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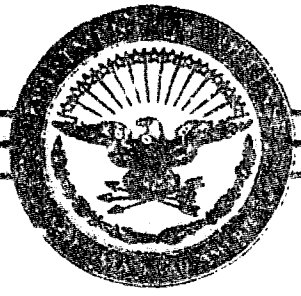

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Battelle
Columbus Division

# Report

AD-B115 356

FINAL REPORT

Contract No. DAMD17-83-C-3129
Multiple Animal Studies for Medical Chemical Defense
Program in Soldier/Patient Decontamination and Drug Development

on

# TASK ORDER 84-1:
# VALIDATION OF A PROTOCOL TO COMPARE THE EFFECTIVENESS OF EXPERIMENTAL DECONTAMINANTS WITH COMPONENT II OF THE M258A1 KIT OR FULLER'S EARTH STANDARD DECONTAMINANTS AGAINST PERCUTANEOUS APPLICATION OF UNDILUTED VESICANT CHEMICAL SURETY MATERIEL TO THE LABORATORY ALBINO RABBIT

Supported by:
U.S. Army Medical Research & Development
Command, Fort Detrick, Frederick,
Maryland 21701-5012

July 24, 1987

by

Dr. Ronald L. Joiner
Dr. H. Hugh Harroff, Jr.
Mr. W. Bruce Keys, Jr.
Dr. Paul I. Feder

Contract No. DAMD17-83-C-3129

BATTELLE
Columbus Division
505 King Avenue
Columbus, Ohio 43201-2693

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (U. S. Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH), Publication No. 85-23, revised 1985).

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Sulfur mustard (HD), Lewisite (L), rabbit, decontaminants, screen, chemical surety materiels, vesicants.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)
A screening protocol was developed and validated to determine the effectiveness of candidate liquid decontaminant materiels when compared to a single component of the U. S. Army M258A1 system. Rabbits were dosed topically on their shaved dorsa with either sulfur mustard (HD) or Lewisite (L) and lesion lengths were analyzed statistically. Materials as good as or better than the single component M258A1 standard will pass to the next tier for further testing.

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16. Surety Materiel to the Laboratory Albino Rabbit

FINAL REPORT

on

# VALIDATION OF A PROTOCOL TO COMPARE THE EFFECTIVENESS OF EXPERIMENTAL DECONTAMINANTS WITH COMPONENT II OF THE M258A1 KIT OR FULLER'S EARTH STANDARD DECONTAMINANTS AGAINST PERCUTANEOUS APPLICATION OF UNDILUTED VESICANT CHEMICAL SURETY MATERIEL TO THE LABORATORY ALBINO RABBIT

Supported by:
U.S. Army Medical Research & Development Command, Fort Detrick, Frederick, Maryland 21701-5012

July 24, 1987

Ronald L. Joiner, Ph.D.
Study Director

H. Hugh Harroff, Jr., D.V.M.
Chief Veterinarian

W. Bruce Keys, Jr.
Study Supervisor

Paul I. Feder, Ph.D.
Biostatistician

Ramona A. Mayer
Quality Control

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## 1.0 INTRODUCTION

A task was initiated at the Medical Research and Evaluation Facility (MREF) in October 1983 to develop a screening protocol to determine the effectiveness of candidate liquid decontaminant materials when compared to a single component of the standard liquid decontaminant currently fielded by the U. S. Army. The protocol was designed to eliminate those candidates that were not as good as the most effective component of the M258A1 field kit in decontaminating rabbits exposed percutaneously to mustard (HD) or Lewisite (L). Materials as good as or better than the single-component M258A1 standard pass to the next tier for further testing.

A draft protocol was submitted in November to the U. S. Army Medical Research and Development Command (USAMRDC) for comment, modification, and subsequent approval for implementation at the MREF. The final protocol, MREF Protocol 1 (entitled "Dermal Study for the Assessment and Validation of Decontaminants in Rabbits Against Mustard and Lewisite"), was signed in early December 1983 and range-finding studies were initiated in March 1984. A copy of the signed protocol is included in Appendix A.

A revision was made to the signed MREF Protocol I in June 1984 to include the use of candidate powder decontaminants versus a standard powder decontaminant designated by the USAMRDC (Fuller's Earth). Developmental work was begun in May 1984 to provide sufficient information to make the final revisions in June 1984. A copy of the revised protocol is included in Appendix B.

# 2.0 EXPERIMENTAL DESIGN

## 2.1 Animals

Albino rabbits were chosen for this study on the basis of the extensive data base available for percutaneous application of toxic materials in this species and on the size of the application area for multiple challenges with neat chemical surety materiel (CSM). Equal numbers of 2.0- to 4.0-kg male and female New Zealand White (albino) rabbits from the Kings Wheel Rabbitry, 8085 Camp Road, Rt. 5, Mt. Vernon, Ohio 43050, were randomly assigned to treatment groups based on body weights. Preselections were made on all rabbits to obtain only those with hair-growth patterns that would allow bilateral, pair-wise comparisons of standard and candidate dosing sites. All animals were quarantined for at least 7 days at Battelle Columbus Laboratories' Animal Resources Facility at 505 King Avenue before being transported to the MREF.

Upon receipt at the Animal Resources Facility, the rabbits were ear tattooed for positive identification, weighed, sexed, and observed for signs or symptoms of disease. At the MREF, animals were acclimated for at least 24 hr prior to being placed on study. At both facilities, housing was individual in stainless steel, slotted cages equipped with automatic watering systems. Humidity was programmed at 50 percent ($\pm$10 percent) and temperature at 70 F ($\pm$5 F). Fluorescent lighting was maintained at a light/dark cycle of 12 hr each per day. Purina Certified Rabbit Chow and water were available at all times during quarantine and holding. During the 24-hr test period, animals were given free access to water but were not given rabbit chow while in the treatment stanchions.

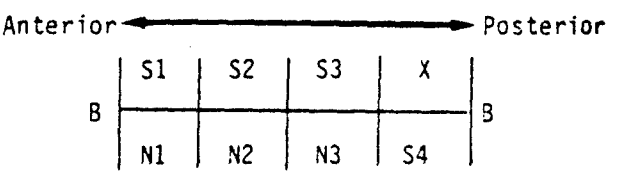
Battelle's Animal Resources Facilities have been registered with the U. S. Department of Agriculture (USDA) as a Research Facility (No. 31-21) since August 14, 1967, and are periodically inspected in accordance with the provisions of the Federal Animal Welfare Act. In addition, animals for use in research are obtained only from laboratory animal suppliers duly licensed by the USDA. Battelle's statement of assurance regarding the Department of Health and Human Services policy on humane care of laboratory animals was

accepted by the Office of Protection from Research Risks, National Institutes of Health on August 27, 1973. Animals at Battelle are cared for in accordance with the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" (DHHS Publication No. (NIH) 78-23) and/or in the regulations and standards as promulgated by the Agricultural Research Service, USDA, pursuant to the Laboratory Animal Welfare Act of August 24, 1966 as amended (P.L. 89-544 and P.L. 91-579).

On January 31, 1978, Battelle's Columbus Division received full accreditation of its animal-care program and facilities from the American Association for Accreditation of Laboratory Animal Care (AAALAC). Battelle's full accreditation status has been renewed after every inspection since the original accreditation. The MREF is a part of the facilities granted full accreditation.

## 2.2 Treatment Design

Groups of eight rabbits (four male and four female) were matched by weight after selecting animals with suitable hair-growth patterns within the dorsal application area. Each animal in the group received a series of 0.5-µl applications of chemical surety materiel (CSM) along the dorsum of the back in the following pattern:




N = 0.5 µl of CSM followed by experimental candidate

S = 0.5 µl of CSM followed by M258A1 II (except S4)

X = No treatment or challenge

B = M258A1 II alone

1 = Decontamination at shortest time period

2 = Decontamination at middle time period

3 = Decontamination at longest time period

4 = CSM without decontamination.

## 2.3 Experimental Compounds

The materials used to validate the original MREF Protocol 1 model were the M258A1 II component of the M258A1 field kit, which was the positive liquid control for effective decontamination, and distilled water, which was selected as a liquid material that would not be effective as a decontaminant against percutaneous application of HD or L. The M258A1 kit consists of two components (I and II) to be used in sequence in field application as specified by the kit use instructions (TM 3-4230-216-10, April 1982). Each component is individually packaged in aluminum foil to maintain activity of the ingredients and to prolong the storage life of the kit.

Component I consists of a nominal 2.75-in. by 5-in. towelette moistened with 3.5-4.9 g, of decontaminating solution (average volume estimated to be 4.25 ml), which is a mixture of ethanol (72 percent, w/w), phenol (10 percent, w/w), sodium hydroxide (5 percent, w/w), ammonia (0.2 percent, w/w), and water (12.8 percent, w/w) (Military Specification DOD-D-51467(EA), 25 February 1980; Military Specification MIL-D-51468(EA), 11 August 1983). Component II consists of a nominal 2.75-in. by 5-in. dry towelette impregnated with 1.0 g of chloramine-B (quantity to produce an active chlorine content of 0.156 g) and three crushable glass vials containing a total of approximately 4.5 ml of a mixture of ethanol (45 percent, w/w), zinc chloride (5 percent, w/w), and water (5 percent, w/w) (Military Specification DOD-D-51467(EA), 25 February 1980; Military Specification DOD-C-51464(EA), 25 February 1980; Military Specification MIL-D-51468(EA), 11 August 1983).

The use of the individually wrapped and already prepared kit components was discarded in favor of obtaining the individual bulk chemicals and towelettes used to manufacture the kit components and making the towelettes and solutions up fresh at the moment of use in the experiment. In this manner, the opening of individual packages and handling of hazardous materials impregnated into or absorbed onto towelettes were avoided. The bulk

liquid solutions were applied directly to the precut towelettes in the hood (to minimize personnel exposure) immediately prior to in-hood use. Thus, handling of the premoistened towelettes and evaporation of the volatile portions of the liquid solutions prior to use were minimized. The volumes of the liquid portions and the sizes of the cloth towelettes used in these experiments were proportional to the military specifications that governed their manufacture (Military Specification DOD-C-51464(EA), 25 February 1980; Military Specification DOD-D-51467(EA), 25 February 1980; Military Specification MIL-D-51468(EA), 11 August 1983).

Thus, the actual prepared, packaged kit components were not used in this screen. Instead, freshly prepared towelettes were made from bulk components immediately prior to use in the experiment. The components of the M258A1 II system were obtained from Chemtronics Corp., Swannanona, North Carolina.

Component II of the M258A1 kit was chosen for use as the standard single-component decontaminant after consultation with LTC Donald Harrington (USAMRICD), LTC(P) Howard Johnson (USAMRICD), and Dr. Millard Mershon (USAMRICD). Dr. Mershon presented unpublished data from his laboratory that showed that component II was the more effective of the two components against HD and L. Consideration was not given to using both components in sequence.

The L and HD were supplied by the USAMRDC, and the following information was obtained from the USAMRDC for each:

| | HD | L |
|---|---|---|
| Purity (%) | 96.6 | 98.0 |
| Density (g/ml) | 1.27 | 1.88 |
| Known impurities | None | None |
| Additives | None | None |
| Color | Colorless | Dark amber to brown |
| Appearance | Clear liquid | Slightly oily liquid |

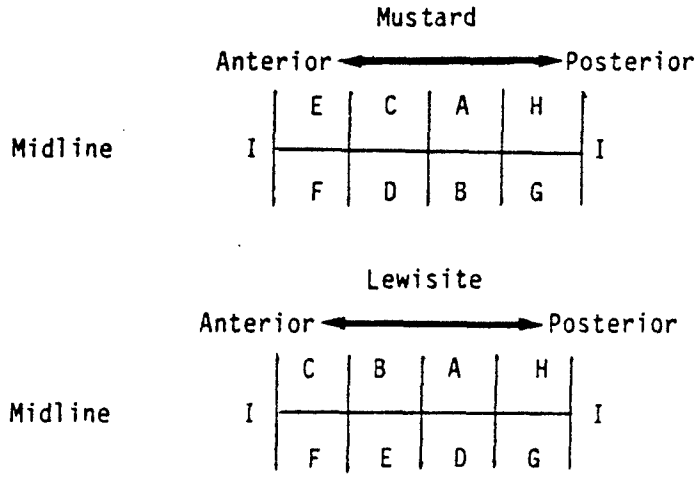
Battelle did not confirm the purity, density, impurities, or additives information supplied by the USAMRDC. Dose analyses were not performed since the CSM was applied undiluted.

The materials used to validate the revised MREF Protocol 1 model were Fuller's Earth, which was the positive powder control selected by the USAMRDC for effective decontamination, and powdered marble dust, which was selected as a powdered material that would not be effective as a decontaminant against percutaneous application of HD or L. Fuller's Earth was obtained from Sigma Chemical Company (St. Louis, MO) as stock number F-200 (mesh 100-200, lot no. 31F-0619). Marble dust was purchased locally; the manufacturer was North County Aggregates Inc. (Governeur, NY), and the sample was identified as "finely ground crystalline marble."

## 2.4 Application of Vesicants

A constant 0.5-µl dose was arbitrarily chosen as an initial dose for the validation runs based on the results of a preliminary qualitative methodology run in which several volumes were tested for ease of applicability and on discussions with Dr. Millard Mershon of the USAMRICD. The 0.5-µl dose was easy to apply, did not run from the dosed site, and produced irritation at the 24-hr evaluation. Time from exposure to decontamination was chosen as the variable to produce differing degrees of response, based on the assumption that it would be easier and more accurate to repetitively deliver the same dose than to deliver different doses with the time being held constant.

Prior to application of the CSM, each rabbit was clipped and anesthetized with a 1.75:1.0 (w/w) mixture of Ketamine and Rompun (8.75 mg/kg and 5.0 mg/kg, respectively) by intramuscular injection. The unconscious animals were then placed in stainless steel stanchions and transported to the hood for dosing. CSM (0.5 µl of HD or L) was applied to each of the seven spots on the back of each rabbit (see Section 2.2) as a small streak (approximately 1 cm in length) with a Hamilton microliter syringe. L was applied with a special Hamilton syringe equipped with a platinum barrel and a tungsten plunger, while HD was applied with a standard stainless steel syringe. The following pattern of application was used on each rabbit:




- Application of CSM proceeds from A-G in alphabetical order to allow proper sequencing of timed decontaminations at each site.
- Application of experimental liquid or powder candidate (without CSM) at site H and standard M258A1 II or Fuller's Earth at site I is done during the dosing regimen at the first available time period.

The duration of exposure before the beginning of decontamination was chosen after consultation with the USAMRDC as the variable of importance to test in this model. A preliminary, qualitative methodology run was made with a dose of 0.5 µl of L to determine the time sequences to use as the initial series in the validation study. The instantaneous corrosivity of L made all times to decontamination sufficient to produce severe, irreversible irritation. The shortest time that could reliably be used between application and initiation of decontamination was estimated to be 30 sec. Thus, the shortest time period was chosen as 30 sec. The second and third times were arbitrarily chosen as 60 sec (2x) and 120 sec (4x).

For HD, 1.25, 5.0, and 10.0 min after exposure were chosen as the decontamination application times based on a similar but slightly different basis. The initial qualitative methodology run showed that a dose of 0.5 µl of HD produced a measurable and moderately severe irritation at 24 hr after a time from application to decontamination of 5 min (initial time chosen after

consultation with Dr. Mershon of the USAMRICD). Arbitrary times of 1.25 min (1/4x) and 10 min (2x) were chosen as initial sequences for the validation tests.

The decontamination process for liquids in MREF Protocol 1 initially consisted of wiping a pad wetted with the decontamination solution over the exposed area for 5 sec in a circular motion. This was subsequently changed to a back and forth motion within the rectangular grid of the outlined area perpendicular to the spine. The cloth pad, cut to approximately one-half of the packaged kit pad, was made from bulk M258A1 II kit cloth that was then taped to a tongue depressor. The size of the pad was proportioned to the surface area relationship between the rabbit back and the soldier's exposed hands and neck. This ratio was approximately 1:6 (rabbit to man), thus the area represented by each outlined area was approximately one-thirtieth of the total area, or one-twelfth of the backs of the hands themselves. Thus, each treatment area represented about the area that the soldier would decontaminate in approximately 5 sec.

The cloth was wetted with 2.25 ml (one-half of the kit volume) of liquid from the bulk component of the kit, which corresponded to the proportion of cloth used to make the pad (each laboratory-made pad represented 0.5 of the surface area of the pad in the M258A1 II kit packet). The decontamination pad for the experimental decontamination solution was made as above from a strip of nonimpregnated cloth that was identical to the M258A1 II cloth except that it did not contain chloramine-B (a component of the M258A1 II system that is impregnated into the cloth as a dry powder to be activated with the addition of the liquid component). The amount of liquid added to the pad (2.25 ml) was equivalent in volume to that of the M258A1 solution applied.

The decontamination process for powders in the revised MREF Protocol 1 consisted of weighing a prescribed amount of the powder (100 mg) into a capped vial for application. The 100-mg amount was chosen after consultation with the USAMRDC. It was based on methodology runs that showed that 100 mg was the smallest amount that completely covered the entire surface area of the treatment site and provided an excess of decontaminant to CSM greater than 20:1 (100 mg of decontaminant versus 0.64 mg of L and 0.94 mg of HD). The 20:1 ratio was arbitrarily chosen after discussions with USAMRDC personnel.

The powder was applied to the appropriate site at the predetermined time sequence and rubbed into the dosed site with a back and forth motion perpendicular to the spine for 5 sec using a cotton-tipped swab. A piece of cardboard was held behind the immediate site of application to minimize contamination of the other dosing sites with the powdered decontaminant. The cardboard was necessary to minimize contamination of adjacent sites, which could be caused by the 165 linear feet per minute of incoming hood air that was passing over the back of the rabbit during the decontamination process. This air velocity was mandated by safety/surety regulations to protect the personnel working in front of the chemical fume hoods.

The standard and test decontaminants were washed off with 5 percent sodium hypochlorite followed by distilled water immediately prior to lesion evaluations.

