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EVALUATION OF THE VESICATING PROPERTIES OF NEUTRALIZED CHEMICAL AGENT IDENTIFICATION SETS

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NON-STOCKPILE CHEMICAL MATERIEL PROGRAM

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Aberdeen Proving Ground, MD 21010-5423

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Vesication and skin irritation studies were conducted in hairless guinea-pigs to determine the vesicant and skin irritation potential of chemically-neutralized Chemical Agent Identification Sets (CAIS). The CAIS are training items that contain agent (HD, HN, or L) and were declared obsolete in 1971. Guinea-pigs were topically dosed with "test article" [neat HD, 10% agent/chloroform solutions, or product solutions (wastestreams)] and evaluated for skin-damaging effects (gross and light microscopic). Product solutions from the chemical neutralization of neat sulfur mustard resulted in microvesicle formation (vesication). All agent-dosed (agent/chloroform solutions or HD) sites exhibited microblisters, as well as other histopathologic lesions of the skin. Wastestreams from the neutalization of agent (agent/chloroform; agent on charcoal) were devoid of microvesicant activity. Dermal irritant effects (erythema and edema) were consistent with the skin-injurious activity associated with the neutralizing reagent [1,3-dichloro-5,5-dimethylhydantoin (DCDMH)].

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GLP COMPLIANCE STATEMENT

The percutaneous dosing of hairless guinea pigs with wastestreams, neutralizing solution and known vesicants, and the gross and histopathologic evaluations of skin lesions in this study were performed by Battelle in compliance with the Environmental Protection Agency's (EPA) Good Laboratory Practice (GLP) Standards (40 CFR Part 792). Likewise, evaluation of the analytical method for HD, HN-1 and L in wastestreams and the determination of HD or HD, HN-1 and L concentrations, as appropriate, in wastestreams was accomplished at Battelle in compliance with EPA GLP Standards. Reports on findings from searches of the literature on HD, HN-1 and L degradation and degradation products and their vesicancy potential as well as analyses of wastestreams for degradation products and residual agent concentrations performed elsewhere than the MREF are excepted from this Good Laboratory Practices Compliance Statement. This study was conducted according to the study protocol, as amended, and Battelle's standard operating procedures. Deviations from the protocol or standard operating procedures are documented in Appendix A. The data presented accurately reflect the results of this study.

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QUALITY ASSURANCE

The analytical data supplied by the U. S. Army Edgewood Research, Development and Engineering Center (ERDEC) in support of this task were generated under the auspices of the Research and Technology Directorate Quality Assurance Program Plan. Accordingly, the data are supported by written methodology, sample identification records, and suitable instrument maintenance and calibration. The data and supporting records are retained by ERDEC.

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REPORT REVIEW

The report entitled, "Vesication Evaluation of Neutralized Chemical Agent Identification Sets (CAIS)" was reviewed for technical accuracy in data analysis and report approach. To the best of our knowledge, the report was considered to be an accurate reflection of the vesication data presented in the original report by Olson et al. (1997) titled "Evaluation of the Vesicating Properties of Neutralized Chemical Agent Identification Set (CAIS) Components".

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LIST OF ACRONYMS AND ABBREVIATIONS

CAIS	Chemical Agent Identification Sets
CASARM	Chemical Agent Standard Analytical Reference Materiel
CFR	Code of Federal Regulations
CI	Chemical Ionization
CW	Chemical Warfare
DCDMH	1, 3-Dichloro-5,5 Dimethylhydantoin
DoD	Department of Defense
DOT	Department of Transportation
EI	Electron Impact
EPA	Environmental Protection Agency
ERDEC	U.S. Army Edgewood Research, Development and Engineering Center
GC/MS	Gas Chromotagraphy/Mass Spectrometry
GLP	Good Laboratory Practice
H	Sulfur Mustard
HD	Sulfur Mustard
HMR	Hazardous Materials Regulations
HMTA	Hazardous Materials Transportation Act
HN	Nitrogen Mustard
HS	Sulfur Mustard
LD_{LO}	Lethal Dose Low
L	Lewisite
LC_{50}	Lethal Concentration 50
LD_{50}	Lethal Dose 50
NSCM	Non-Stockpile Chemical Materiel
NSCMP	Non-Stockpile Chemical Materiel Program
PMCD	Program Manager for Chemical Demilitarization
PMCSD	Project Manager for Chemical Stockpile Disposal
PMNSCM	Project Manager for Non-Stockpile Chemical Materiel
ppm	Parts per Million
RRS	Rapid Response System
t-BuOH	Tertiary-butyl Alcohol
TD_{LO}	Toxic Dose Low
TSDF	Treatment, Storage, and Disposal Facility

