Scope: The preparation of cadavers resulting from lethal exposure to chemical agents (CA) on the battlefield includes thorough skin decontamination prior to transfer in body bags. There may be more effective alternatives than the current practice of using soapy water and a 2 percent aqueous solution of sodium hypochlorite (bleach). The U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) is investigating the decontamination efficacy of Modec Decon Formulation 200 (DF-200) for 2,2'-dichlorodiethyl sulfide (sulfur mustard; HD) and O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX). This task evaluates the effect on appearance and integrity of skin with the concurrent use of DF-200 and embalming fluid using pig and harvested human skin tissue.

II. Background

Study Rationale: Information is needed on the effect DF-200 and embalming solutions on the appearance and integrity of human skin.

III. Objective/Hypothesis

The objective of the proposed research is to use porcine carcass skin and human breast skin tissue (or other human skin harvested during plastic surgery) to evaluate the effect of DF200 on skin appearance and integrity under expected human remains storage conditions.

IV. Military Relevance

An important mission of the Military Mortuary Affairs Units is to search for, recover, and prepare for transfer any military members lost as a result of combat. This includes ensuring that the skin surface is free of lingering CA that would pose a threat to handlers, shipment recipients, and funeral participants. These studies are needed to determine whether the use of DF-200 can replace the current method for decontaminating casualties.

V. Materials and Methods

1. Test Materials

- A. DF-200 is supplied by USAMRICD or its designee. It is the responsibility of USAMRICD to ensure that appropriate identification (batch number and/or lot number), expiration date (if available), safety data, and storage requirements are supplied. DF-200 is prepared on the day of use according to manufacturer's instructions.
- B. Rex 36 Arterial Embalming fluid, Gold Crest Chemical Company. Rex 36 will be diluted and used according to directions from the sponsor.
- C. Hexaphene MA64Cavity embalming fluid. Hexaphene will be used undiluted according to sponsor specifications.
- D. Polar Cavity Firminindex 53, ESCO Proline. Polar Cavity Firminindex 53 embalming fluid will be used undiluted according to sponsor specifications.

2. Test System

- A. Full-thickness porcine dorsal surface skin is obtained from a local abattoir.
- B. Human breast skin (or other human skin harvested during plastic surgery procedures) will be obtained from approved suppliers.

3. Methods

- A. Experimental Plan (Appendix A)

The experiment will be divided into two sections: porcine skin and human skin exposures. Three different embalming fluids will be tested on each type of skin. For each embalming fluid, embalming fluid alone will be compared to embalming fluid plus DF-200. In addition, different embalming fluid fixation times for each fluid (3, 6, 12, 24, and 48h) will be evaluated.

Treatment Group: Porcine or human skin will be transported in a cooler from the abattoir/supplier and refrigerated overnight, if necessary, at approximately 4C. Skin tissue samples will then be covered with DF-200 for 2 h (Day 0). The DF-200 will then be poured off and the tissue refrigerated overnight at approximately 4C. The next day (Day 1), skin samples will be exposed for up to 5 different fixation times to embalming fluid. At the end of the fixation time, embalming fluid will be poured from the container. The container then will be covered and the tissue left in a chemical fume hood for the remainder of the experiment.

Control Group: Porcine or human skin will be transported in a cooler from the abattoir/supplier and refrigerated overnight, if necessary, at approximately 4C. Unlike the treatment group, skin tissue samples will not be covered in DF-200 for 2 h but will be left in the hood for a 2 h period in a covered container (Day 0). The tissue then will be refrigerated overnight at approximately 4C. The next day (Day 1), skin samples will be exposed for up to 5 different fixation times to embalming fluid. At the end of the fixation time, embalming fluid will be poured from the container. The container then will be covered and the tissue left in a chemical fume hood for the remainder of the experiment.