## 2.5 Lesion Evaluation

The model contained two evaluation schemes for determining the effectiveness of the experimental liquid or powder decontamination material as compared to the standard M258A1 II or Fuller's Earth system. The first evaluation scheme was a modification of the Draize method for evaluation of primary irritation of the skin (Draize, J. H. 1979. Dermal Toxicity. In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Edited and published by The Association of Food and Drug Officials of the United States, pp. 46-59). The degree of irritation at 20-24 hr after exposure was estimated by scoring separately two skin reaction responses: erythema and edema formation. The "primary irritation index" was calculated by adding the score values for erythema and edema formation for similar treatment sites and dividing by the total number of similar treatment sites. The scoring was guided by the following:

| Erythema Formation | |
|---|---|
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well-defined erythema | 2 |

| | |
|---|---|
| Moderate to severe erythema | 3 |
| Severe erythema (beet redness) to slight eschar formation (injuries in depth) | 4 |
| Highest possible erythema score | 4 |
| Edema Formation | |
| No edema | 0 |
| Very slight edema (barely perceptible) | 1 |
| Slight edema (edges of area well-defined by definite raising) | 2 |
| Moderate edema (raised 1 mm but not extending beyond area of exposure) | 3 |
| Severe edema (raised more than 1 mm and extending beyond area of exposure) | 4 |
| Highest possible edema score | 4 |
| Highest possible "primary irritation index" score | 8 |

Descriptive words were correlated with values of the primary irritation index as follows:

0-2 = Mildly irritating
>2-5 = Moderately irritating
>5-6 = Moderately to severely irritating
>6-8 = Severely irritating.

The second evaluation scheme was based on a visual estimate of the length (size) of the lesion at each application site. The lesion length was estimated on the backs of anesthetized rabbits by matching the diameter of the long axis of the affected area with a series of reference circles with known diameters. The long axis was chosen arbitrarily as the initial estimator of lesion involvement. The transverse axis or some relationship between the two axes may also provide a means of discrimination between effective and noneffective decontamination systems.

The estimated lesion length from each experimental compound site was compared with the contralateral estimated lesion length at the corresponding

time interval for the standard decontamination system. The ease of estimation of lesion length was enhanced by the intramuscular injection of 1 ml of a 3 percent suspension of trypan blue dye in saline to each thigh of the rabbit 2-4 hr prior to lesion length evaluations. Photographs were taken of the lesions to supplement the estimates if necessary.

After lesion evaluation at 24-28 hr after exposure, the rabbits were killed by administering T-61. The lesions were reread and additional photographs were taken if necessary.

## 2.6 Necropsy and Histopathology

No tissue samples were saved and all animal carcasses were decontaminated and discarded.

## 2.7 Statistical Analysis

The lesion size data analyses included graphical displays, summary statistics, and application of analysis of variance methodology to estimate biologically important contrasts among the measured lesion sizes (lengths) and to determine the degree of their statistical significance. In the skin irritation evaluations (i.e., Draize scoring), each animal served as its own control. Thus, comparisons of the effectiveness of candidate versus standard decontaminants were based on internal comparisons of contralateral sites within each animal. Separate analyses were carried out for each decontaminant.

### 2.7.1 Lesion Length Analysis

The preliminary scatter plots and summary statistics displayed the average lesion lengths associated with the standard decontaminant and with the candidate decontaminant at each of the three times of application. These displays provided direct visual comparisons of the effectiveness of the candidate decontaminant relative to the standard decontaminant at each time of application, as well as comparisons with the average lesion length obtained

when no decontaminant was used. The data and analyses do not provide a comparison of the potential effects of decontaminants over the three time periods because of the possibility of an anterior-posterior positional effect.

The analysis of variance methodology utilized a multifactor analysis of variance model. The analysis of variance model incorporated the factors: day (replicate), animal (tested within day), decontamination treatment (candidate, standard, none), time to decontamination (early, intermediate, late), and within animal variability. The model reflected two sources of experimental variation: animal-to-animal variation and within-animal variation. Since comparisons between candidate and standard decontamination treatments and comparisons across time to decontamination were based on contrasts within animals, the precision and statistical significance of each of these comparisons were based only on the within-animal variability. Since different animals were placed on study on each of the 3 replicate days, comparisons among days were based on comparisons across animals; they thus incorporated animal-to-animal variation as well as within-animal variation.

The analysis of variance model tested for the possibility of overall day (replicate) effects by comparing the day effect with the variability observed among animals within each day. The day effect was calculated by averaging all of the lesion lengths (seven) within each animal and then averaging over the eight animals tested on each day. These daily averages were compared to one another, using the variability among animals within each day as an error yardstick. Significant differences among the daily averages indicated overall day-to-day differences in either the animals, the experimental conditions, or both; these differences may be confounded by an anterior-posterior positional effect.

Comparisons between the candidate and standard decontamination treatments were based on contrasts within animals. To determine whether it was appropriate to pool the data across days to estimate these contrasts, we first tested whether there were any significant day-to-day differences in the contrasts. This was done by testing for the presence of interaction between measurement and day, using the within-animal variability as an error yardstick. The measurement-by-day interaction was based on contrasts among the seven measurements within each animal. These contrasts were averaged over

the animals tested on each day. The average values of these contrasts on each day were compared. If no significant differences existed among days, we pooled the test results across all 3 days for further analyses of the contrasts. If a significant measurement-by-day interaction existed, we evaluated the reason for the significance of the interaction to determine whether the data could be pooled across replicates.

Decisions concerning how to pool the data across days were made on a case-by-case basis. For example, the significant measurement-by-day interaction might be due to a single outlying animal or to several individual outlying measurements. The outlying animal(s) or the individual outlying measurements might then be deleted if there was appropriate reason to do so. Alternatively, there might be systematic day-to-day differences that need to be accounted for in the subsequent analyses.

If the measurement-by-day interaction was not significant, subsequent comparisons between the candidate and the standard decontamination treatments were based on the results observed in all animals, pooled across all replicates at contralateral sites. Contrasts between the standard and the candidate decontaminants that were of particular interest were:

Standard less Candidate - earliest decontamination time
Standard less Candidate - intermediate decontamination time
Standard less Candidate - latest decontamination time
Standard less Candidate - composite (average over the three decontamination times).

For each of these contrasts (and any others of interest), the average value (over the 24 animals) and its standard error were calculated. A (one-sample) t-ratio and its (two-sided) significance level were also calculated.

If the standard less candidate-composite contrast was negative and statistically significant ($P < 0.05$), the candidate decontaminant was considered to be inferior to the standard decontaminant and was screened out from further testing. Otherwise, the candidate was passed on for further stages of testing.

A crude ranking of the efficacy of different candidate decontaminants can be based on the results of these analyses. Namely, the

candidates can be grouped, based on the standard less candidate contrast, as follows:

1. Negative $P < 0.05$
2. Negative $P > 0.05$
3. Positive $P > 0.05$
4. Positive $0.01 < P < 0.05$
5. Positive $P < 0.01$.

Candidates in the first category are deleted from further testing. Candidates in the remaining categories are continued on for further testing, with increasing priority given to the higher numbered categories.

### 2.7.2 Comparison of Measurements Made Before and After Euthanasia

Comparisons of the differences in lesion length between the measurements taken after anesthesia but before euthanasia ("before death") with those taken immediately after euthanasia ("after death") were carried out to determine whether there were any substantial differences in lesion lengths before and after death. If the two sets of estimates were essentially the same, then just one set could be used for determination of decontaminant effects. For the mustard-liquid, mustard-powder, L-liquid, and L-powder comparisons, the "before death" lesion lengths associated with the candidate decontaminants were compared with the corresponding "after death" lesion lengths associated with the candidate decontaminants. For each animal, differences ("before" minus "after") were calculated for the early, intermediate, and late times to decontamination and for the average over all three times to decontamination.

# 3.0 RESULTS

Tables are presented in Appendix C and Figures are presented in Appendix D.

## 3.1 Mortality

None of the rabbits in the HD validation studies died or were terminated in moribund condition during the study. One rabbit (A1606M) in the L validation study for liquid decontaminants died shortly after dosing from what appeared to be suffocation caused by the pressure of the stanchion on the trachea. Another rabbit (A1660M) in the same validation study died shortly after taking the Draize irritation readings from what appeared to be nontreatment-related suffocation, possibly due to the anesthesia or positioning of the rabbit in the stanchion after lesion evaluation.

## 3.2 Clinical Observations

Skin lesions occurred in all rabbits at every site of application of HD or L within the 24- to 28-hr study period for both the liquid and powder decontamination studies. The lesions varied in shape from a line just extending along the dosing line to the formation of an irregular, elliptical or elongated lesion involving more area than the dosing site to a fuller, irregular, circular-like lesion extending well away from the dosing site. Edema and erythema were always present in association with the lesion, and in the case of L, the edema on the backs of the rabbits was so severe that it caused an edema cap over the entire treated area. Eschar formation was present at the application sites for both HD and L, but was more involved for L than HD. In the L animals, necrotic areas extended well beyond the dosing line, in some cases forming an almost circular lesion around the dosing area.

Loss of skin tone and elasticity was observed with both HD and L, the most pronounced effects occurring following treatment with L.

## 3.3 Draize Irritation Evaluation

The estimations of irritation caused by the application of HD are given in Tables 3.3.1 (liquids) and 3.3.2 (powders) and those for L are given in Tables 3.3.3 (liquids) and 3.3.4 (powders). Photographs are presented in Plates 1-14 in Appendix E. The erythema and edema estimates for L in both liquid and powder studies were always 4 (most severe) at each time period and for each liquid and powder decontamination material, including the M258A1 II and Fuller's Earth standard.

The erythema and edema estimates for distilled water as a test liquid decontaminant for HD ranged from 3.6 to 4.0 and were not statistically different ($P > 0.05$) from the 3.9 to 4.0 for the undecontaminated HD control. The estimates for erythema for the M258A1 II standard liquid decontamination system against HD ranged from 3.5 to 3.9 (grand average of 3.7) and were not statistically different ($P > 0.05$) from the 3.9 to 4.0 for the undecontaminated control. The edema average values for the M258A1 II standard liquid decontamination system against HD ranged from 1.9 at the 1.25-min after exposure decontamination time to 3.4 at the 10 min decontamination time period. The values increased at each time interval and those at the 1.25- and 5.0-min time periods were statistically different ($P < 0.05$) from the nondecontaminated controls at the same time period. No erythema or edema was noted at any time period for the water application control site (site H).

The erythema and edema estimates for HD for both the Fuller's Earth and powdered marble dust were 3.9 or 4.0 (most severe) for every time period and were not statistically different ($P > 0.05$) from the 3.9-4.0 scores for the undecontaminated mustard control.

## 3.4 Lesion Length Evaluation

Lesion lengths were estimated at 24-28 hr after exposure following trypan blue dye administration and anesthesia (before death) and again shortly after euthanasia with T-61 (after death). Estimates for lesion length determinations for HD versus liquid decontamination materials before death and after death are given in Tables 3.4.1 and 3.4.2, respectively, and for

powdered decontamination materials in Tables 3.4.3 and 3.4.4, respectively. Lesion length estimates for L versus liquid decontamination materials made before euthanasia and after euthanasia are given in Tables 3.4.5 and 3.4.6, respectively, and for powdered decontamination materials in Tables 3.4.7 and 3.4.8, respectively.

Averages for HD estimates are given in Table 3.4.9 (liquids) and in Table 3.4.10 (powders) and for L in Table 3.4.11 (liquids) and in Table 3.4.12 (powders). The data are presented graphically in Figures 3.4.1 and 3.4.2 for liquid decontamination of HD, in Figures 3.4.5 and 3.4.6 for liquid decontamination of L, and in Figures 3.4.7 and 3.4.8 for powdered decontamination of L.

### 3.4.1 Lesion Lengths

There was a significant difference ($P < 0.01$) between the lesion lengths from decontamination with M258A1 II versus distilled water at each time period for both HD and L. In every case, the M258A1 II treatment was significantly better than distilled water or no treatment at all, and the shortest time interval between exposure and decontamination (or the anterior-most site if there is a positional effect) produced the smallest lesion length (although all time periods produced severe lesions). There appeared to be a time-related or position-related response in lesion length for the M258A1 II standard decontamination for both HD and L. Water was not effective as a liquid decontamination material, although it appeared that the mechanical action of applying the water did produce a slight lessening of the involved area when compared to the nondecontaminated control.

There was a significant different ($P < 0.01$) between the lesion lengths from decontamination of L with Fuller's Earth versus marble dust for all time periods. The Fuller's Earth treatment was significantly better than no treatment at all time periods and the effectiveness appeared to be directly related to the time period between decontamination and application or to the position of treatment on the backs of the animals. Marble dust provided little protection against the effects of L and the lesions produced were similar to lesions produced with no decontamination.

Fuller's Earth appeared to be slightly but not statistically ($P = 0.06$) better than marble dust as a decontamination material for HD. Both powders were better than no treatment at the two shorter time periods but were judged not effective at the 10-min time period after HD application.

There appeared to be a time-related or position-related response in lesion length for both the single-component M258A1 II liquid decontamination system and Fuller's Earth powder for both HD and L.

### 3.4.2 Measurement Time

The average values for lesion lengths measured prior to and after administration of T-61 for both HD and L exposures and subsequent decontamination with M258A1 II or water were not statistically different ($P > 0.05$) at any time period for liquid or powdered decontamination systems. The lesion lengths measured after euthanasia appeared to be the most variable, possibly due to the loss of hyperemic tissue definition surrounding the necrotic area of the lesion, although statistically the two estimations of lesion lengths were not different ($P > 0.05$).

The evaluations were carried out as described previously, with differences ("before" minus "after") for each animal calculated for the early, intermediate, and late times to decontamination and for the average over all three time periods. The distributions of these differences are summarized below (there are 16 cases: 2 CSM x 2 decontaminant types x 4 times to decontamination):

LESIONS AFTER HD APPLICATION AND WATER DECONTAMINATION
(BEFORE DEATH MINUS AFTER DEATH)

| | 1.25 Min | 5 Min | 10 Min | Average |
|---|---|---|---|---|
| Number of Rabbits | 24 | 24 | 24 | 24 |
| Mean | 0.17 | -0.83 | -0.75 | -0.47 |
| Standard Deviation | 1.43 | 1.43 | 1.78 | 1.16 |
| Standard Error | 0.29 | 0.29 | 0.36 | 0.24 |
| T-Significance Level | 0.57 | 0.009** | 0.05* | 0.06 |
| 5th Percentile | -3 | -3.75 | -4.5 | -3.17 |
| 25th Percentile | 0 | -2 | -2 | -1.25 |
| 50th Percentile | 0 | 0 | -1 | -0.5 |
| 75th Percentile | 1 | 0 | 0 | 0 |
| 95th Percentile | 2.75 | 1.75 | 2.75 | 1.83 |

LESIONS AFTER HD APPLICATION AND MARBLE DUST DECONTAMINATION
(BEFORE DEATH MINUS AFTER DEATH)

| | 1.25 Min | 5 Min | 10 Min | Average |
|---|---|---|---|---|
| Number of Rabbits | 24 | 24 | 24 | 24 |
| Mean | -0.08 | 0.42 | 0.75 | 0.36 |
| Standard Deviation | 2.80 | 2.57 | 2.19 | 2.05 |
| Standard Error | 0.57 | 0.52 | 0.45 | 0.42 |
| T-Significance Level | 0.89 | 0.44 | 0.11 | 0.40 |
| 5th Percentile | -5.5 | -4 | -3.5 | -3.17 |
| 25th Percentile | -2 | -2 | 0 | -1.33 |
| 50th Percentile | 0 | 0 | 0 | 0 |
| 75th Percentile | 2 | 2 | 2 | 2 |
| 95th Percentile | 5.5 | 6 | 5.5 | 4.33 |

LESIONS AFTER L APPLICATION AND WATER DECONTAMINATION
(BEFORE DEATH MINUS AFTER DEATH)

| | 30 Sec | 60 Sec | 120 Sec | Average |
|---|---|---|---|---|
| Number of Rabbits | 22 | 22 | 22 | 22 |
| Mean | -1.45 | -0.14 | -0.32 | -0.64 |
| Standard Deviation | 2.15 | 2.01 | 1.91 | 1.25 |
| Standard Error | 0.46 | 0.43 | 0.41 | 0.27 |
| T-Significance Level | 0.005** | 0.75 | 0.44 | 0.03* |
| 5th Percentile | -5.7 | -3 | -5.55 | -3.23 |
| 25th Percentile | -3 | -1.25 | -1 | -1.42 |
| 50th Percentile | -1 | 0 | 0 | -0.83 |
| 75th Percentile | 0 | 0 | 0.25 | 0.08 |
| 95th Percentile | 3.4 | 3.85 | 2.85 | 1.57 |

LESIONS AFTER L APPLICATION AND MARBLE DUST DECONTAMINATION
(BEFORE DEATH MINUS AFTER DEATH)

| | 30 Sec | 60 Sec | 120 Sec | Average |
|---|---|---|---|---|
| Number of Rabbits | 24 | 24 | 24 | 24 |
| Mean | -0.25 | -0.17 | 0.08 | -0.11 |
| Standard Deviation | 1.70 | 1.31 | 1.50 | 1.05 |
| Standard Error | 0.35 | 0.27 | 0.31 | 0.21 |
| T-Significance Level | 0.48 | 0.54 | 0.79 | 0.61 |
| 5th Percentile | -3.5 | -3.5 | -2 | -1.83 |
| 25th Percentile | -2 | 0 | -1.5 | -0.67 |
| 50th Percentile | 0 | 0 | 0 | 0 |
| 75th Percentile | 0 | 0 | 2 | 0.5 |
| 95th Percentile | 3.5 | 2 | 2 | 2 |

* = $P < 0.05$.
** = $P < 0.01$.

The average differences were within 1 mm in 15 of the 16 cases (the exception, L-$H_2O$-30 seconds, was 1.45 mm). The average differences were negative in 11 of the 16 cases, indicating that the before death lesion averages were smaller than the after death lesion averages. The middle 50 percent of differences (between the 25th percentile and the 75th percentile) was within 2 mm in 15 of 16 cases. The middle 80 percent of differences (between the 10th percentile and the 90th percentile) was within 3 mm in 11 of 16 cases and was within 4 mm in all cases (not shown). The extreme 10 percent of differences (below the 5th percentile or above the 95th percentile) varied by as much as 5-6 mm.