PREFACE

The work described in this report was authorized under the Chemical Demilitarization Program. This work was started in November 1995 and completed in August 1997.

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Evaluation of the Vesicating Properties of Neutralized Chemical Agent Identification Sets

1. INTRODUCTION

The U.S. Army Program Manager for Chemical Demilitarization (PMCD) has been designated as the single agency within the Department of Defense (DoD) to destroy all chemical warfare-related materiel. Destruction of chemical weapons which are part of the unitary stockpile is the responsibility of the Project Manager for Chemical Stockpile Disposal (PMCSD). The demilitarization/destruction of non-stockpile chemical materiel (NSCM), among those items are Chemical Agent Identification Sets (CAIS), is the responsibility of the Project Manager for Non-Stockpile Chemical Materiel (PMNSCM). CAIS may contain agent in chloroform (HD, HN or L), agent (HD, HN or L) adsorbed on charcoal, and agent (HD) in neat form - all packed in glass ampules. CAIS may also contain miscellaneous materiel/industrial chemicals. CAIS were declared obsolete in 1971.

The PMNSCM is developing the Rapid Response System (RRS) for processing CAIS. The RRS is a system of trailer-mounted equipment designed to support on-site characterization and primary treatment of recovered CAIS. The system is designed for unpacking, identification of chemicals, segregation of CAIS components, neutralization of the chemical agents, repackaging of industrial chemicals. The chemical agent wastes and repacked industrial chemicals will be provided to a hazardous waste treatment, storage, and disposal facility (TSDF) for ultimate disposal.

The RRS is designed to provide a safe and environmentally secure work area to chemically treat mustard and lewisite agents, and to repackage industrial chemicals. The containers of neutralized mustards and lewisite, dunnage, neutralents, and the repacked industrial compounds will be transferred to a TSDF for disposal. It should be noted that the primary objective of the RRS chemical neutralization process is to convert CAIS chemical agents to less toxic products to minimize the health hazard associated with the handling and transportation of demilitarized non-stockpile chemical materiel. Given the chemistry and toxicity characteristics of the chemical agents/degradation products, and the reactants used in the neutralization process, the wastestreams designated for transportation to TSDFs are expected to be complex mixtures exhibiting various degrees of acute dermal toxicities such as irritation; however, other skin-injurious effects (i.e. vesication) are possible.

In preparation for the RRS test to be conducted at Deseret Chemical Depot, Utah, the PMNSCM has conducted dermal toxicity evaluations (vesication testing) of wastestreams resultant from the chemical neutralization of CAIS to determine reduction of agent and/or agent degradation product vesicancy. This document is a synopsis of a technical report (Olson et al, 1997) on results of vesicancy studies conducted in hairless guinea-pigs.

1.1 PROCESS OVERVIEW

Process chemistry developed for chemical agent "detoxification", referred to as the neutralization process, focused on chemical methods that were capable of converting chemical agents to products/by-products with marked reduction in agent characteristics (i.e. vesication). Thus, chemical neutralization processes were sought which: (1) achieved process simplicity, (2) resulted in marked reduction in agent characteristics, and (3) generated wastestreams having reduced toxicity characteristics that can be handled and disposed of in a manner similar to industrial chemicals and/or wastes. The process combines chemical agent(s) with a treatment solution, and oxidizing chemical dissolved in an organic/aqueous solvent mixture. The final product of the chemical neutralization process (wastestream) is a complex mixture composed of reaction products, by-products, unreacted excess reactants, and residual chemical agent(s).