This procedure will be conducted for each embalming fluid. Pictures of each control and treatment group skin will be taken on a daily basis beginning prior to exposure to DF-200 (day 0). The appearance of skin samples will also be described daily by a scoring system (Table 1). Comments about appearance will be recorded when the treatment VOS is different than its associated control VOS. On the last day of the experiment (Day 7), each tissue sample will be evaluated for integrity by bending and /or twisting (Table 2).

Results from the porcine skin experiment will be used to determine the parameters (time points, number of groups, etc) to be tested for the human skin samples. It is anticipated that a reduced number of samples and fixation time points will be used in the testing of human skin tissue.

B. Preparation of skin samples.

For the porcine skin, on each test day, 90 approximately 2.5-cm diameter disks of skin (Appendix A) are cut with an arc punch, and each disk is

placed in a petri dish or other appropriately labeled container. Adjacent pieces of tissue will be used for control and treatment samples to reduce possible variation due to sample location. If available, both light and dark skin will be used. Alternatively, equal size strips of skin may be substituted for disks.

For the human skin samples, the size of each sample will depend on the availability of tissue. It is anticipated that less samples (reduced time points, reduced group numbers, etc) will be used since the human skin testing will be used primarily to confirm results obtained in porcine skin. Adjacent pieces of tissue will be used for control and treatment samples to reduce possible variation due to sample location. Either disks or strips of tissue may be used.

- C. Disposal of Fluids: All skin samples, DF-200 and embalming fluids are placed into waste containers and disposed of in accordance with Battelle SOPs.
- D. Data Analysis

Each tissue will be evaluated using a subjective scoring system at predetermined time intervals (Table 2). Friedman's two-way nonparametric analysis of variance (ANOVA) will be used to evaluate the effects of embalming time and length of follow-up on visual observation scores.

VI. Records to be Maintained

Test articles inventory.

Dosage preparation and administration of test articles.

Data calculations and statistical analyses.

VII. Final Report

Battelle will prepare a final report within 30 workdays of receiving USAMRICD comments on a draft final report.

VIII. Biohazard Safety

No biological organisms or CA will be used on study. Safety procedures for the use of chemicals are thoroughly outlined in MREF facility plans.

IX. Assurances:

As the primary investigator on this protocol, I provide the following assurances:

Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

Michael Sub 11/15/02
Study Director Date

Table 1. Visual Observation Score (VOS)

Score	Tissue Observation*
0	No observable tissue differences
1	Very minor tissue differences seen
3	Moderate tissue differences seen
5	Severe tissue differences seen

*VOS scoring will be relative to the control tissue at each observation time.

Table 2. Tissue Integrity Evaluation (TIE)

Score	Tissue Bending	Tissue Twisting
Yes	Loss of tissue integrity	Loss of tissue integrity
No	(cracking/ breaking etc) while bending.	(cracking/ breaking etc) while twisting.

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APPENDIX A
Experimental Plan

Group	Tissue	Sample Number	Exposure Time (h) *	Time refrigerator	Embalming fluid	Embalming time (hours)	Observation time**	Procedures	Tissue evaluation (day 7 only)
Treatment	skin	3	DF-200 for 2h	overnight	A	3	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	A	6	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	A	12	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	A	24	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	A	48	daily for 7 days	Picture/description	Bending, twisting
Control	skin	3	R. T. for 2 h	overnight	A	3	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	A	6	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	A	12	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	A	24	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	A	48	daily for 7 days	Picture/description	Bending, twisting
Treatment	skin	3	DF-200 for 2h	overnight	B	3	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	B	6	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	B	12	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	B	24	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	B	48	daily for 7 days	Picture/description	Bending, twisting
Control	skin	3	R. T. for 2 h	overnight	B	3	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	B	6	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	B	12	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	B	24	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	B	48	daily for 7 days	Picture/description	Bending, twisting
Treatment	skin	3	DF-200 for 2h	overnight	C	3	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	C	6	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	C	12	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	C	24	daily for 7 days	Picture/description	Bending, twisting
	skin	3	DF-200 for 2h	overnight	C	48	daily for 7 days	Picture/description	Bending, twisting
Control	skin	3	R. T. for 2 h	overnight	C	3	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	C	6	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	C	12	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	C	24	daily for 7 days	Picture/description	Bending, twisting
	skin	3	R. T. for 2 h	overnight	C	48	daily for 7 days	Picture/description	Bending, twisting