Tables 3.4.9 to 3.4.12 show that the same conclusions concerning the significance of differences between the candidate decontamination lesion lengths and the standard decontamination lesion lengths were arrived at in all four cases (HD and L with liquid and powder), irrespective of whether measurements were made before or after euthanasia. Since comparisons between candidate and standard decontaminants were based on averages across animals and since the average differences between the estimates taken before and after death were within 1 mm in seven of eight cases for the candidate decontaminants and in all cases for the standard decontaminants, the comparisons between test and standard were essentially the same, irrespective of which set of lesion lengths was used. Thus, only one set need be used in future evaluations. We recommend that the measurements be made prior to euthanasia so that remeasuring can be done if necessary.

## 4.0 DISCUSSION

The model chosen for this study has been successfully validated against HD and L exposures followed by decontamination with the component II of the currently fielded liquid decontamination material, the M258A1 kit system, and a powder decontamination material designated by the USAMRDC, Fuller's Earth. Distilled water and marble dust served as experimental decontaminants to test the model's ability to reject a liquid or powder decontaminant that was not as effective as the standards. At all time periods

for both CSM, the single-component M258A1 II system was significantly ($P < 0.05$) better than the water and the water was rejected for further testing in higher-tier models on the basis of these results. At the shorter time periods for L and HD, the Fuller's Earth was better than the marble dust, but was equivalent to the marble dust at the longest time period.

The Draize skin irritation evaluations were not useful as a screening parameter for L with the current protocol conditions. Every time period for decontamination after exposure produced the maximum readings for erythema (4.0), edema (4.0), and primary irritation index (8.0). The shortest interval between L application and decontaminant, 30 sec, was the minimum time period in which accurate and reproducible application of CSM and subsequent application of decontaminant could be accomplished mechanically in the hood. Thus, the Draize irritation evaluation is not effective as a screening parameter for L in which the dose is held constant at 0.5 µl and the time is the variable. Therefore, we recommend that this series of evaluations be deleted from MREF Protocol 1 for L.

The two series of lesion length determinations, those made before euthanasia and those made after euthanasia, produced similar readings for both HD and L for both liquid and powdered decontamination materials. In only 1 of 16 cases were the average differences between the two readings larger than 1 mm (and that average was 1.45 mm). Thus, there were essentially no differences at any of the time periods between the average lesion lengths measured before or after euthanasia. Therefore, we recommend that only one set of lesion length determinations be taken for MREF Protocol 1 studies with HD or L. Since there is the opportunity for taking additional measurements if the initial measurements are made prior to euthanasia, we recommend that the single determination of lesion size be taken before euthanasia; this also allows for the possible need for observation of the lesions by additional researchers (which is limited when the readings are taken after the administration of the euthanasia drug).

Photographing the lesions for both CSM was a cumbersome and imprecise process because of the requirement to keep the treated rabbits within the hood and at least 20 cm from the hood face. The restrictions in the positioning of the rabbits, because of the hood requirement and the

necessity to handhold the 35-mm camera (with bellows and macro lens) to provide focusing and shot selection, produced slides that were often unusable. Unless the rabbits can be removed from the hood prior to photographing the lesions or unless a camera system can be permanently dedicated to each hood bank so that a fixed stand and light assembly can be used, we recommend that the photography currently included in MREF Protocol 1 be eliminated as a recording process and be included for visual reference only.

The validation studies pointed out several area where possible protocol modifications or different procedures should be considered in addition to those given above. The model described in MREF Protocol 1 used a fixed dose concept and varied the time-to-decontamination to discern between effective and ineffective candidates. For L in particular (and mustard to a lesser degree), the constant dose produced lesions that were irreversible within 10-15 sec and at doses as low as 0.25 µl of L (preliminary methodology studies not included in this report). Shorter time periods are not possible because of mechanical and time constraints in making the DCM and decontaminant application to the same area within the time allotted. Reducing the dose volume may be feasible, but a large reduction may cause mechanical problems in reproducing the application of such a small volume on the backs of rabbits. Therefore, a meaningful irritation screen involving L and candidate liquid or powdered dermal decontaminants may not be practical, and the only parameter for discerning effective versus ineffective materials may be lesion length evaluations. We recommend that an experiment be conducted with neat L (and HD) to hold the time-to-decontamination constant and vary the dose between practical application limits to ascertain if Draize irritation evaluations can be an effective discriminator in this screen.

The lesion length evaluations are based on the estimation of the length of the lesion along the application line. Other useful parameters for measurement may be the width of the lesion (that distance perpendicular to the application line) or some mathematical manipulation (gross estimate of lesion "area") of these two measurements. We collected the data for both length and width for the L validation series with Fuller's Earth and marble dust to provide input for possible analysis. These data should be analyzed and their usefulness in comparing lesion involvement between treatments assessed.

Appropriate recommendations for modifications to the current protocol can then be made if warranted.

The relationship between positional effect and time dependency in this protocol should be evaluated. The data from these studies indicate that either a positional or time effect (or both) could exist for both the single-component M258A1 II system and Fuller's Earth for L and for the M258A1 II system for HD. The contralateral, paired comparison scheme for the analysis of acquired data suggests a time-related effect, but does not rule out a positional effect or at least a positional contribution to a time-related effect.

The comparisons between standard and candidate decontaminants that are of current primary interest are contralateral comparisons of lesion lengths, corresponding to equal times to decontamination, and as such, they are unaffected by positional effects. It would be of interest to study the extent of changes in lesion length with increases in time to decontamination, either for the candidate or the standard decontaminant. Such comparisons, however, are confounded with positional effects. Since the lesions corresponding to longer times to decontamination are farther toward the back of the animal, any systematic trends in skin sensitivity from front to back may interfere with the observed trends in lesion sizes due to time to decontamination. There is no way to separate these effects with the current protocol.

A special study is being undertaken as a part of another phase of Task 84-1 to examine the influence of position on lesion estimation (length and width). If this study reveals the absence of positional effects on lesion length, then the effects of time to decontamination with each individual decontaminant can be determined from MREF Protocol 1 if the study reveals the presence of positional effects, then MREF Protocol 1 cannot be used to determine positional effects with individual decontaminants.

The evaluations of irritation and lesion involvement could be facilitated greatly if the rabbits could be removed from the hood. Photography would be enhanced as previously mentioned. We recommend an experiment be performed to evaluate the time period in which active CSM is still on the backs of the rabbits and in which off-gassing occurs. The

experiment could include several groups of rabbits at time periods of 1, 2, 4, 6, and 24 hr, for example. Previous information available from Dr. Mershon at USAMRDC indicated that off-gassing of HD applied as a vapor with the use of a "vapor-cup" assembly was undetectable as early as 2 hr after dermal application and that after 4 hr, it was safe to remove rabbits from the hoods without decontamination and to house them in conventional caging. Although the application methods are different, the results of Dr. Mershon's experiment indicate that it may be possible to remove HD-treated rabbits from the hood at some time interval prior to 24 hr after dosing.

The current design calls for use of equal numbers of male and female animals. Gender, however, is not currently included in the statistical model used to analyze the data. The previously obtained lesion data could be reanalyzed, and the results used to suggest how this facto could be treated in future tests, both with respect to experimental design and data analysis.

The significance levels currently reported for the paired t-tests on the contrasts are two-sided levels. That is, they indicate whether the differences (standard less candidate) are significantly positive or negative. It has been suggested that a 5 percent, one-sided level be used as a decision criterion. Under this criterion, a candidate decontaminant would be significantly worse than the standard decontaminant ($P = 0.05$, one-sided) if the contrast was negative and the reported significance level was less than 0.10 (two-sided). This would result in screening out greater numbers of decontaminants than with use of the two-sided, 5 percent criterion. Use of the one-sided criterion would be essentially the same as that currently being used, except that the decision point would be reduced. Candidate decontaminants passing the modified screen criterion could be ranked exactly in the manner discussed previously.

The previously described analyses compare average lesion lengths associated with the candidate and the standard decontaminants. It would also be of interest to study variability of the lesion lengths. If two decontaminants result in equivalent average lengths, the one with the lesser variability would be preferable.

The present analysis approach treats each comparison of a candidate decontaminant versus standard decontaminant as a completely separate analysis.

No consideration is given to relationships among the formulations of the different candidate decontaminants. If appropriate combinations of formulations were available, the result from the lesion length comparison tests with the different candidates could be analyzed together to determine which candidate constituents were most strongly associated with reductions in lesion lengths. This can be accomplished, but modifications may be necessary in experimental design and data analysis to determine which combinations of test formulations would be needed to best separate the effects of the individual constituents.

## 5.0 RECORD ARCHIVES

Records pertinent to the conduct of this study are contained in Battelle Laboratory Record Book Nos. MREF-6, MREF-10, and MREF-16. All prestudy animal quarantine and observations are on file at the MREF. All original data, as well as the original final report, will be maintained in the secured files of the MREF until forwarded to the USAMRDC at the conclusion of the project or until microfiched and permanently archived at Battelle.

## 6.0 ACKNOWLEDGMENTS

The names, role in the study, and highest degree of the principal contributors in this study are presented in the following list:

| Name | Title | Degree |
|---|---|---|
| Dr. Ronald L. Joiner | Study Director | Ph.D. |
| Dr. H. Hugh Harroff, Jr. | Chief Veterinarian | D.V.M. |
| W. Bruce Keys, Jr. | Study Supervisor | B.A. |
| Dr. Paul I. Feder | Biostatistician | Ph.D. |

# APPENDIX A

MREF Protocol 1 --- "Dermal Study for the Assessment and Validation of Decontaminants in Rabbits Against Mustard and Lewisite"

# Dermal Study for the Assessment and Validation of Decontaminants in Rabbits Against Mustard and Lewisite

Study Performed by Battelle Columbus Laboratories,
505 King Avenue, Columbus, Ohio 43201

1. Study Director: Ronald L. Joiner, Ph.D.

2. Veterinarian: H. Hugh Harroff, Jr., D.V.M.

3. Sponsor: U.S. Army Medical Research and Development Command

4. Sponsor Monitor: LTC Howard Johnson, USAMRICD

5. Objective:

To develop and validate a quantitative animal model and experimental method for screening and testing decontaminants against mustard (HD) and lewisite (L) exposure.

6. Experimental Design:

A. Test System

Albino rabbits were chosen for this study on the basis of the extensive data base available for this species and on the size of the application area for multiple challenges with neat agent.

(1) Strain -- New Zealand White (albino) rabbits (male and female), supplied by Kings Wheel Rabbitry.

(2) Initial Weight -- 2.0 to 4.0 kilograms.

(3) Selection -- Animals selected after a minimum 7-day quarantine period are in good physical condition. Rabbits are weighed and assigned to groups based on body weight, sex, and hair growth cycle stage.

(4) Acclimation -- All animals are held at the Medical Research and Evaluation Facility for at least 24 hours prior to study initiation.

(5) Animal Identification -- All animals are ear tagged to retain positive identification for all care involving animal handling and observations. Cage cards are color-coded by group.

(6) Housing -- Animals are housed individually in stainless steel, slotted cages equipped with automatic watering systems.

(7) Lighting -- Fluorescent lighting, light/dark cycle is 12 hours each per day.

(8) Temperature -- Maintained at 70F ($\pm$5F).

(9) Humidity -- Maintained at 50% ($\pm$ 10%).

(10) Diet -- Purina Certified Rabbit Chow pellets are available at all times during animal quarantine and holding. No contaminants are known to be present in the feed which would interfere or affect the results of the study.

(11) Water Supply -- Water is supplied from the public water system and given ad libitum during quarantine and holding. No contaminants are known to be present in the water which would affect the results of the study.

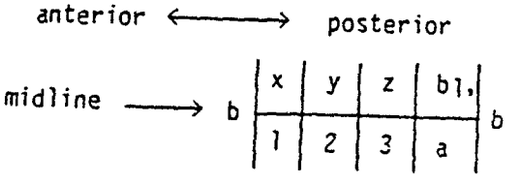
(12) Animal Care During Test -- All animals are housed in stanchions for treatment in individual restraint cages for the remainder of the test period. No food is provided during the 28 hours of the test.

B. Test Groups

(1) Size -- Routine screening tests are performed with groups of 8 animals. Group matching is based on individual and total group weight, sex, and hair growth cycle stage.

(2) Number -- Two groups of animals are used for each series of exposures. One test group receives HD and the appropriate decontamination solutions and the second group receives L and the decontamination solutions.

(3) Sequence -- Each animal in each group receives a series of 0.5 µl doses of HD or L along the dorsum of the back in the following pattern:




where a = agent control without decontamination,
b = control for standard decontamination material,
b1 = control for test decontamination material,
x = agent plus standard decontamination material after minimum time period,
y = agent plus standard decontamination material after middle time period,
z = agent plus standard decontamination material after maximum time period,
1 = agent plus test decontamination material after minimum time period,
2 = agent plus test decontamination material after middle time period,
3 = agent plus test decontamination material after maximum time period.

(4) Dose -- The volume applied for each agent at each position is 0.5 µl.

C. Test Material

The M-258A1 II slurry or suspension is used as the standard decontamination system against HD and L exposure. Distilled water is used as the test decontamination material for comparison of effects.

(1) The M-258A1 II kit materials are supplied by the USAMRDC/ICD.

(2) Lewisite and HD are supplied by the USAMRDC/ICD. Purity, appropriate identification (batch number, lot number, state), and stability data are supplied by the USAMRDC/ICD. Purity and stability are confirmed periodically by Battelle for material stored at the Hazardous Materials Laboratory.

(3) Surety, security, and safety procedures for the use of L and HD are thoroughly outlined in facility plans, in personnel requirements for qualifications to work with agents, and in agent storage and use standard operating procedures. Specific procedures have been included in this document to ensure the safety of the personnel conducting this experiment.

D. Preparation of Animals for Exposure to Vesicants

(1) Hair Clipping -- All animals are acclimated in approved cages at the MREF for at least one day before use. Study animals are closely clipped from withers to rump with care to avoid skin damage. An Oster Model A-2 animal clipper with a No. 40 blade, or equivalent, is used to clip animals at least 24 hours prior to the intended use.

Clipped backs of rabbits are graded for relative vascularization and hair density to indicate estimated percent maximal hair growth. For example, a back with a large island of dense white hair stubble on the whole exposure area and a surrounding margin (of purple-pink color) that is noticeably elevated above surrounding normal (thin, yellowish pink) skin that shows superficial venation is graded 100 percent growth. When thin hair, thin pale skin, venation, and absence of vascularized areas within the exposure zone are the observed signs, the skin is graded 0. If 6 of 6 exposure sites can be asymmetrically located so that the exposure area is over skin without appreciable hair growth, the grading is 20 percent. If large islands of growing hair and vascularized skin lie among areas having resting follicles, such back area is unsatisfactory, and the rabbit is rejected as unsuitable for use in such tests. Skin sites uniformly covered with growing hair but with moderate vascularization may be graded 50 percent on the basis that growth is only half as vigorous as on active growth sites.

This grading method is used to assign rabbits into groups of 8 that have equivalent of paired variations in matched test sites. Animals are clipped two days prior to intended use and graded at or after 24 hours in which to grow hair. Rabbits are reclipped, if necessary, after anesthesia has been induced to prevent shielding of exposure sites by hair stubble.

(2) Paired Selection -- The selection of paired animals is based on the stage of hair growth cycle and the condition of the skin in the mid-dorsal area of the animal's back. Routine screening is performed with groups of 8 animals matched by individual weight, sex, and hair growth.

(3) Anesthesia -- Test rabbits are anesthetized prior to treatment by the intermuscular administration of a mixture of Ketamine and Rompun. The unconscious rabbits are placed in prone position in metal stanchions. Animals are then positioned inside exposure hoods and the hood sash positioned to maintain average air flow of 165 ft/min past the rabbit noses.

E. Application of Vesicants

(1) Agent (0.5 µl of L or HD) is applied to each of 7 spots on the back of each rabbit as a small streak (approximately 1 cm in length) with a microliter syringe. Lewisite is delivered using a Hamilton microliter syringe equipped with a special platinum needle (barrel) and a standard tungsten plunger. Standard stainless microliter syringes are used to apply HD.

(2) Agent and decontaminants are applied by the sequence given in 6.8(3).

F. Decontamination Techniques

(1) The test decontaminating solution to be applied to animals exposed to lewisite and mustard is distilled water. The standard decontaminating material is the M-258A1 II slurry.

(2) The duration of exposure before the onset of decontamination is the most critical factor to decontamination procedures. Decontamination for lewisite is a timed sequence that begins at 30

seconds and progresses to 60 and 120 seconds. For mustard, a sequence of 2.5, 5.0, and 10.0 minutes are used. Modifications may have to be made to the initial time sequences to provide a series of graded readings approximating slight, moderate, and severe irritation.

(3) The mechanical method used to remove agent is the second most critical factor in skin decontamination. The decontamination process consists of wiping a pad wetted with the decontamination solution on the exposed area for a period of 5 seconds in a circular motion within the exposure grid area.

(4) The standard decontamination pad is made by cutting the M-258A1 kit cloth into strips and taping the cut cloth segment to a tongue depressor. The cloth is then wetted with the amount of liquid from the kit that corresponds to the proportion of cloth used to make the decontamination pad. The kit contains the prescribed volume of M-258A1 II slurry given in the MIL specifications for the M-258A1 kit.

(5) The wetted cloth is wiped briskly but not harshly for 5 seconds in a circular motion within each grid area.

(6) The pad is deposited into decontaminating solution immediately after use.

(7) The decontamination pad for the test decontamination solution is made as above from a strip of non-impregnated cloth that is the precursor of the M-258A1 kit material (the cloth does not contain the chloramine B but is of identical texture).

G. Lesion Evaluation

(1) Irritation Determination -- On the morning following exposure to HD or L (at approximately 20 to 24 hours post-exposure), lesions are evaluated and scored by a modification of the Draize method for primary irritation of the skin (Draize, J.H. 1979. Dermal Toxicity. In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Edited and published by The Association of Food and Drug Officials of the United States, pp 46-59). The degree of irritation at 20-24 hours post-exposure is estimated by scoring two individual sets of skin reactions: erythema and

edema formation (see table below for scores). The primary irritation index is calculated by adding the values for erythema and edema formation for similar treatment sites and dividing by the total number of similar treatment sites. For example, all sites treated with 0.5 µl HD for 60 seconds before decontamination with the standard decontamination material are scored individually for edema and erythema formation; the resulting scores are summed for each site; scores from all other treatment sites with 0.5 µl of HD for 60 seconds prior to standard decontamination are summed; and the total is divided by the total number of sites included in the summation. The resulting combined average is the primary irritation index for 0.5 µl of HD for 60 seconds prior to decontamination with standard decontamination material.