A moderate oxidizing agent, 1, 3-dichloro-5, 5-dimethylhydantoin (DCDMH), was used in all RRS process chemistries. The reaction conditions varied depending on stoichiometry, sequence of addition of reacting mixtures, and physical condition of the chemical agent - whether neat, dissolved in chloroform, or adsorbed on charcoal.

In the nomenclature of the RRS, chemical treatment of neat HD with DCDMH in organic/aqueous solvent is referred to as the modified "Blue" process. Chemical neutralization of dilute chloroform solutions containing HD, HN-1 or L with DCDMH in organic/aqueous solvent is identified as the modified "Red" process. Chemical neutralization of HD, HN-1 or L adsorbed on charcoal with DCDMH in organic/aqueous solvent is referred to as the "Charcoal" process. Toxicity characteristics of wastestreams resulting from these neutralization reactions were expected to differ from those of chemical agents and/or severe irritant oxidant/solvent systems used in these reactions. Consequently, the changed toxicity characteristics of the resultant wastestreams are primarily attributed to reduction in the concentration of chemical agent(s). However, the vesicancy potential of the wastestreams would depend on the degradation product(s) profile - presence of vesicating moieties such as HD sulfone and divinyl sulfone.

1.2 STUDY PLAN

Evaluation of the vesicancy potential of neutralized CAIS was conducted using a validated animal model (hairless guinea-pig) to assess vesication. Animals were dosed with test article, and treated skin evaluated for microblister formation using light microscopy. The objective was to determine the efficacy of the neutralization process in reducing the vesicating properties of agent (HD, HN or L) as well as forming product solutions with minimal vesicant potential.

2. Materials and Methods

2.1 Chemicals

2.1.1 Agents

Sulfur mustard [2,2'-dichlorodiethyl sulfide (HD), CAS #505-60-2] furnished from Medical Research and Evaluation Facility (MREF) stocks was used neat (undiluted) as a positive control article for vesication[1]. Lewisite [dichloro-2-chlorovinyl arsine (L)] CAS #541-25-3 was also furnished from MREF stock. U.S. Army Edgewood Research, Development and Engineering Center (ERDEC) provided a 20 percent solution of nitrogen mustard [bis (2-chloroethyl) ethylamine (HN-1), CAS #538-07-8] in chloroform. Ten percent solutions of HD, HN-1, or L in chloroform (Pretreated - CAIS which also served as control articles in the Phase II portion (dose-ranging) of the vesication studies) were prepared at the MREF laboratory and evaluated for vesicant activity and other histopathology following dermal application.

2.1.2 Chemical Agent Identification Sets (Synthesized)

Actual ampules from CAIS kits were not used; however "CAIS components" were prepared from agent stocks to contain 10 percent agent in chloroform (Chatfield, *et al.* 1995). Chemical Agent Standard Analytical Reference Material (CASARM) grade HD CAS# 505-60-2 (97.5 mole %), nitrogen mustard [bis (2-chloroethyl) ethylamine (HN-1)] CAS #538-07-8 (≥97% by weight), and CASARM grade lewisite [dichloro-2-chlorovinyl arsine (L)] CAS #541-25-3 (97.8 % by weight) from stocks maintained by the Operations Directorate, ERDEC were used in the preparation of synthesized CAIS. CASARM for HN-1 is not available.

2.1.3 Neutralized Chemical Agent Identification Sets (Wastestreams)

Wastestreams were provided by ERDEC, Aberdeen Proving Ground, MD. Wastestreams from the chemical neutralization of "CAIS components" prepared from agent stocks were tested for vesicancy potential. These wastestreams were prepared by ERDEC as follows:

---

[1]The chemical agents found in CAIS include sulfur mustard, nitrogen mustard, or lewisite. Sulfur mustard was used as representative vesicant for these blistering agents.

- Wastestreams from the neutralization of neat HD with 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) in $CHCl_3$/t-BuOH/3% H_2O ("Blue" process).

- Wastestreams from the neutralization of 10% HD, HN, or L (agent in $CHCl_3$) with DCDMH in $CHCl_3$/t-BuOH/3% H_2O ("Red" process).
- Wastestreams from neutralization of HD, HN, or L (agent on charcoal) with DCDMH in $CHCl_3$ (HD, HN samples) and with DCDMH in $CHCl_3$/t-BuOH/3% H_2O (L sample) ("Charcoal" process).