Samples 90

* R.T. (room temperature)

** Observation time begins at time of exposure to embalming fluid (day 1)

**EFFECT OF DF-200 ON SKIN INTEGRITY UNDER EXPECTED HUMAN REMAINS
STORAGE CONDITIONS**

MREF Protocol Amendment No. (1)

Change No. (1).

Section 3.A. (Experimental Plan) provides for both porcine and human skin to be tested under this protocol. A decision has been made not to pursue the human skin testing at this time. If human skin testing is needed, it will be tested under another TASK or other appropriate funding channel.

Reason for Change:

The data that has been generated under this TASK may be sufficient to provide the needed information for decision making purposes.

Impact on Study:

No impact on previously conducted work.

Effective Date: 6 December 2002

Change No. (2).

Because of the unexpected "bleaching effect" in the treated tissue, two unplanned experiments were conducted with the remaining unused porcine skin. A time course (15, 30, 45 and 60 min) for porcine skin exposed to DF-200 was performed. Briefly, 1 ml of DF-200 was placed on top of approximately 2 square inch pieces of porcine skin (n=1). The DF-200 was removed at time intervals of 15, 30, 45 and 60 min. Pictures were taken after the last time point.

In a separate 24 hr experiment, water, 2% bleach and 5% bleach was also tested with each embalming fluid (n=1) to evaluate tissue appearance and integrity. The experiment was conducted under the same parameters as the DF-200 experiment with water and bleach being used as the decon materials (n=1).

Reason for Change:

Because of the severe bleaching effect of the DF-200, it was anticipated that the client would need time and effect data in order to make a use decision on the decon. It was also anticipated that the client might ask how the effects of DF-200 on skin compared to bleach.

Impact on Study:

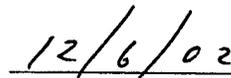
Provided additional information that the client needed to adequately evaluate DF-200.

Effective Date: 6 December 2002

Approved By:



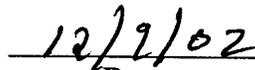
Michael C. Babin, D.V.M., Ph.D.
Study Director



Date

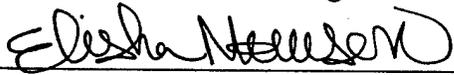


David Lenz, Ph.D.
Contract Officer Representative

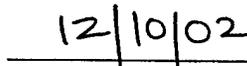


Date

Quality Assurance Review and Registration:



Elisha N. Morrison, M.S.
Quality Assurance Officer



Date

APPENDIX G
Statistics Report

Internal Distribution

Rosebrough/Project Files (Melling)
NA Niemuth
MC Matthews
RMO

s:\niem\MREF\Task 20\Priority 3 Memo.doc

Date December 18, 2002
To **Mike Babin**
From Nancy Niemuth
Subject **MREF Task 20: Statistical Analysis of VOS
and Tissue Evaluation Scores on Porcine
Skin**

This memorandum summarizes the statistical analysis of tests conducted under MREF Task 20 to examine the effects of DF-200 and embalming time on the appearance and integrity of skin, for 3 embalming fluids.

Summary of Study Design: Three pairs of porcine skin samples were tested at each of five embalming times (3, 6, 12, 24, and 48 hours), for each of three embalming fluids. Within each pair, one skin sample was treated with DF-200 decontamination fluid for 2 hours and the other control sample was not treated. Both samples were refrigerated overnight, then treated with embalming fluid for the specified time interval. Visual observation scores (VOS) that compared the DF-200 treated sample to the matched control sample were recorded on day 0 prior to DF-200 treatment, day 1 prior to embalming fluid treatment, and days 2, 3, 6, and 7. The 48-hr embalming groups were not observed on Day 2, as the embalming treatment was not complete. On Day 7, the samples were evaluated for tissue integrity when bent or twisted. VOS were defined as follows: 0= No observable difference seen; 1= Very minor observable difference seen; 3= Moderate to serious observable difference seen; 5= Severe tissue destruction. Tissue integrity was evaluated as yes/no for loss of tissue integrity (cracking/breaking, etc.) while bending or twisting.