Erythema Formation

| | |
|---|---|
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well-defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (beet redness) to slight eschar formation (injuries in depth) | 4 |
| Highest possible erythema score | 4 |

Edema Formation

| | |
|---|---|
| No edema | 0 |
| Very slight edema (barely perceptible)1 | |
| Slight edema (edges of area well-defined by definite raising) | 2 |
| Severe edema (raised more than 1 mm and extending beyond area of exposure) | 4 |
| Highest possible edema score | 4 |
| Highest possible total score | 8 |

Descriptive words are correlated with values of the primary irritation index as follows:

>0 -2 = Mildly irritating

>2 -5 = Moderately irritating
>5 -6 = Moderately to severely irritating
>6 -8 = Severely irritating

(2) Dye Injection -- Following the irritation evaluation, each animal is given a 1-ml intramuscular injection (in each thigh) of a 3% suspension of trypan blue dye in saline. The dye requires at least two hours to translocate throughout the damaged vessels of the exposed areas. The dye forms a dark blue marking of the lesion against the contrasting pale blue of adjacent normal skin. A pink halo may extend for 2-4 mm wider than the blue zone, which presumably is indicative of active hyperemia.

(3) Anesthesia -- Approximately 2-4 hours after administration of the dye, the test animals are anesthetized by a dose of ketamine/rompun.

(4) Lesion Size Determination -- After anesthesia (at approximately hour 24 to hour 28), the lesion at each test area is visually compared with the lesion at the corresponding time interval for the standard M-258A1 II decontamination slurry. The observer estimates the size of the affected area by matching the total involved area with a series of reference spots with known areas. A Medical Nikon 35 mm or similar camera is used to record the lesions.

(5) Euthanasia -- After photographing the lesions, the test animals are killed by administering T-61. The lesions are re-read and additional photographs are taken, if necessary.

(6) Extended Observation Period -- In some instances, it may be necessary to extend the observation period beyond the initial period at hour 28. The lesion is decontaminated with 5% sodium hypochlorite and washed with water prior to quantitating the lesion. The animals are placed into holding cages after the initial observation and held for 2-7 days for observation of necrosis, healing, etc.

H. Disposal of Experimental Animals

(1) Decontamination -- After euthanasia, each vesicant exposure area is thoroughly rubbed with a saturated gauze pad or other applicator saturated with 5% sodium hypochlorite, which is then

discarded into a beaker of the same decontaminating fluid.

(2) Packaging for Disposal -- The decontaminated animals are placed into double plastic bags and sealed before removal from the hood. All animals are certified as decontaminated by appropriate monitoring methods before disposal.

(3) Disposal -- All animals and materials contaminated with HD are incinerated after confirmation of decontamination. All animals and materials contaminated with L are packaged for burial in a hazardous materials landfill following confirmation of decontamination.

I. Specific Procedures

(1) Exposure and decontamination timing is controlled by one investigator who also maintains the laboratory notebook. A second investigator prepares the materials and delivers them to a third, operating investigator in proper sequence and timing. The third operating investigator applies the agent and performs decontaminating procedures while wearing approved protective gloves and an apron. A fourth investigator maintains a supply of rabbits from the preparation area to the exposure hoods and reports signs or death of exposed rabbits to the reporting investigator.

(2) All animals are inspected after the agent has been applied to the last animal. Animals are observed for signs of toxicity after dosing until the end of the workday.

7. Necropsy and Histopathology:

No tissue samples are to be saved and all animals carcasses are to be decontaminated and discarded.

8. Records to be Maintained:

A. CSM accountability log and inventory

B. Preparation of reagents and dosage administration

C. Animal data

D. Experimental parameters and test conditions

E. Lesion observations and evaluations

F. Results of decontamination monitoring

G. Confirmation of disposal

9. Statistical Methods:

The evaluation of the relative effectiveness of the timed sequence of test decontaminating solutions is done by comparing those results with the corresponding standard M-258A1 II decontaminating solution controls. At the same time intervals, the corresponding lesion intensity, as estimated by the Draize primary irritation index, for the test decontamination solution plus agent is divided by the primary irritation index of the M-258A1 II decontamination slurry plus agent. If the answer is equal to or less than 1.0, the test material is as or more effective at that time interval than the standard solution and may warrant a second series of tests at different application intervals after exposure or with different decontaminating time sequences. If the answer is greater than 1.0, the test solution is not equivalent to the M-258A1 II slurry and should be discarded.

10. Reports:

A. Monthly Progress Reports

Each letter report contains a brief narrative description of the accomplishments, problems, plans, expenditures, and levels of effort in relation to the research task. It is submitted to the U.S. Army project officer (COTR) within seven working days after the end of the month.

B. Final Report

A final report is prepared and submitted within 30 days after completion of the task. It includes at least the following:

1. Signature page for key study individuals and their respondibilities
2. Experimental design
3. Animal supplier
4. Test animal selection criteria
5. Test material description and preparation
6. Application procedures
7. Description of clinical observations
8. Tabulation of response data by dose
9. Statistical methodology used.
10. Discussion
11. Photographs

11. Approval Signatures

Ronald L Joiner — Study Director — 12/5/83 — Date

W. Hugh Hamrick, DVM — Chief Veterinarian — 12/8/83 — Date

Howard C Johnson — USAMRDC Monitor — 5 Dec 83 — Date

APPENDIX B

MREF Protocol 1 --- "Dermal Study for the Assessment and Validation of Decontaminants in Rabbits Against Mustard and Lewisite"
Revised 1 June 1984

# Dermal Study for the Assessment and Validation of Decontaminants in Rabbits Against Mustard and Lewisite

Study Performed by Battelle Columbus Laboratories,
505 King Avenue, Columbus, Ohio 43201

1. Study Director: Ronald L. Joiner, Ph.D.

2. Veterinarian: H. Hugh Harroff, Jr., D.V.M.

3. Sponsor: U.S. Army Medical Research and Development Command

4. Sponsor Monitor: LTC Howard Johnson, USAMRICD

5. Objective:

   To develop and validate a quantitative animal model and experimental method for screening and testing decontaminates against mustard (HD) and lewisite (L) exposure.

6. Experimental Design:

   A. Test System

      Albino rabbits were chosen for this study on the basis of the extensive data base available for this species and on the size of the application area for multiple challenges with neat agent.

      (1) Strain -- New Zealand White (albino) rabbits (male and female), supplied by Kings Wheel Rabbitry.

(2) Initial Weight -- 2.0 to 4.0 kilograms.

(3) Selection -- Animals selected after a minimum 7-day quarantine period are in good physical condition. Rabbits are weighed and randomized into groups based on body weight and sex, having been previously selected for having the least amount of hair growth.

(4) Acclimation -- All animals are held at the Medical Research and Evaluation Facility for at least 24 hours prior to study initiation.

(5) Animal Identification -- All animals are ear tattooed to retain positive identification for all care involving animal handling and observations. Cage cards are color-coded by sex.

(6) Housing -- Animals are housed individually in stainless steel, slotted cages equipped with automatic watering systems.

(7) Lighting -- Fluorescent lighting, light/dark cycle is 12 hours each per day.

(8) Temperature -- Maintained at 70F ($\pm$5F).

(9) Humidity -- Maintained at 50% ($\pm$ 10%).

(10) Diet -- Purina Certified Rabbit Chow pellets are available at all times during animal quarantine and holding. No contaminants are known to be present in the feed which would interfere or affect the results of the study.

(11) Water Supply -- Water is supplied from the public water system and given ad libitum during quarantine and holding. No contaminants are known to be present in the water which would affect the results of the study.

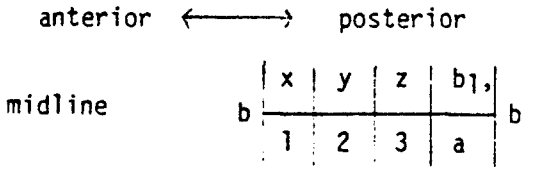
(12) Animal Care During Test -- All animals are housed in stanchions for treatment in individual restraint cages for the remainder of the test period. No food is provided during the 28 hours of the test.

B. Test Groups

(1) Size -- Routine screening tests are performed with groups of 8 animals. Group matching is based on individual and total group weight and sex.

(2) Number -- Two groups of animals are used for each series of exposures. One test group receives HD and the appropriate decontamination solutions or powders and the second group receives L and the decontamination solutions or powders.

(3) Sequence -- Each animal in each group receives a series of 0.5 1 doses of HD or L along the dorsum of the back in the following pattern:




where a = agent control without decontamination,
b = control for standard decontamination material,
b1 = control for test decontamination material,
x = agent plus standard decontamination material after minimum time period,
y = agent plus standard decontamination material after middle time period,
z = agent plus standard decontamination material after maximum time period,
1 = agent plus test decontamination material after minimum time period,
2 = agent plus test decontamination material after middle time period,
3 = agent plus test decontamination material after maximum time period.

(4) Dose -- The volume applied for each agent at each position is 0.5 1.

Revised June 1, 1984

C. Test Material

The M-258A1 II slurry or Fuller's Earth powder is used as the standard decontamination system against HD and L exposure. Distilled water or the test decontamination material is used for comparison of effects.

(1) The M-258A1 II kit and bulk materials are supplied by the USAMRDC/ICD. Fuller's Earth is available commercially.

(2) Lewisite and HD are supplied by the USAMRDC/ICD. Purity, appropriate identification (batch number, lot number, state), and stability data are supplied by the USAMRDC/ICD. Purity and stability are confirmed periodically by Battelle for material stored at the Hazardous Materials Laboratory.

(3) Surety, security, and safety procedures for the use of L and HD are thoroughly outlined in facility plans, in personnel requirements for qualifications to work with agents, and in agent storage and use standard operating procedures. Specific procedures have been included in this document to ensure the safety of the personnel conducting this experiment.

D. Preparation of Animals for Exposure to Vesicants

(1) Hair Clipping -- All animals are acclimated in approved cages at the MREF for at least one day before use. Study animals are closely clipped from withers to rump with care to avoid skin damage. An Oster Model A-2 animal clipper with a No. 40 blade, or equivalent, is used to clip animals at least 24 hours prior to the intended use.

Animals are clipped at least 24 hours prior to intended use and reclipped, if necessary, after anesthesia has been induced to prevent shielding of exposure sites by hair stubble.

(2) Anesthesia -- Test rabbits are anesthetized prior to treatment by the intermuscular administration of a mixture of Ketamine and Rompun. The unconscious rabbits are placed in prone position in metal stanchions. Animals are then positioned inside exposure hoods and the hood sash positioned to maintain average air flow of 165 ft/min past the rabbit noses.

E. Application of Vesicants

(1) Agent (0.5 1 of L or HD) is applied to each of 7 spots on the back of each rabbit as a small streak (approximately 1 cm in length) with a microliter syringe. Lewisite is delivered using a Hamilton microliter syringe equipped with a special platinum needle (barrel) and a standard tungsten plunger. Standard stainless microliter syringes are used to apply HD.

(2) Agent and decontaminates are applied by the sequence given in 6.B(3).

F. Decontamination Techniques

(1) The test decontaminating solution or distilled water is applied to animals exposed to lewisite and mustard. The standard decontaminating material is the M-258A1 II slurry or Fuller's Earth powder.

(2) The duration of exposure before the onset of decontamination is the most critical factor to decontamination procedures. Decontamination for lewisite is a timed sequence that begins at 30 seconds and progresses to 60 and 120 seconds. For mustard, a sequence of 1.25, 5.0, and 10.0 minutes are used. Modifications may have to be made to the initial time sequences to provide a series of graded readings approximating slight, moderate, and severe irritation.

(3) The mechanical method used to remove agent is the second most critical factor in skin decontamination. The decontamination process consists of wiping a pad wetted with the decontamination solution or a cotton swab to spread the powder on the exposed area for a period of 5 seconds in a back and forth motion, perpendicular to the spine, within the exposure grid area.

(4) The standard decontamination pad for solutions is made by cutting the M-258A1 kit cloth into strips and taping the cut cloth segment to a tongue depressor. The cloth is then wetted with the amount of liquid from the kit that corresponds to the proportion of cloth used to make the decontamination pad. The kit contains the prescribed volume of M-258A1 II slurry given in the MIL specifications for the M-258A1 kit. The standard applicator for powders is a cotton swab.

(5) The wetted cloth or cotton swab is wiped briskly but not harshly for 5 seconds in a back and forth motion, perpendicular to the spine, within each grid area.

(6) The pad or swab is deposited into decontaminating solution immediately after use.

(7) The decontamination pad for the test decontamination solution is made as above from a strip of non-impregnated cloth that is the precursor of the M-258A1 kit material (the cloth does not contain the chloramine B but is of identical texture).

(8) When using powdered decontaminants, a prescribed amount is placed in vials for application. At the appropriate time sequences, the powder is applied on the appropriate site and rubbed into the dosing site for 5 seconds using a cotton-tipped swab. A piece of cardboard is held behind the immediate site of application to prevent contamination of other dosing sites with the powdered decontaminant.

G. Lesion Evaluation

(1) When using powdered decontaminants, the powdered decontaminants are removed at 20-24 hours post-exposure by washing the backs with 5% sodium hypochlorite solution and then rinsing the back 2 or 3 times with distilled water.

(2) Irritation Determination -- On the morning following exposure to HD or L (at approximately 20 to 24 hours post-exposure), animals are anesthetized and lesions are evaluated and scored by a modification of the Draize method for primary irritation of the

skin (Draize, J.H. 1979. Dermal Toxicity. In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Edited and published by The Association of Food and Drug Officials of the United States, pp 46-59). The degree of irritation at 0-24 hours post-exposure is estimated by scoring two individual sets of skin reactions: erythema and edema formation (see table below for scores). The primary irritation index is calculated by adding the values for erythema and edema formation for similar treatment sites and dividing by the total number of similar treatment sites. For example, all sites treated with 0.5 1 of L for 60 seconds before decontamination with the standard decontamination material are scored individually for edema and erythema formation; the resulting scores are summed for each site; scores from all other treatment sites with 0.5 1 of of L for 60 seconds prior to standard decontamination are summed; and the total is divided by the total number of sites included in the summation. The resulting combined average is the primary irritation index for 0.5 1 of L for 60 seconds prior to decontamination with standard decontamination material.

Erythema Formation

| | |
|---|---|
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well-defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (beet redness) to slight eschar formation (injuries in depth) | 4 |
| Highest possible erythema score | 4 |

Edema Formation

| | |
|---|---|
| No edema | 0 |
| Very slight edema (barely perceptible) | 1 |
| Slight edema (edges of area well-defined by definite raising) | 2 |
| Moderate edema (raised 1 mm but not extending beyond area of exposure | 3 |
| Severe edema (raised more than 1 mm and extending beyond area of exposure) | 4 |

| | |
|---|---|
| Highest possible edema score | 4 |
| Highest possible total score | 8 |

Descriptive words are correlated with values of the primary irritation index as follows:

0 -2 = Mildly irritating
2 -5 = Moderately irritating
5 -6 = Moderately to severely irritating
6 -8 = Severely irritating

(2) Dye Injection -- Following the irritation evaluation, each animal is given a 1-ml intramuscular injection (in each thigh) of a 3% suspension of trypan blue dye in saline. The dye requires at least two hours to translocate throughout the damaged vessels of the exposed areas. The dye forms a dark blue marking of the lesion against the contrasting pale blue of adjacent normal skin. A pink halo may extend for 2-4 mm wider than the blue zone, which presumably is indicative of active hyperemia.

(3) Anesthesia -- Approximately 2-4 hours after administration of the dye, the test animals are anesthetized by a dose of ketamine/rompun.

(4) Lesion Size Determination -- After anesthesia (at approximately hour 24 to hour 28), the lesion at each test area is visually compared with the lesion at the corresponding time interval for the standard M-258A1 II decontamination slurry. The observer estimates the size of the affected area by matching the total involved area with a series of reference spots with known areas. A Medical Nikon 35 mm or similar camera is used to record the lesions.

(5) Euthanasia -- After photographing the lesions, the test animals are killed by administering T-61. The lesions are re-read and additional photographs are taken, if necessary.

(6) Extended Observation Period -- In some instances, it may be necessary to extend the observation period beyond the initial period at hour 28. The lesion is decontaminated with 5% sodium hypochlorite and washed with water prior to quantitating the

lesion. The animals are placed into holding cages after the initial observation and held for 2-7 days for observation of necrosis, healing, etc.

H. Disposal of Experimental Animals

(1) Decontamination -- After euthanasia, each vesicant exposure area is thoroughly rubbed with a saturated gauze pad or other applicator saturated with 5% sodium hypochlorite, which is then discarded into a beaker of the same decontaminating fluid.

(2) Packaging for Disposal -- The decontaminated animals are placed into double plastic bags and sealed. All animals are certified as decontaminated by appropriate monitoring methods before disposal.

(3) Disposal -- All animals and materials contaminated with HD or L are incinerated after confirmation of decontamination.

I. Specific Procedures

(1) Exposure and decontamination timing is controlled by one investigator who also maintains the laboratory notebook. A second investigator prepares the materials and delivers them to a third, operating investigator in proper sequence and timing. The fourth operating investigator applies the agent, and the third investigator performs decontaminating procedures while wearing approved protective gloves and an apron. A fifth investigator maintains a supply of rabbits from the preparation area to the exposure hoods and reports signs or death of exposed rabbits to the reporting investigator.

(2) All animals are inspected after the agent has been applied to the last animal. Animals are observed for signs of toxicity after dosing until the end of the workday.

7. Necropsy and Histopathology:

No tissue samples are to be saved and all animals carcasses are to be decontaminated and discarded.

Revised June 1, 1984

8. Records to be Maintained:

A. CSM accountability log and inventory

B. Preparation of reagents and dosage administration

C. Animal data

D. Experimental parameters and test conditions

E. Lesion observations and evaluations

F. Results of decontamination monitoring

G. Confirmation of disposal

9. Statistical Methods:

The evaluation of the relative effectiveness of the timed sequence of test decontaminating solutions or powders is done by comparing those results with the corresponding standard M-258A1 II decontaminating solution or Fuller's Earth controls. At the same time intervals, the corresponding lesion intensity, as estimated by the area of lesion involvement, for the test decontamination solution or powder plus agent is compared to the size of the lesion from the M-258A1 II decontamination slurry or Fuller's Earth plus agent. Significant differences in the areas of involvement can be used to classify a test decontamination material as less effective than the M258A1 standard decontamination solution or Fuller's Earth standard or equal to or more effective. If the test material is as or more effective than the standard, it may warrant a second series of tests at different application intervals after exposure or with different decontaminating time sequences.