Two wastestreams ("archived"[2] and "fresh"[3]) were prepared for each process - "Blue", "Red", and "Charcoal" - and samples sent to the MREF for analysis of agent content and for vesicancy testing. The stability of the wastestreams under conditions of administration were not determined by MREF personnel. Test articles were "archived" and "fresh" "Blue", "Red", and "Charcoal" wastestreams.

2.1.4 Neutralization Solution

Neutralizing solution was prepared at the MREF to determine the effect on the skin of dosing this solution alone. For testing vesicating potential, a 0.555M 1,3-dichloro-5,5-dimethylhydantoin (FW 197.02) control article neutralizing solution was prepared by adding 10.9g DCDMH to a 50:50 tertiary butanol:chloroform with 3 percent water solution in a 100-mL volumetric flask and adding sufficient volume of the butanol/chloroform/water solution to bring the volume to the 100-mL mark. DCDMH (CAS #118-52-5) was purchased from Aldrich Chemical Company (St. Louis, MO). Chloroform (CAS #67-66-3; GC/Spectro grade) was purchased from Burdick and Jackson (Muskegon, MI), and tertiary-butyl alcohol (CAS #75-65-0; ACS Reagent grade) from J.T. Baker (Phillipsburg, NJ). Distilled water was further purified using a Millipore (Bedford, MA) reverse osmosis system.

2.2 Chemical Neutralization of CAIS

RRS chemical neutralization technologies were developed for neutralization of chemical agents HD, HN and L. The primary objective was to develop processes that convert chemical agents to products/by-products that do not exhibit the highly toxic properties of the agents. Additionally, it was desirous to also reduce agent characteristics (i.e. vesication) of the final product solution (wastestream). However, reduction in vesication is <u>not</u> considered a

[2] "Archived" "Blue" and "Red" wastestreams were initially analyzed at ERDEC (Oct 95) and re-analyzed for agent residual at the MREF and tested for vesicancy (March 96; August 96). "Charcoal" wastestream initially analyzed at ERDEC (Nov 95) was re-analyzed and test for vesicancy at the MREF (March 96; August 96).

[3] "Fresh" wastestreams were prepared and initially analyzed at ERDEC (June 96) and re-analyzed and tested for vesicancy at the MREF (June 96; August 96).

requirement per the Hazardous Materials Regulations (HMR). All process chemistries used 1, 3-dichloro-5, 5-dimethylhydantoin (DCDMH) as neutralizing reagent. The solvent system used in the process chemistries was $CHCl_3$/t-BuOH/3% H_20). Formulations of treatment reagent/solvent systems for the chemical neutralization of CAIS are presented in Table 1. The principal differences in the chemical composition of wastestreams, originating from the RRS process chemistry, are primarily due to the physichochemical characteristics of the reactants in the reaction mixtures and the agent undergoing chemical treatment. In the presence of oxidizing agent (DCDMH), the chemical agent(s) undergo oxidation, chlorination, substitution, and/or elimination reactions to yield a mixture of products/by-products. Depending on volume, composition, and reaction conditions -- residual chemical agent, products/by-products, and varying amounts of unreacted excess DCDMH may also be present in the wastestreams (Olajos et al, 1996).

TABLE 1. OXIDIZER/SOLVENT SYSTEM STOICHIOMETRY UTILIZED IN THE MODIFIED "BLUE", "RED", AND "CHARCOAL" PROCESS CHEMISTRIES

• 1 volume of neat HD treated with 20 volumes of 0.555M 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) in $CHCl_3$/t-butanol (50/50) with 3% water by volume ("Blue" Process).
• 1 volume of each 10% HD in $CHCl_3$, 10% HN in $CHCl_3$, and 10% L in $CHCl_3$ treated with 4 volumes of 0.555M 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) in 50/50 $CHCl_3$/t-butanol with 3% water by volume ("Red" Process).
• 45% by weight HD and HN-1 on charcoal treated with excess 1,3-dichloro-5,5-dimethylhydantoin in $CHCl_3$ combined with 43% by weight L with excess 1,3-dichloro-5,5-dimethylhydantoin in $CHCl_3$/t-butanol (50/50) with 3% water by volume ("Charcoal" Process).