Statistical Methods: A nonparametric analysis of variance (ANOVA) technique was used to evaluate the effects of embalming time and length of follow-up on visual observation scores. The nonparametric method entailed fitting a standard parametric model to the ranks of the VOS data. A separate model was fitted to the data for each embalming fluid. McNemar's test was used to assess whether there was an association in tissue integrity evaluations between pairs of skin samples. Because the sample size was small, the data for all embalming times were pooled for the tissue integrity evaluation. All statistical analyses were conducted using the RANK, GLM, and FREQ procedures in Version 8.2 of SAS.

Results: For all embalming fluids, embalming times, and observation days, at least minor differences were observed between pairs of DF-200 treated and control tissues, with most comparisons scored as 3. Therefore, it can be concluded, in general, that clinically significant differences were observed between DF-200 treated and control tissues.

For embalming fluid A, the effects of embalming time ($p=0.0001$) and length of follow-up ($p=0.0158$) and their interaction (0.0020) were statistically significant. These effects were evident for the 24-hr embalming time on days 2 and 3, where less substantial differences were

observed between the treatment and control samples. For embalming fluid B, the model could not be fit, as all scores were identical and equal to 3 for all embalming times and observation times. For embalming fluid C, the effects of embalming time and length of follow-up were not statistically significant at the 0.05 level.

For the tissue integrity evaluations on day 7, no loss of tissue integrity was observed while bending or twisting for embalming fluid A, nor while twisting for embalming fluids B and C. For embalming fluids B and C, loss of tissue integrity while bending was observed in some of the treatment and control samples. The statistical test for association using McNemar's test did not indicate that loss of tissue integrity was associated with the DF-200 treatment, for either embalming fluid.

NAN:llj

For Review and Approval

	Name	Initials	Date
Originator	Nancy Niemuth	N	12/18/02
Concurrence	Claire Matthews	CM	12-18-02
Approved	Bill Rosebrough	WR	12/18/02

Sent via: Interoffice Mail

APPENDIX H
Protocol Deviation

**MEDICAL RESEARCH AND EVALUATION FACILITY
DEVIATION FORM**

Deviation No. (Assigned by QAU): DR-1252

CAQ No. (Assigned by QAU): NA

Standard or Procedure Deviated:

- Protocol (Number): 293
- SOP (Number):
- Method (Number):
- GLP (Section):
- Other:

Type of Deviation (check one):

Facility

Study (fill out study info) Study Number: 293-G472520

Study Title: Effect of DF-200 on Skin Integrity under Expected Human Remains Storage Conditions

Record Affected (describe Title, Binder name, location, Form no. etc.): TASK 20-3. Data collection on observation forms (MREF Obs sht-001-00) for observation days 4 and 5.

Date of Deviation(s): 6 December 2002

Description of Deviation:

The concentration of bleach used in the study was incorrectly described in Amendment 1 of Protocol 293.

Cause of Deviation:

In change No. 2 of Amendment 1, bleach was incorrectly described as 2.0% instead of the planned 0.5% bleach solution used in the experiment.

Corrective Action:

None needed, 0.5%, as planned, was used in the experiment and will be reported out as 0.5% in the final report.

Impact of Deviation: None

If deviation is planned, effective date: NA

Deviation form Prepared by/Date:

Michael C. Bohm 3/4/04

Deviation Reviewed and Corrective Action Accepted by/Date:

Michael C. Bohm 3/4/04

Deviation Reviewed and Registered by QAU/Date:

Wright 3/4/04