10. Reports:

A. Monthly Progress Reports

Each letter report contains a brief narrative description of the accomplishments, problems, plans, expenditures, and levels of effort in relation to the research task. It is submitted to the U.S. Army project officer (COTR) within seven working days after the end of the month.

B. Final Report

A final report is prepared and submitted within 30 days after completion of the task. It includes at least the following:

1. Signature page for key study individuals and their responsibilities
2. Experimental design
3. Animal supplier
4. Test animal selection criteria
5. Test material description and preparation
6. Application procedures
7. Description of clinical observations
8. Tabulation of response data by dose
9. Statistical methodology used.
10. Discussion
11. Photographs

11. Approval Signatures:

Ronald B Jones
Study Director

November 9, 1984
Date

H. Hugh Hamoff, Jr DVM
Chief Veterinarian

11-13-84
Date

Howard C Johnson
USAMRDC Monitor

15 Nov 84
Date

# APPENDIX C

Tables

TABLE 3.3.1 DRAIZE IRRITATION SCORES FOR M258A1 II AND DISTILLED WATER DECONTAMINANTS AGAINST 0.5 µl OF HD

| Animal No. | M258A1 II | | | | | | Distilled Water | | | | | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.25 Min | | 5 Min | | 10 Min | | 1.25 Min | | 5 Min | | 10 Min | | | |
| | R* | E** | R | E | R | E | R | E | R | E | R | E | R | E |
| A1453M | 3 | 2 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1421F | 4 | 4 | 3 | 2 | 3 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1456M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1422F | 4 | 1 | 4 | 2 | 4 | 2 | 4 | 1 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1476M | 3 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1436F | 3 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1363M | 4 | 1 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1283F | 3 | 2 | 3 | 2 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 3.5 | 2.5 | 3.6 | 2.9 | 3.5 | 3.4 | 4.0 | 3.6 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| A1462M | 3 | 3 | 4 | 4 | 3 | 3 | 4 | 3 | 3 | 2 | 4 | 3 | 4 | 4 |
| A1333F | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1365M | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1431F | 4 | 3 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1475M | 4 | 1 | 4 | 2 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1424F | 4 | 1 | 3 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1458M | 4 | 2 | 4 | 2 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1448F | 4 | 2 | 4 | 2 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 3.9 | 2.0 | 3.9 | 2.5 | 3.9 | 3.2 | 4.0 | 3.9 | 3.9 | 3.8 | 4.0 | 3.9 | 4.0 | 4.0 |

TABLE 3.3.1 (Continued)

| Animal No. | M258A1 II | | | | | | Distilled Water | | | | | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.25 Min | | 5 Min | | 10 Min | | 1.25 Min | | 5 Min | | 10 Min | | | |
| | R* | E** | R | E | R | E | R | E | R | E | R | E | R | E |
| A1693M | 4 | 1 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1636F | 4 | 2 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1663M | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1648F | 4 | 1 | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 3 | 4 | 4 | 3 | 4 |
| A1549M | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1817F | 3 | 1 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1669M | 3 | 1 | 3 | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1672F | 3 | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 3.6 | 1.5 | 3.6 | 3.1 | 3.6 | 3.8 | 4.0 | 3.8 | 4.0 | 3.9 | 4.0 | 4.0 | 3.9 | 4.0 |
| Grand Average | 3.7 | 1.9 | 3.7 | 2.8 | 3.7 | 3.4 | 4.0 | 3.8 | 4.0 | 3.9 | 4.0 | 4.0 | 4.0 | 4.0 |

* R, erythema.
** E, edema.

TABLE 3.3.2 DRAIZE IRRITATION SCORES FOR FULLER'S EARTH AND POWDERED MARBLE DUST DECONTAMINANTS AGAINST 0.5 µl OF HD

| Animal No. | Fuller's Earth | | | | | | Marble Dust | | | | | | Unchallenged Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.25 Min | | 5 Min | | 10 Min | | 1.25 Min | | 5 Min | | 10 Min | | | |
| | R* | E** | R | E | R | E | R | E | R | E | R | E | R | E |
| A3348M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 |
| A3438F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3352M | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3340F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3345M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3277F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3382M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3294F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 3.9 | 3.9 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 3.9 |
| A3342M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3431F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3358M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3334F | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| A3377M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3295F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3386M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3320F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 |

TABLE 3.3.2 (Continued)

| Animal No. | Fuller's Earth | | | | | | Marble Dust | | | | | | Unchallenged Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.25 Min | | 5 Min | | 10 Min | | 1.25 Min | | 5 Min | | 10 Min | | | |
| | R* | E** | R | E | R | E | R | E | R | E | R | E | R | E |
| A3222M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3406F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3214M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3425F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3242M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3393F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3235M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3419F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Grand Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 3.9 |

* R, erythema.
** E, edema.

TABLE 3.3.3 DRAIZE IRRITATION SCORES FOR M258A1 II AND DISTILLED WATER DECONTAMINANTS AGAINST 0.5 µl OF L

| Animal No. | M258A1 II | | | | | | Distilled Water | | | | | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 Sec | | 60 Sec | | 120 Sec | | 30 Sec | | 60 Sec | | 120 Sec | | | |
| | R* | E** | R | E | R | E | R | E | R | E | R | E | R | E |
| A1590M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1522F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1611M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1510F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1548F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1566M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1506F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| A1572M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1511F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1451M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1517F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1581M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1509F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A562M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1519F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |

TABLE 3.3.3 (Continued)

| Animal No. | M258A1 II | | | | | | Distilled Water | | | | | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 Sec | | 60 Sec | | 120 Sec | | 30 Sec | | 60 Sec | | 120 Sec | | | |
| | R* | E** | R | E | R | E | R | E | R | E | R | E | R | E |
| A1660M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1784F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1676M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1639F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1673M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1623F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1678M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A1627F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Grand Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |

* R, erythema.
** E, edema.

TABLE 3.3.4 DRAIZE IRRITATION SCORES FOR FOR FULLER'S EARTH AND POWDERED MARBLE DUST DECONTAMINANTS AGAINST 0.5 µl OF L

| Animal No. | Fuller's Earth | | | | | | Marble Dust | | | | | | Unchallenged Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 Sec | | 60 Sec | | 120 Sec | | 30 Sec | | 60 Sec | | 120 Sec | | 24 Hr | |
| | R* | E** | R | E | R | E | R | E | R | E | R | E | R | E |
| A3359M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3428F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3370M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3404F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3366M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3396F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3388M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3432F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| A3508M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3481F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3500M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3489F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3270M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3454F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3503M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3482F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |

TABLE 3.3.4 (Continued)

| Animal No. | Fuller's Earth | | | | | | Marble Dust | | | | | | Unchallenged Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 Sec | | 60 Sec | | 120 Sec | | 30 Sec | | 60 Sec | | 120 Sec | | 24 Hr | |
| | R* | E** | R | E | R | E | R | E | R | E | R | E | R | E |
| A3217M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3456F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3516M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3488F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3518M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3442F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3496M | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| A3448F | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Grand Average | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |

* R, erythema.
** E, edema.

TABLE 3.4.1 LESION LENGTHS (in mm) MEASURED BEFORE EUTHANASIA USING DISTILLED WATER AND M258A1 II STANDARD KIT MATERIAL TO VALIDATE THE PROTOCOL 1 SCREEN AGAINST 0.5 µl OF HD

| Replicate | Animal No. | M258A1 II | | | Unchallenged Control | Distilled Water | | |
|---|---|---|---|---|---|---|---|---|
| | | 1.25 Min | 5.0 Min | 10.0 Min | | 1.25 Min | 5.0 Min | 10.0 Min |
| 1 | A1453M | 8 | 10 | 15 | 19 | 19 | 17 | 15 |
| 1 | A1421F | 9 | 10 | 12 | 19 | 10 | 12 | 14 |
| 1 | A1456M | 10 | 12 | 15 | 19 | 17 | 17 | 18 |
| 1 | A1422F | 9 | 9 | 12 | 18 | 14 | 12 | 19 |
| 1 | A1476M | 10 | 10 | 12 | 18 | 14 | 14 | 14 |
| 1 | A1436F | 10 | 9 | 14 | 19 | 17 | 17 | 17 |
| 1 | A1363M | 10 | 12 | 19 | 19 | 15 | 17 | 19 |
| 1 | A1283F | 12 | 12 | 12 | 17 | 14 | 14 | 14 |
| 2 | A1462M | 12 | 18 | 15 | 22 | 19 | 12 | 18 |
| 2 | A1333F | 9 | 10 | 15 | 18 | 15 | 17 | 18 |
| 2 | A1365M | 9 | 10 | 15 | 31 | 22 | 22 | 22 |
| 2 | A1431F | 9 | 10 | 15 | 19 | 17 | 18 | 18 |
| 2 | A1475M | 12 | 12 | 12 | 15 | 12 | 12 | 10 |
| 2 | A1424F | 7 | 12 | 12 | 17 | 12 | 14 | 17 |
| 2 | A1458M | 10 | 12 | 12 | 18 | 14 | 15 | 15 |
| 2 | A1448F | 9 | 10 | 10 | 17 | 12 | 15 | 15 |
| 3 | A1693M | 14 | 15 | 18 | 19 | 24 | 19 | 17 |
| 3 | A1636F | 12 | 14 | 17 | 22 | 18 | 22 | 24 |
| 3 | A1663M | 10 | 15 | 19 | 19 | 19 | 17 | 22 |
| 3 | A1648F | 12 | 18 | 18 | 25 | 31 | 22 | 22 |
| 3 | A1549M | 12 | 14 | 15 | 15 | 15 | 14 | 15 |
| 3 | A1817F | 12 | 18 | 19 | 25 | 19 | 22 | 22 |
| 3 | A1669M | 14 | 15 | 18 | 22 | 24 | 22 | 24 |
| 3 | A1672F | 14 | 14 | 17 | 19 | 24 | 18 | 19 |

TABLE 3.4.2 LESION LENGTHS (in mm) MEASURED AFTER EUTHANASIA USING DISTILLED WATER AND M258A1 II STANDARD KIT MATERIAL TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF HD

| Replicate | Animal No. | M258A1 II | | | Unchallenged Control | Distilled Water | | |
|---|---|---|---|---|---|---|---|---|
| | | 1.25 Min | 5.0 Min | 10.0 Min | | 1.25 Min | 5.0 Min | 10.0 Min |
| 1 | A1453M | 10 | 12 | 15 | 19 | 17 | 17 | 17 |
| 1 | A1421M | 9 | 10 | 14 | 17 | 10 | 12 | 14 |
| 1 | A1456M | 9 | 10 | 14 | 22 | 18 | 18 | 18 |
| 1 | A1422F | 9 | 9 | 10 | 18 | 12 | 12 | 19 |
| 1 | A1476M | 9 | 10 | 12 | 18 | 14 | 14 | 14 |
| 1 | A1436F | 10 | 12 | 17 | 22 | 15 | 17 | 18 |
| 1 | A1363M | 10 | 12 | 28 | 19 | 15 | 15 | 17 |
| 1 | A1283F | 12 | 12 | 12 | 14 | 14 | 14 | 14 |
| 2 | A1462M | 12 | 17 | 15 | 28 | 19 | 15 | 19 |
| 2 | A1333F | 10 | 10 | 14 | 19 | 14 | 17 | 19 |
| 2 | A1365M | 10 | 10 | 15 | 31 | 19 | 22 | 19 |
| 2 | A1431F | 9 | 12 | 15 | 19 | 17 | 17 | 17 |
| 2 | A1475M | 10 | 18 | 12 | 14 | 12 | 12 | 12 |
| 2 | A1424F | 8 | 12 | 14 | 18 | 12 | 15 | 17 |
| 2 | A1458M | 10 | 10 | 12 | 18 | 14 | 17 | 17 |
| 2 | A1448F | 9 | 10 | 12 | 19 | 12 | 15 | 17 |
| 3 | A1693M | 14 | 15 | 17 | 18 | 22 | 22 | 18 |
| 3 | A1636F | 14 | 12 | 15 | 22 | 19 | 25 | 25 |
| 3 | A1663M | 10 | 15 | 19 | 19 | 18 | 18 | 25 |
| 3 | A1648F | 15 | 22 | 19 | 25 | 31 | 22 | 24 |
| 3 | A1549M | 12 | 14 | 17 | 17 | 18 | 15 | 15 |
| 3 | A1817F | 12 | 17 | 17 | 25 | 22 | 24 | 25 |
| 3 | A1669M | 14 | 17 | 19 | 22 | 24 | 24 | 22 |
| 3 | A1672F | 15 | 15 | 17 | 22 | 25 | 22 | 24 |

TABLE 3.4.3 LESION LENGTHS (in mm) MEASURED BEFORE EUTHANASIA USING FULLER'S EARTH AND MARBLE DUST TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF HD

| Replicate | Animal No. | Fuller's Earth | | | Unchallenged Control | Marble Dust | | |
|---|---|---|---|---|---|---|---|---|
| | | 1.25 Min | 5.0 Min | 10.0 Min | | 1.25 Min | 5.0 Min | 10.0 Min |
| 1 | A3348M | 18 | 20 | 20 | 14 | 20 | 18 | 20 |
| 1 | A3438F | 16 | 14 | 22 | 22 | 22 | 26 | 24 |
| 1 | A3352M | 8 | 16 | 20 | 22 | 16 | 16 | 20 |
| 1 | A3340F | 20 | 18 | 20 | 16 | 14 | 16 | 12 |
| 1 | A3345M | 16 | 16 | 16 | 16 | 14 | 10 | 18 |
| 1 | A3277F | 6 | 16 | 22 | 18 | 14 | 16 | 16 |
| 1 | A3382M | 16 | 20 | 22 | 22 | 24 | 24 | 20 |
| 1 | A3294F | 16 | 16 | 16 | 18 | 12 | 12 | 18 |
| 2 | A3342M | 16 | 14 | 16 | 20 | 20 | 18 | 20 |
| 2 | A3431F | 18 | 16 | 20 | 22 | 18 | 20 | 22 |
| 2 | A3358M | 16 | 16 | 20 | 20 | 12 | 14 | 14 |
| 2 | A3334F | 16 | 14 | 20 | 18 | 10 | 14 | 14 |
| 2 | A3377M | 16 | 18 | 16 | 20 | 16 | 18 | 20 |
| 2 | A3295F | 10 | 12 | 16 | 20 | 10 | 12 | 18 |
| 2 | A3386M | 16 | 18 | 20 | 20 | 18 | 20 | 22 |
| 2 | A3320F | 18 | 18 | 18 | 14 | 18 | 18 | 20 |
| 3 | A3222M | 16 | 24 | 22 | 22 | 20 | 22 | 22 |
| 3 | A3406F | 16 | 18 | 20 | 14 | 18 | 20 | 20 |
| 3 | A3214M | 16 | 20 | 20 | 20 | 20 | 22 | 20 |
| 3 | A3425F | 18 | 16 | 18 | 18 | 20 | 20 | 18 |
| 3 | A3242M | 18 | 18 | 22 | 22 | 18 | 22 | 22 |
| 3 | A3393F | 20 | 20 | 16 | 20 | 22 | 18 | 20 |
| 3 | A3235M | 20 | 16 | 20 | 22 | 16 | 18 | 22 |
| 3 | A3419F | 14 | 14 | 18 | 20 | 24 | 18 | 18 |

TABLE 3.4.4 LESION LENGTHS (in mm) MEASURED AFTER EUTHANASIA USING FULLER'S EARTH AND MARBLE DUST TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF HD

| Replicate | Animal No. | Fuller's Earth | | | Unchallenged Control | Marble Dust | | |
|---|---|---|---|---|---|---|---|---|
| | | 1.25 Min | 5.0 Min | 10.0 Min | | 1.25 Min | 5.0 Min | 10.0 Min |
| 1 | A3348M | 18 | 18 | 18 | 12 | 14 | 16 | 14 |
| 1 | A3438F | 20 | 16 | 20 | 22 | 22 | 20 | 20 |
| 1 | A3352M | 8 | 16 | 20 | 22 | 12 | 14 | 20 |
| 1 | A3340F | 10 | 16 | 18 | 16 | 16 | 18 | 12 |
| 1 | A3345M | 18 | 18 | 18 | 16 | 18 | 10 | 16 |
| 1 | A3277F | 12 | 16 | 16 | 14 | 18 | 18 | 16 |
| 1 | A3382M | 16 | 24 | 22 | 20 | 22 | 22 | 18 |
| 1 | A3294F | 20 | 18 | 16 | 18 | 12 | 12 | 20 |
| 2 | A3342M | 14 | 14 | 16 | 18 | 16 | 16 | 20 |
| 2 | A3431F | 20 | 16 | 18 | 22 | 18 | 14 | 18 |
| 2 | A3358M | 18 | 18 | 18 | 22 | 14 | 16 | 18 |
| 2 | A3334F | 16 | 18 | 18 | 18 | 16 | 18 | 14 |
| 2 | A3377M | 16 | 16 | 16 | 18 | 16 | 16 | 18 |
| 2 | A3295F | 14 | 14 | 16 | 18 | 12 | 16 | 18 |
| 2 | A3386M | 18 | 18 | 20 | 22 | 20 | 20 | 24 |
| 2 | A3320F | 20 | 20 | 18 | 16 | 16 | 16 | 18 |
| 3 | A3222M | 20 | 20 | 20 | 20 | 20 | 22 | 22 |
| 3 | A3406F | 16 | 16 | 18 | 12 | 16 | 18 | 18 |
| 3 | A3214M | 18 | 20 | 20 | 20 | 20 | 22 | 20 |
| 3 | A3425F | 18 | 16 | 18 | 16 | 18 | 18 | 18 |
| 3 | A3242M | 18 | 18 | 22 | 22 | 20 | 22 | 20 |
| 3 | A3393F | 20 | 20 | 18 | 22 | 22 | 20 | 22 |
| 3 | A3235M | 18 | 14 | 18 | 20 | 18 | 18 | 20 |
| 3 | A3419F | 14 | 14 | 18 | 18 | 22 | 20 | 18 |