2.3 Analytical Methodologies

2.3.1 GC-MS Spectroscopy

Chemically-treated (neutralized) CAIS were analyzed for agent residue levels using full scanning gc-ms spectroscopy. GC-MS spectroscopy was conducted at ERDEC on all wastestreams provided to the MREF, and confirmatory gc-ms analysis was also performed at the MREF prior to conducting the bioassays.

Instrumentation used in the ERDEC analysis of "archived" wastestreams (non-quenched samples) was a Hewlett-Packard 5989B MS engine with Chemstation Data System. Analyses

conducted at both ERDEC and the MREF on "fresh" wastestreams, using quenching and derivatization techniques, utilized a Hewlett-Packard Model 5970B Mass Selective Detector (MSD) with an HP 5890A GC and HP 61034 CMS. For procedural details, the reader is referred to Lucas (1997), Lucas (1996), as provided in the report by Olson et al 1997, and Rosso (1995), as provided in ERDEC-TR-372 (Olajos, et al. 1996). Quantitation was based on internal standardization (internal standard = 1,2,4, 5-tetrachlorobenzene). Calibration standards were as follows: HD (purity 97.%%), HN-1 (purity (96.5%), and L (purity 97.8%).

Product identification of the CAIS wastestreams (archived) was accomplished using GC/MS spectroscopy (EI and CI modes). These studies were performed at ERDEC per procedures described by Rosso and co-workers (Rosso et al. 1995) and documented in the report by Olajos et al. (Olajos et al. 1996).

2.3.2 NMR Spectroscopy

Nuclear magnetic resonance (nmr) spectroscopy analyses of "fresh" wastestreams were conducted at ERDEC as an adjunct to gc-ms analyses. These analyses were performed using a Varian Fourier Transform (FT) nmr spectrometer operated at 200 MHZ for ^{1}H observation and at 50 MHZ for ^{13}C observation. Quantitative data were obtained by digital integration of peak areas.

2.4 Vesicancy Testing

2.4.1 Experimental Design

Studies were conducted which utilized a validated animal model to assess agent-induced vesication of skin (Marlow et al, 1990 and Mershon et al, 1990). Microblister formation in the hairless guinea-pig is analogous to the changes seen in humans (Papirmeister et al, 1984). The degree of vesication was assessed before and after neutralization of agents.

Thirty-five male, hairless guinea-pigs were used in a multiphase study (Phase I analytical; Phase II dose-range; Phase III vesicant assessment of wastestreams) to ascertain the vesicant potential of sulfur mustard (HD), agent/chloroform solutions, and product solutions (wastestreams) from chemically-neutralized CAIS. A synopsis of the experimental design is given in Table 2.

Phase II. Experiments were conducted to ascertain the biological effects of dosing

Table 2. Synopsis of Toxicology Procedures and Number of Animals[a]

Treatment[b] Group	Exposure Duration (hr)	Number of Animals	Toxicologic Evaluation[c]	
			Skin Irritation	Histopathology
Phase II[d]		(11)	(11)	(11)
Agent/Chloroform[e]				
(10% HD, HN or L)				
5 μL	2	(2/11)	(2/11)	(2/11)
10 μL	2	(4/11)	(4/11)	(4/11)
50 μL	2	(2/11)	(2/11)	(2/11)
Neat HD (1 μL)[f]	2	(4/11)	(4/11)	(4/11)
Neat HD (1 μL)[f]	1	(7/11)	(7/11)	(7/11)
Oxidant/Solvent (20 μL)				
(DCDMH/$CHCl_3$/t-BuOH)	1	(5/11)	(5/11)	(5/11)
Phase III[g]		(24)	(24)	(24)
Agent/Chloroform[e]				
(10% HD, HN or L)				
5 μL	1	(20/24)	(20/24)	(20/24)
10 μL	1	(4/24)	(4/24)	(4/24)
Neat HD (1 μL)[f]	1	(16/24)	(16/24)	(16/24)
Wastestreams				
"Archived" "Blue"[h]				
25 μL	1	(8/24)	(8/24)	(8/24)
10 μL	1	(4/24)	(4/24)	(4/24)
"Archived" "Red"[h]				
25 μL	1	(8/24)	(8/24)	(8/24)
10 μL	1	(4/24)	(4/24)	(4/24)
"Archived" "Charcoal"[h]				
25 μL	1	(8/24)	(8/24)	(8/24)
10 μL	1	(4/24)	(4/24)	(4/24)