TABLE 3.4.5 LESION LENGTHS (in mm) MEASURED BEFORE EUTHANASIA USING DISTILLED WATER AND M258A1 II STANDARD KIT MATERIAL TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF L

| Replicate | Animal No. | M258A1 II | | | Unchallenged Control | Distilled Water | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 Sec | 60 Sec | 120 Sec | | 30 Sec | 60 Sec | 120 Sec |
| 1 | A1590M | 12 | 14 | 12 | 28 | 18 | 18 | 18 |
| 1 | A1522F | 15 | 17 | 17 | 25 | 19 | 22 | 25 |
| 1 | A1611M | 12 | 12 | 14 | 25 | 22 | 19 | 24 |
| 1 | A1510F | 14 | 15 | 15 | 28 | 25 | 22 | 25 |
| 1 | A1548F | 10 | 12 | 15 | 25 | 22 | 22 | 24 |
| 1 | 1566M | 12 | 14 | 18 | 28 | 19 | 19 | 22 |
| 1 | A1506F | 15 | 14 | 19 | 31 | 24 | 28 | 28 |
| 1 | A1606M | * | * | * | * | * | * | * |
| 2 | A1572M | 15 | 14 | 18 | 28 | 22 | 22 | 22 |
| 2 | A1511F | 14 | 17 | 18 | 28 | 25 | 28 | 25 |
| 2 | A1451M | 14 | 17 | 15 | 25 | 31 | 28 | 31 |
| 2 | A1517F | 12 | 14 | 15 | 28 | 35 | 28 | 24 |
| 2 | A1581M | 12 | 10 | 14 | 25 | 25 | 22 | 24 |
| 2 | A1509F | 12 | 14 | 15 | 28 | 19 | 28 | 25 |
| 2 | A1562M | 12 | 14 | 19 | 25 | 22 | 22 | 31 |
| 2 | A1519F | 17 | 15 | 14 | 25 | 19 | 25 | 24 |
| 3 | A1660M | ** | ** | ** | ** | ** | ** | ** |
| 3 | A1784F | 15 | 15 | 15 | 25 | 25 | 25 | 25 |
| 3 | A1676M | 14 | 18 | 19 | 31 | 19 | 19 | 25 |
| 3 | A1639F | 15 | 15 | 17 | 28 | 22 | 24 | 25 |
| 3 | A1673M | 15 | 17 | 15 | 25 | 19 | 22 | 22 |
| 3 | A1623F | 15 | 15 | 15 | 25 | 25 | 28 | 25 |
| 3 | A1768M | 14 | 15 | 18 | 19 | 24 | 22 | 22 |
| 3 | A1627F | 14 | 15 | 18 | 25 | 22 | 24 | 28 |

TABLE 3.4.6 LESION LENGTHS (in mm) MEASURED AFTER EUTHANASIA USING DISTILLED WATER AND M258A1 II STANDARD KIT MATERIAL TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF L

| Replicate | Animal No. | M258A1 | | | Unchallenged Control | Distilled Water | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 Sec | 60 Sec | 120 Sec | | 30 Sec | 60 Sec | 120 Sec |
| 1 | A1590M | 12 | 15 | 14 | 28 | 22 | 19 | 29 |
| 1 | A1522F | 15 | 17 | 15 | 25 | 22 | 19 | 24 |
| 1 | A1611M | 12 | 12 | 15 | 28 | 22 | 19 | 24 |
| 1 | A1510F | 14 | 15 | 15 | 28 | 25 | 22 | 25 |
| 1 | A1548F | 12 | 14 | 15 | 25 | 22 | 22 | 22 |
| 1 | A1566M | 12 | 14 | 17 | 28 | 22 | 19 | 19 |
| 1 | A1506F | 15 | 15 | 19 | 31 | 24 | 28 | 28 |
| 1 | A1606M | * | * | * | * | * | * | * |
| 2 | A1572M | 18 | 14 | 18 | 31 | 22 | 19 | 22 |
| 2 | A1511F | 15 | 17 | 17 | 25 | 25 | 24 | 24 |
| 2 | A1451M | 14 | 17 | 15 | 25 | 31 | 25 | 31 |
| 2 | A1517F | 14 | 15 | 17 | 28 | 31 | 31 | 25 |
| 2 | A1531M | 12 | 12 | 15 | 25 | 31 | 22 | 22 |
| 2 | A1509F | 12 | 15 | 15 | 28 | 22 | 28 | 25 |
| 2 | A1562M | 14 | 15 | 19 | 25 | 22 | 24 | 31 |
| 2 | A1519F | 17 | 15 | 14 | 25 | 19 | 28 | 24 |
| 3 | A1660M* | 12 | 14 | 15 | 31 | 19 | 28 | 28 |
| 3 | A1784F | 17 | 17 | 19 | 28 | 25 | 25 | 28 |
| 3 | A1676M | 15 | 19 | 19 | 28 | 22 | 22 | 25 |
| 3 | A1639F | 15 | 15 | 17 | 31 | 25 | 25 | 31 |
| 3 | A1673M | 17 | 18 | 19 | 25 | 22 | 22 | 22 |
| 3 | A1623F | 17 | 17 | 17 | 28 | 28 | 28 | 25 |
| 3 | A1678M | 15 | 18 | 19 | 19 | 25 | 25 | 24 |
| 3 | A1627F | 14 | 18 | 19 | 25 | 24 | 24 | 31 |

* A1660 died shortly after Draize irritation phase from what appeared to be suffocation. This is without blue dye and will not be calculated into the mean average of the lesion sizes.

TABLE 3.4.7 LESION LENGTHS (in mm) MEASURED BEFORE EUTHANASIA USING FULLER'S EARTH AND MARBLE DUST TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF L

| Replicate | Animal No. | Fuller's Earth | | | Unchallenged Control | Marble Dust | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 Sec | 60 Sec | 120 Sec | | 30 Sec | 60 Sec | 120 Sec |
| 1 | A3359M | 16 | 20 | 20 | 28 | 32 | 24 | 24 |
| 1 | A3428F | 22 | 20 | 22 | 24 | 24 | 24 | 22 |
| 1 | A3370M | 20 | 22 | 20 | 22 | 22 | 24 | 22 |
| 1 | A3404F | 20 | 18 | 20 | 24 | 24 | 24 | 22 |
| 1 | A3366M | 20 | 22 | 20 | 22 | 22 | 22 | 18 |
| 1 | A3396F | 16 | 18 | 20 | 24 | 22 | 22 | 22 |
| 1 | A3388M | 16 | 20 | 22 | 24 | 22 | 22 | 22 |
| 1 | A3432F | 20 | 24 | 20 | 26 | 20 | 22 | 24 |
| 2 | A3508M | 20 | 20 | 22 | 28 | 24 | 24 | 22 |
| 2 | A3481F | 16 | 18 | 20 | 28 | 22 | 22 | 24 |
| 2 | A3500M | 20 | 20 | 20 | 24 | 26 | 22 | 20 |
| 2 | A3489F | 20 | 20 | 24 | 24 | 16 | 22 | 22 |
| 2 | A3270M | 20 | 20 | 20 | 22 | 20 | 22 | 20 |
| 2 | A3454F | 20 | 22 | 22 | 22 | 22 | 20 | 22 |
| 2 | A3503M | 22 | 20 | 24 | 24 | 20 | 20 | 22 |
| 2 | A3482F | 22 | 20 | 20 | 26 | 26 | 22 | 26 |
| 3 | A3217M | 18 | 22 | 26 | 24 | 24 | 24 | 20 |
| 3 | A3456F | 20 | 24 | 26 | 26 | 28 | 24 | 22 |
| 3 | A3516M | 22 | 22 | 20 | 28 | 26 | 26 | 22 |
| 3 | A3488F | 18 | 16 | 26 | 26 | 26 | 22 | 24 |
| 3 | A3518M | 18 | 20 | 20 | 22 | 24 | 24 | 24 |
| 3 | A3442F | 20 | 20 | 26 | 24 | 24 | 26 | 26 |
| 3 | A3496M | 18 | 20 | 22 | 24 | 22 | 22 | 26 |
| 3 | A3448F | 22 | 22 | 22 | 24 | 22 | 24 | 28 |

TABLE 3.4.8 LESION LENGTHS (in mm) MEASURED AFTER EUTHANASIA USING FULLER'S EARTH AND MARBLE DUST TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF L

| Replicate | Animal No. | Fuller's Earth | | | Unchallenged Control | Marble Dust | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 Sec | 60 Sec | 120 Sec | | 30 Sec | 60 Sec | 120 Sec |
| 1 | A3359M | 18 | 20 | 20 | 26 | 28 | 24 | 22 |
| 1 | A3428F | 24 | 18 | 22 | 24 | 24 | 24 | 24 |
| 1 | A3370M | 20 | 22 | 20 | 22 | 20 | 22 | 20 |
| 1 | A3404F | 22 | 16 | 18 | 24 | 24 | 24 | 24 |
| 1 | A3366M | 22 | 22 | 20 | 22 | 22 | 22 | 20 |
| 1 | A3396F | 14 | 18 | 20 | 24 | 22 | 22 | 22 |
| 1 | A3388M | 18 | 20 | 22 | 24 | 24 | 24 | 22 |
| 1 | A3432F | 22 | 24 | 18 | 26 | 22 | 22 | 22 |
| 2 | A3508M | 20 | 20 | 22 | 26 | 22 | 22 | 22 |
| 2 | A3481F | 16 | 18 | 20 | 26 | 24 | 22 | 22 |
| 2 | A3500M | 20 | 20 | 20 | 22 | 26 | 22 | 20 |
| 2 | A3489F | 20 | 20 | 22 | 22 | 20 | 22 | 22 |
| 2 | A3270M | 20 | 20 | 20 | 20 | 20 | 22 | 20 |
| 2 | A3454F | 22 | 22 | 20 | 22 | 22 | 20 | 20 |
| 2 | A3503M | 22 | 20 | 26 | 24 | 22 | 18 | 24 |
| 2 | A3482F | 24 | 20 | 20 | 26 | 26 | 22 | 24 |
| 3 | A3217M | 22 | 22 | 24 | 22 | 24 | 24 | 20 |
| 3 | A3456F | 20 | 24 | 26 | 24 | 28 | 24 | 22 |
| 3 | A3516M | 22 | 24 | 22 | 28 | 26 | 26 | 24 |
| 3 | A3488F | 20 | 20 | 26 | 26 | 26 | 26 | 26 |
| 3 | A3518M | 18 | 20 | 20 | 20 | 22 | 24 | 22 |
| 3 | A3442F | 22 | 20 | 26 | 26 | 26 | 28 | 26 |
| 3 | A3496M | 20 | 20 | 22 | 26 | 24 | 24 | 26 |
| 3 | A3448F | 22 | 22 | 22 | 22 | 22 | 24 | 28 |

TABLE 3.4.9 AVERAGE LESION LENGTHS PER REPLICATE USING DISTILLED WATER AND COMPONENT II OF THE M258A1 STANDARD KIT TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF HD

| Replicate | M258A1 II | | | Control | Distilled Water | | |
|---|---|---|---|---|---|---|---|
| | 1.25 Min | 5.0 Min | 10.0 Min | | 1.25 Min | 5.0 Min | 10.0 Min |
| | | | | Readings Before Euthanasia | | | |
| 1 | 9.8 | 10.5 | 13.9 | 18.5 | 15.0 | 15.0 | 16.2 |
| 2 | 9.6 | 11.8 | 13.2 | 19.6 | 15.4 | 15.6 | 16.6 |
| 3 | 12.5 | 15.4 | 17.6 | 20.8 | 21.8 | 19.5 | 20.6 |
| Average (SD)[1] | 10.6 (1.9) | 12.5 (2.8) | 14.9 (2.7) | 19.6 (3.5) | 17.4* (4.9) | 16.7* (3.4) | 17.8* (3.5) |
| | | | | Readings After Euthanasia | | | |
| 1 | 9.8 | 10.9 | 15.2 | 18.6 | 14.4 | 14.9 | 16.4 |
| 2 | 9.8 | 12.4 | 13.6 | 20.8 | 14.9 | 16.2 | 17.1 |
| 3 | 13.2 | 15.9 | 17.5 | 21.2 | 22.4 | 21.5 | 22.2 |
| Average (SD)[1] | 10.98 (2.1) | 13.0 (3.3) | 15.5 (3.6) | 20.2 (4.0) | 17.2* (5.0) | 17.5* (4.0) | 18.6* (3.8) |

[1]SD = Standard Deviation.
* Significantly different ($P < 0.01$) from M258A1 II standard decontamination estimates at corresponding time periods.

TABLE 3.4.10 AVERAGE LESION LENGTHS PER REPLICATE USING FULLER'S EARTH AND MARBLE DUST TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF HD

| Replicate | Fuller's Earth | | | Unchallenged Agent | Marble Dust | | |
|---|---|---|---|---|---|---|---|
| | 1.25 Min | 5.0 Min | 10.0 Min | | 1.25 Min | 5.0 Min | 10.0 Min |
| | | | | Readings Before Euthanasia | | | |
| 1 | 14.5 | 17.0 | 19.7 | 18.5 | 17.0 | 17.2 | 18.5 |
| 2 | 15.7 | 15.7 | 18.3 | 19.2 | 15.2 | 16.8 | 18.8 |
| 3 | 17.2 | 18.3 | 19.5 | 19.7 | 19.7 | 20.0 | 20.3 |
| Average (SD)[1] | 15.8 (3.4) | 17.0 (2.6) | 19.2 (2.2) | 19.2 (2.7) | 17.3 (4.0) | 18.0 (3.9) | 19.2 (2.9) |
| | | | | Readings After Euthanasia | | | |
| 1 | 15.2 | 17.7 | 18.5 | 17.5 | 16.8 | 16.3 | 17.0 |
| 2 | 17.0 | 16.8 | 17.5 | 19.2 | 16.0 | 16.5 | 18.5 |
| 3 | 17.7 | 17.2 | 19.0 | 18.3 | 19.5 | 20.0 | 19.7 |
| Average (SD)[1] | 16.7 (3.3) | 17.2 (2.4) | 18.3 (1.7) | 18.5 (3.1) | 17.4 (3.2) | 17.6 (3.2) | 18.4 (2.7) |

[1]SD = Standard Deviation.

TABLE 3.4.11 AVERAGE LESION LENGTHS PER REPLICATE USING DISTILLED WATER AND COMPONENT II OF THE M258A1 STANDARD KIT MATERIAL TO VALIDATE THE MREF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF L

| Replicate | M258A1 II | | | Control | Distilled Water | | |
|---|---|---|---|---|---|---|---|
| | 30 Sec | 60 Sec | 120 Sec | | 30 Sec | 60 Sec | 120 Sec |
| | | | | Readings Before Euthanasia | | | |
| 1 | 12.9 | 14.0 | 15.7 | 27.1 | 21.3 | 21.4 | 23.7 |
| 2 | 13.5 | 14.4 | 16.0 | 26.5 | 24.8 | 25.4 | 25.8 |
| 3 | 14.6 | 15.7 | 16.7 | 26.3 | 22.0 | 23.7 | 25.4 |
| Average (SD)[1] | 13.6 (1.6) | 14.7 (1.9) | 16.1 (2.0) | 26.6 (2.0) | 22.8* (4.1) | 23.6* (3.3) | 25.0* (2.9) |
| | | | | Readings After Euthanasia | | | |
| 1 | 13.1 | 14.6 | 15.7 | 27.6 | 22.7 | 21.1 | 23.0 |
| 2 | 14.5 | 15.0 | 16.2 | 26.5 | 25.4 | 25.1 | 25.5 |
| 3 | 15.7 | 17.4 | 18.4 | 26.3 | 24.4 | 24.4 | 26.6 |
| Average (SD)[1] | 14.5 (1.9) | 15.6 (1.9) | 16.8 (1.9) | 26.8 (2.7) | 24.2* (3.3) | 23.6* (3.5) | 25.0* (3.6) |

[1]SD = Standard Deviation.
* Significantly different ($P < 0.01$) from M258A1 II standard decontamination estimates at corresponding time periods.

TABLE 3.4.12 AVERAGE LESION LENGTHS PER REPLICATE USING FULLER'S EARTH AND MARBLE DUST TO VALIDATE THE MRLF PROTOCOL 1 SCREEN AGAINST 0.5 µl OF L

| Replicate | Fuller's Earth 30 Sec | Fuller's Earth 60 Sec | Fuller's Earth 120 Sec | Unchallenged Agent | Marble Dust 30 Sec | Marble Dust 60 Sec | Marble Dust 120 Sec |
|---|---|---|---|---|---|---|---|
| **Readings Before Euthanasia** | | | | | | | |
| 1 | 19.8 | 20.5 | 20.5 | 24.3 | 23.5 | 23.0 | 22.0 |
| 2 | 20.0 | 20.0 | 21.5 | 24.8 | 22.0 | 21.7 | 22.3 |
| 3 | 19.5 | 20.8 | 23.5 | 24.8 | 24.5 | 24.0 | 24.0 |
| Average (SD)[1] | 19.4 (2.0) | 20.4 (1.9) | 21.8 (2.3) | 24.6 (2.0) | 23.3* (3.2) | 22.9* (1.6) | 22.8* (2.3) |
| **Readings After Euthanasia** | | | | | | | |
| 1 | 20.0 | 20.0 | 20.0 | 24.0 | 23.2 | 23.0 | 22.0 |
| 2 | 20.5 | 20.0 | 21.2 | 23.5 | 22.8 | 21.2 | 21.7 |
| 3 | 20.8 | 21.5 | 23.5 | 24.3 | 24.8 | 25.0 | 24.3 |
| Average (SD)[1] | 20.4 (2.4) | 20.5 (2.0) | 21.6 (2.4) | 23.9 (2.2) | 23.6* (2.4) | 23.1* (2.0) | 22.7* (2.3) |

[1]SD = Standard Deviation.
* Significantly different ($P < 0.01$) from Fuller's Earth standard decontamination estimates at corresponding time periods.

# APPENDIX D

Figures

FIGURE 3.4.1 MEAN LESION LENGTHS (mm) FOR 0.5 μl OF HD DECONTAMINATED WITH EITHER M258A1 II STANDARD OR DISTILLED WATER MEASURED BEFORE EUTHANASIA.

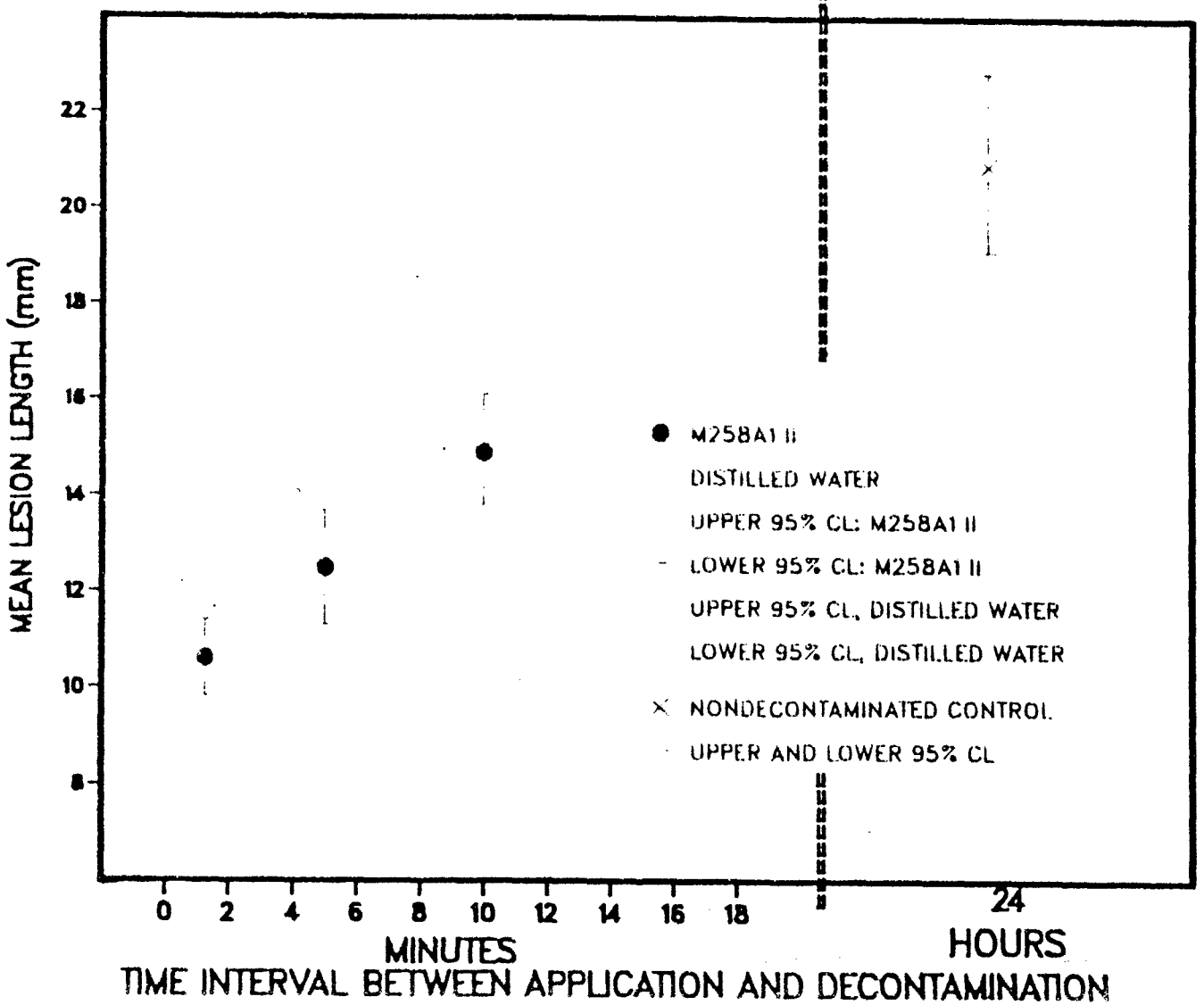


FIGURE 3.4.2 MEAN LESION LENGTHS (mm) FOR 0.5 $\mu l$ OF HD DECONTAMINATED WITH EITHER M258A1 II STANDARD OR DISTILLED WATER MEASURED AFTER EUTHANASIA

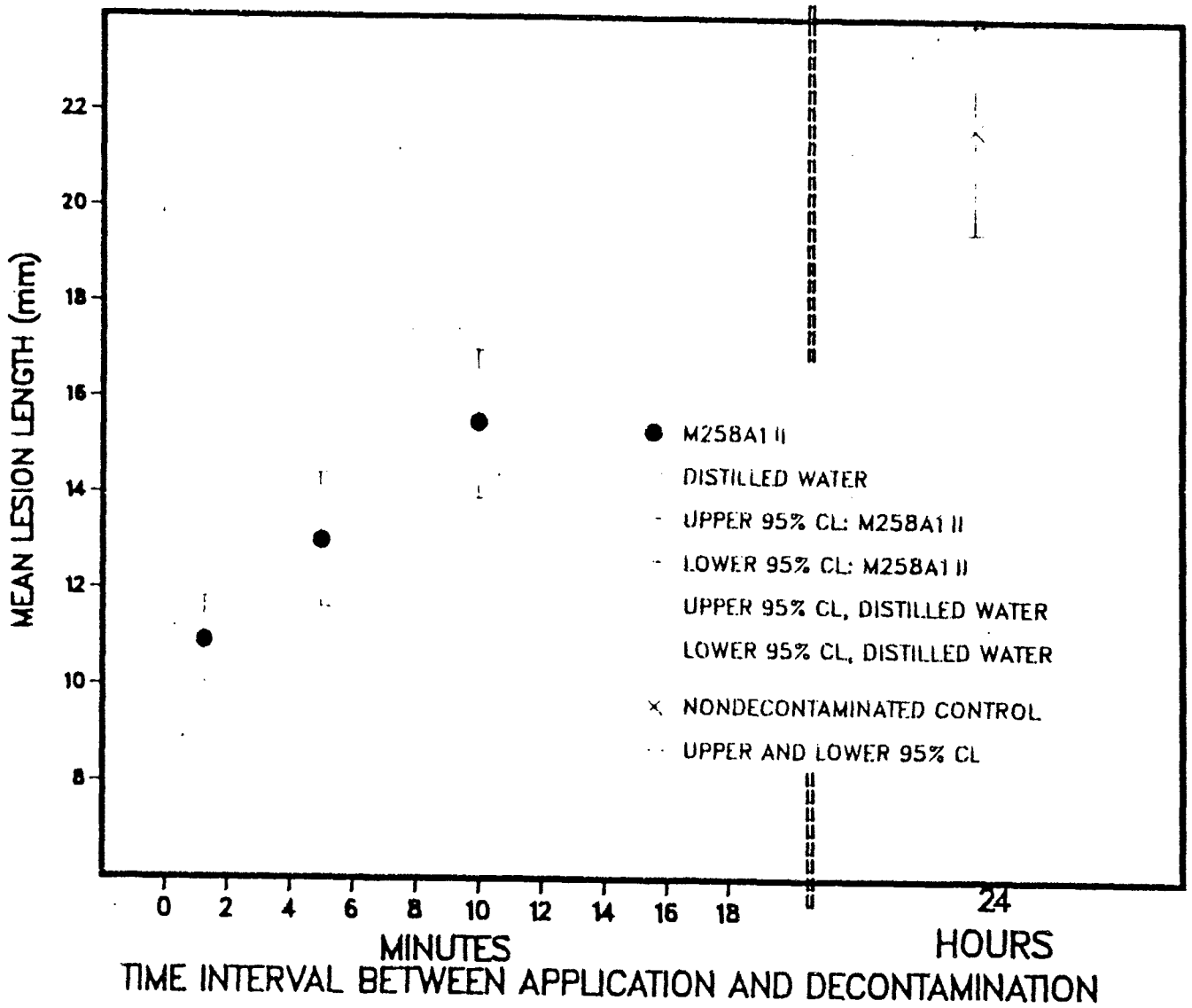


FIGURE 3.4.3 MEAN LESION LENGTHS (mm) FOR 0.5 $\mu$l OF HD DECONTAMINATED WITH EITHER FULLERS EARTH STANDARD OR MARBLE DUST MEASURED BEFORE EUTHANASIA

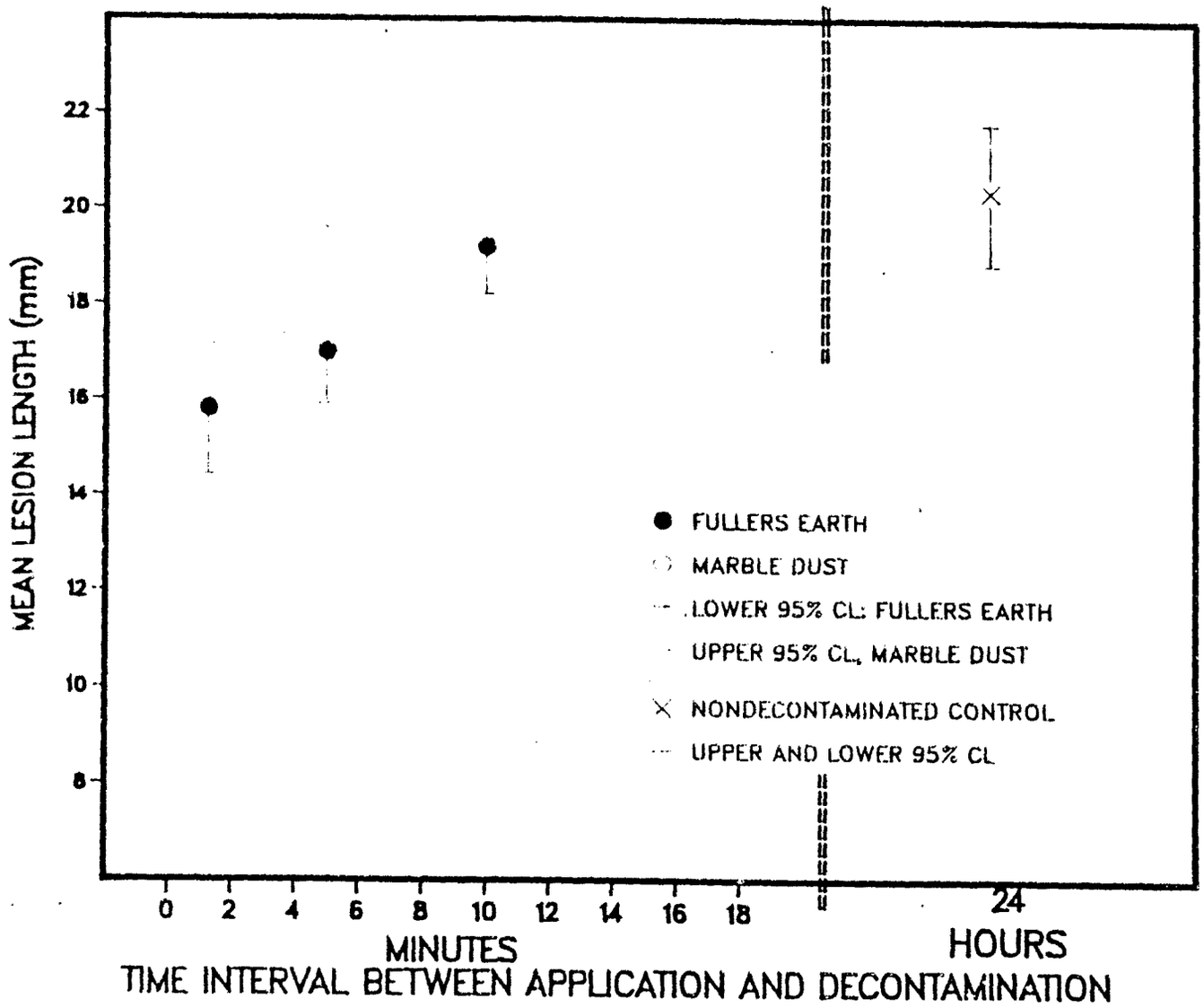


FIGURE 3.4.4 MEAN LESION LENGTHS (mm) FOR 0.5 $\mu$l OF HD DECONTAMINATED WITH EITHER FULLERS EARTH STANDARD OR MARBLE DUST MEASURED AFTER EUTHANASIA

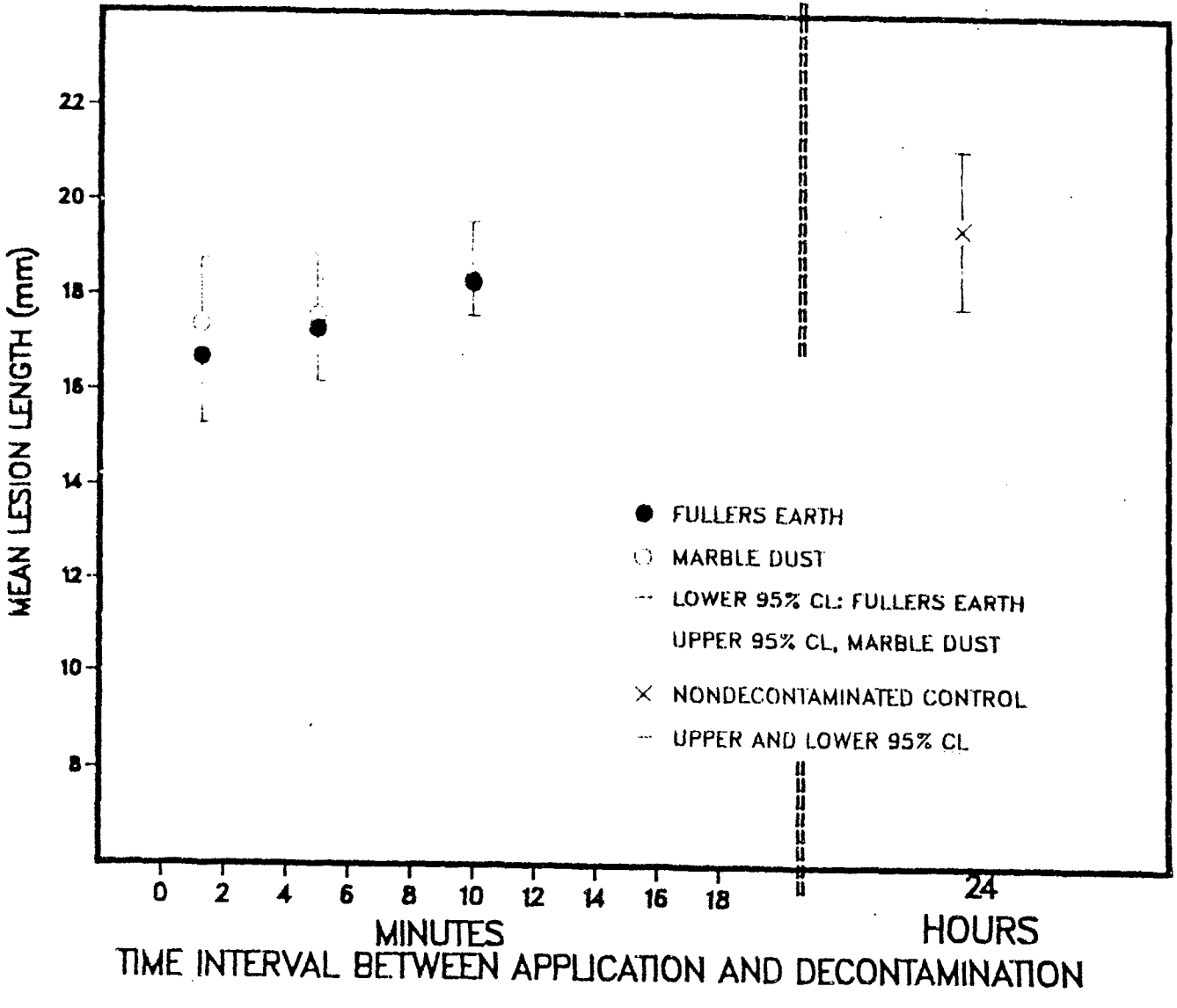


FIGURE 3.4.5 MEAN LESION LENGTHS (mm) FOR 0.5 $\mu l$ OF L DECONTAMINATED WITH EITHER M258A1 II STANDARD OR DISTILLED WATER MEASURED BEFORE EUTHANASIA

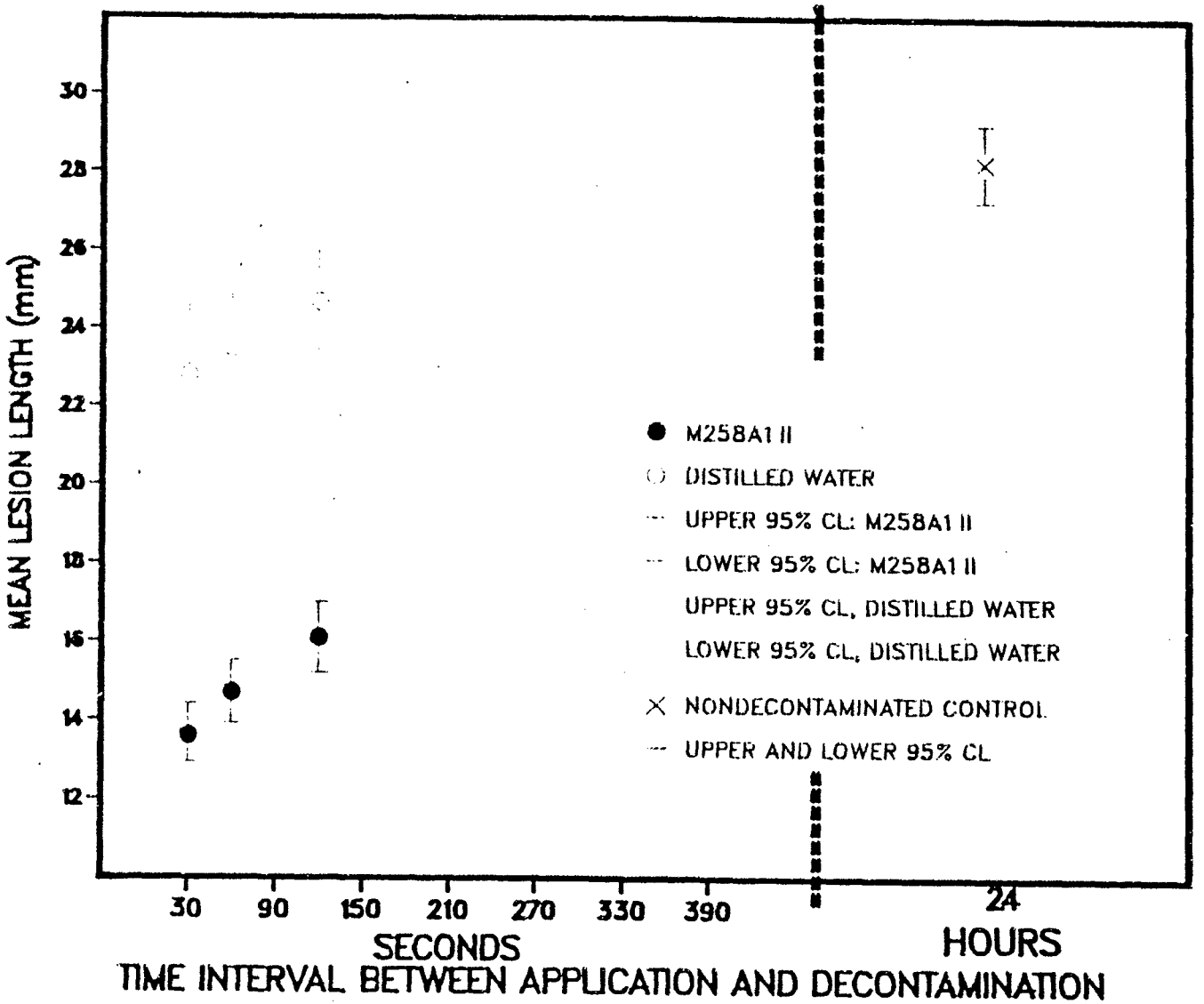


FIGURE 3.4.6 MEAN LESION LENGTHS (mm) FOR 0.5 $\mu$l OF L DECONTAMINATED WITH EITHER M258A1 II STANDARD OR DISTILLED WATER MEASURED AFTER EUTHANASIA

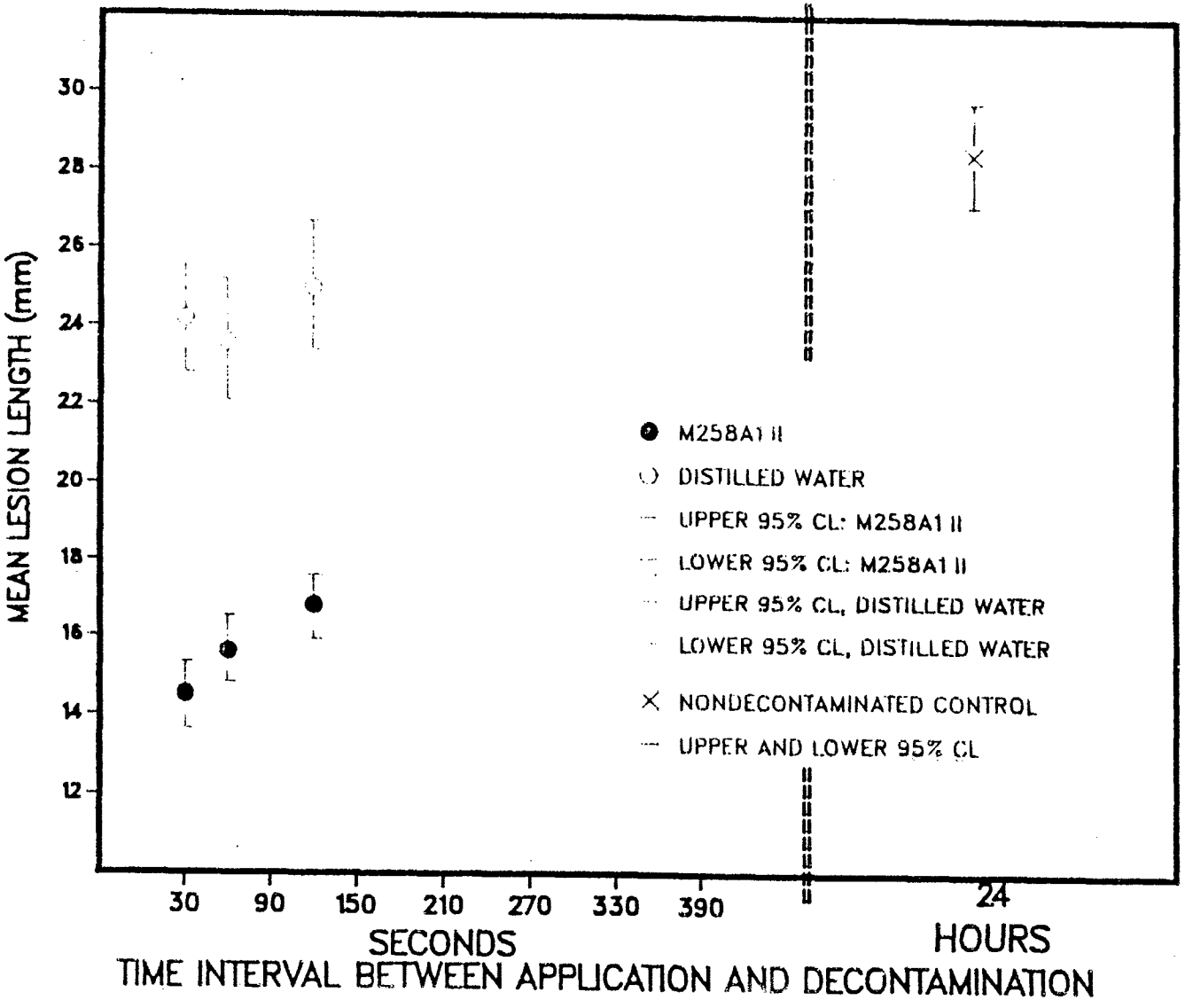


FIGURE 3.4.7 MEAN LESION LENGTHS (mm) FOR 0.5 $\mu$l OF L DECONTAMINATED WITH EITHER FULLERS EARTH STANDARD OR MARBLE DUST MEASURED BEFORE EUTHANASIA

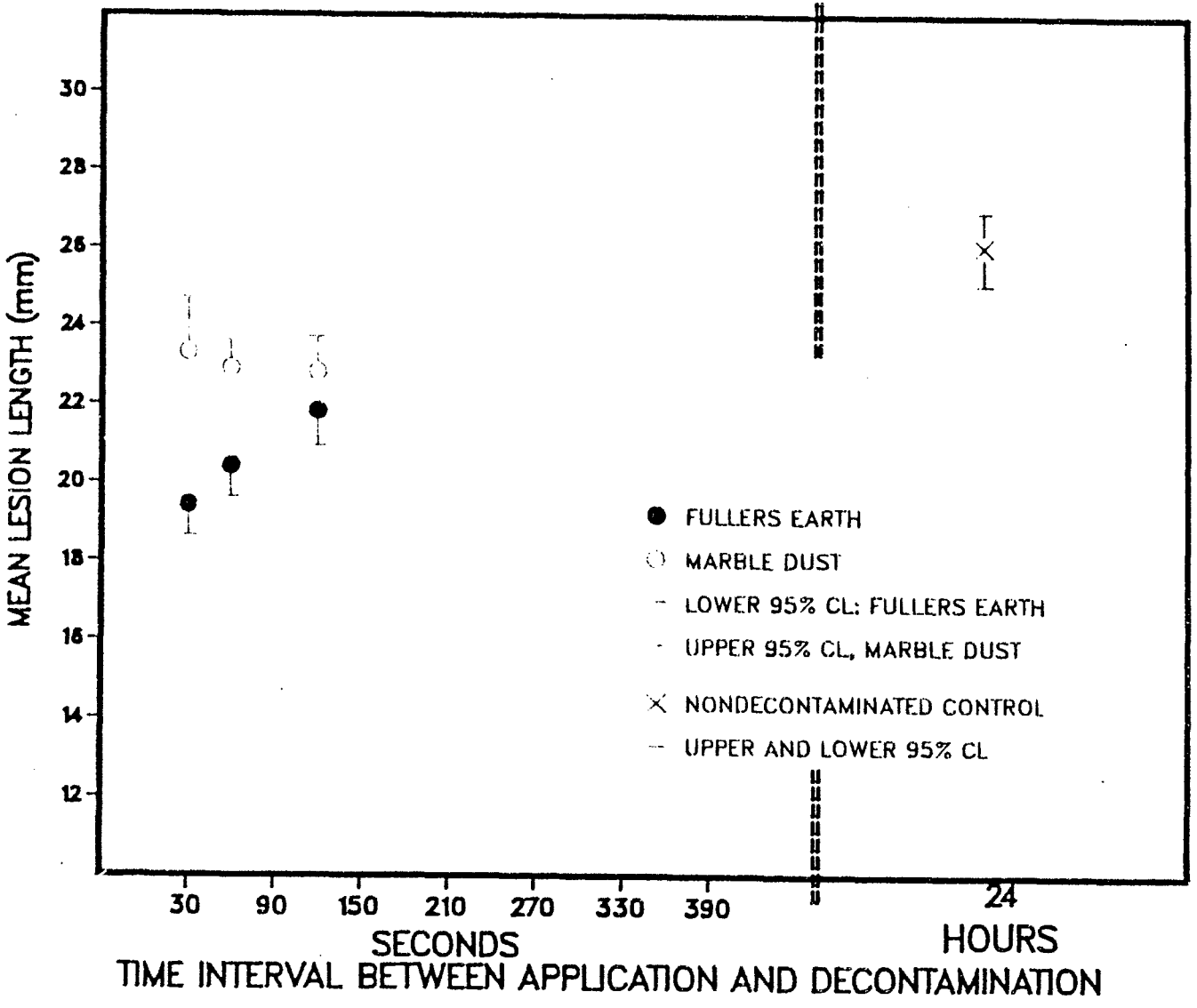
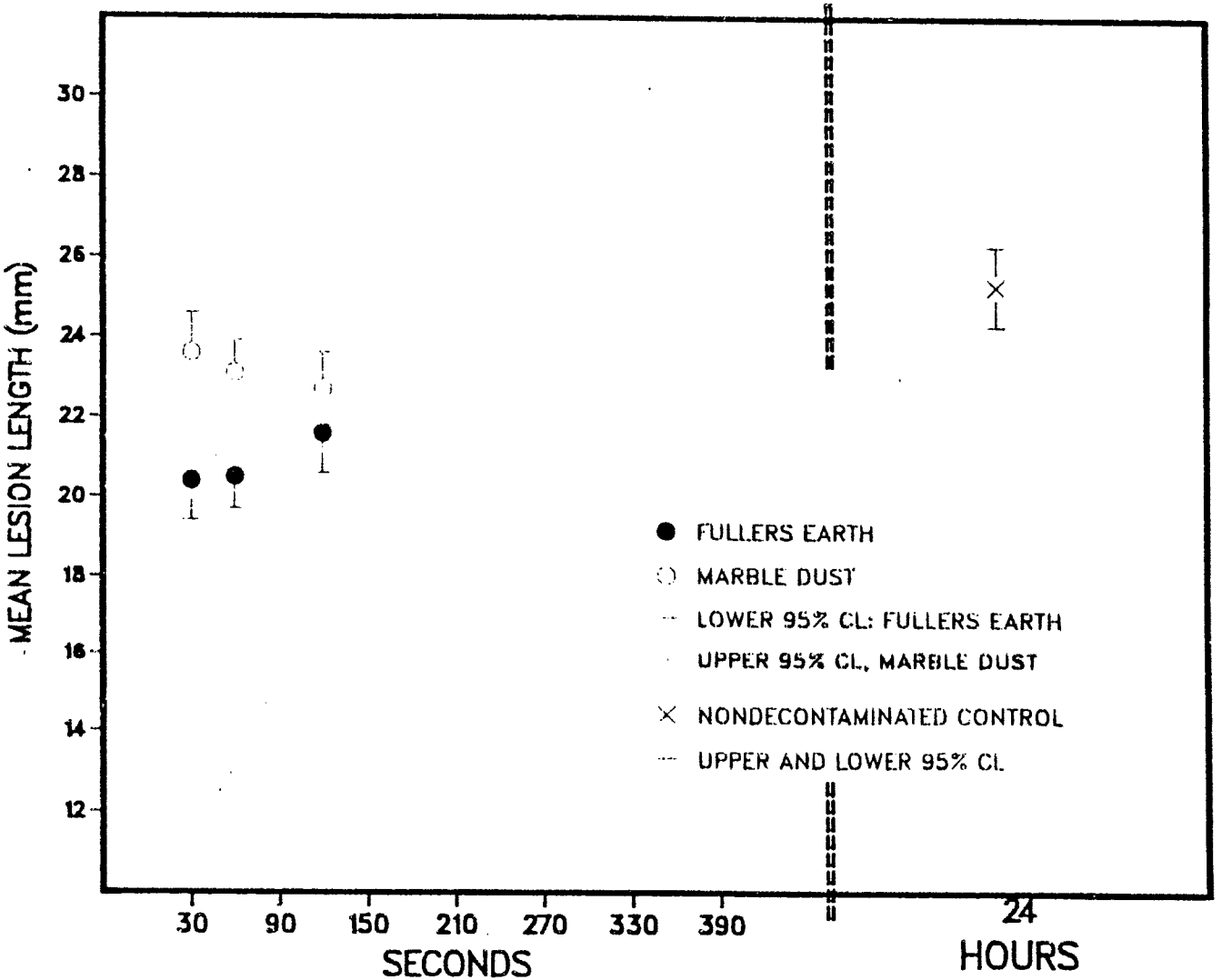


FIGURE 3.4.8 MEAN LESION LENGTHS FOR 0.5 $\mu$l OF L DECONTAMINATED WITH EITHER FULLERS EARTH STANDARD OR MARBLE DUST MEASURED AFTER EUTHANASIA



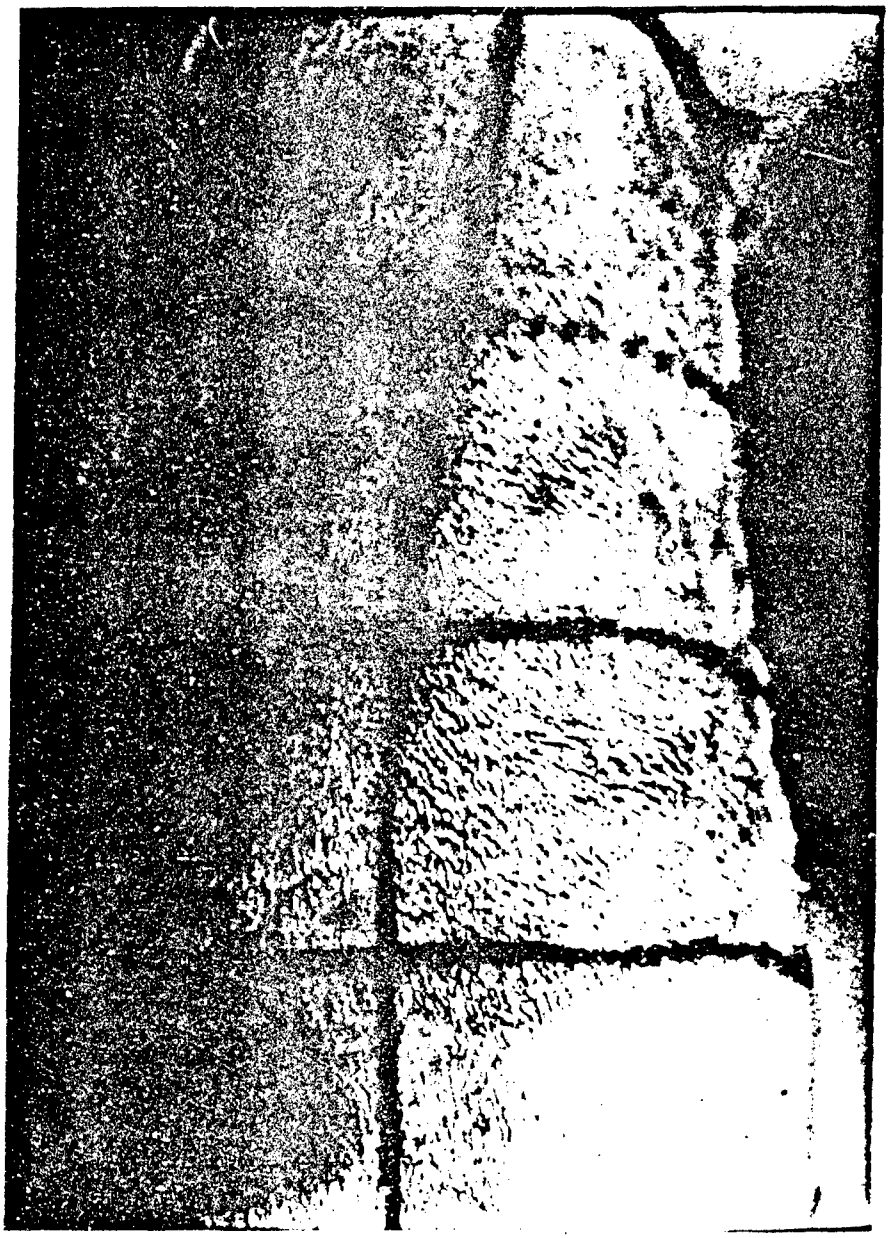

TIME INTERVAL BETWEEN APPLICATION AND DECONTAMINATION

# APPENDIX E

Photographs

Entry #1; Picture #8; HD; logbook 10; pg 1; 4-24-84; A1669M; whole back, distilled $H_2O$ and M258A1 II

Entry #2; Picture #6; HD; logbook 10; pg 1; 4-24-84; A1669M; sites B, D, F, G; distilled $H_2O$



Entry #3; Picture #7; HD; logbook 10; pg 1; 4-24-84; A1817F; whole back; distilled $H_2O$ and M258A1 II

Entry #4; Picture #9; L; logbook 10; pg 1; 4-24-84; A16395,
whole back; distilled $H_2O$ and M258A1 II

Entry #5; Picture #10; L; logbook 10; pg 1; 4-24-84; A1623F;
whole back; distilled $H_2O$ and M250A1 II

Entry #6; Picture #11; L; logbook 10; pg 1; 4-24-84; A1660M; whole back; distilled $H_2O$ and M258A1 I!

Entry #7; Picture #12; L. loghook 10; og 1: 4-24-84; A1627F:
whole back; distilled $H_2O$ and M258A1 II