Table 2. Synopsis of Toxicology Procedures and Number of Animals[a] (cont'd)

Treatment[b] Group	Exposure Duration (hr)	Number of Animals	Toxicologic Evaluation[c]	
			Skin Irritation	Histopathology
Wastestreams				
"Fresh" "Blue" (25 μL)[i]	1	(8/24)	(8/24)	(8/24)
"Fresh" "Red" (25 μL)[i]	1	(8/24)	(8/24)	(8/24)
"Fresh" "Charcoal" (25 μL)[i]	1	(4/24)	(4/24)	(4/24)

(a) Toxicology studies comprised of two phases (Phase II and Phase III) of a multiphase effort: Phase II (dosing-ranging/optimization); Phase III (Vesicancy testing of wastestreams). The total number of animals on test was thirty five [Phase II studies (11); Phase III studies (24)].

(b) Multiple dosing sites per animal (refer to Fig 1). The number of animals per particular treatment ("test article"/dosage/exposure duration) and toxicologic evaluation is given as (#/#).

(c) Toxicologic evaluation consisted of gross changes (erythema/edema) and light microscopic examination. Evaluation, (scoring of gross lesions) based on observations at 24-hr post-dosing. Following euthanasia, skin samples were taken and processed for microscopic examination.

(d) In Phase II studies, each animal was dosed dermally with neat HD, agent/$CHCl_3$ solution, and oxidant/solvent. Dosage of neat HD (1.0 μL), dosage of agent/$CHCl_3$ solution (5, 10 and 50 μL), and dosage of oxidant/solvent (20 μL). "Test article" was allowed to remain in contact with the skin for either one or two hours. Dosages of "test article" rotated among skin exposure sites to control for differences in skin thickness.

(e) Actual ampoules from CAIS kits were not used. Instead, "CAIS" (agent/chloroform solutions) were prepared from agent (HD, HN or L) stocks to the following specifications (10% HD, HN or L).

(f) Chemical agents found in CAIS include HD, HN or L. Sulfur mustard (HD) was used as representative vesicant for the blistering agents.

(g) In Phase III studies, each animal was dosed dermally with neat HD, agent/$CHCl_3$ solution, and wastestreams. Dosage of neat HD (1.0 μL), dosages of agent/$CHCl_3$ solution (5 and 10 μL), and wastestreams (10 and 25 μL). Exposure duration was for one hour. Dosage of "test article" rotated among skin exposure sites to control for differences in skin thickness.

(h) "Archived" "Blue" and "Red" wastestreams initially analyzed Oct 95 and re-analyzed and tested for vesicancy (Mar/Aug 96). "Charcoal" wastestream initially analyzed Nov 95 and re-analyzed and tested for vesicancy in (Mar/Aug 96).

(i) "Fresh" indicates that chemical analysis of wastestreams matched in time with bioassay.

volume and exposure duration, the uniformity and reproducibility of responses, and the skin-injurious effects of oxidant/solvent solution. Eleven animals were dermally dosed with neat HD (1 ul) and with 10 percent agent (HD, HN or L) in chloroform. Dosing volumes ranged from 5 to 50 ul, and exposure times were 1 or 2 hour durations. Five guinea-pigs were treated with oxidant/solvent solution. The exposed skin was examined 24-hr post-exposure for presence of gross and microscopic changes.

Phase III. This phase was designed to ascertain the vesicant potential of neutralized CAIS ("archived" and "fresh" wastestreams) and that of agent/chloroform solutions. The exposed skin was examined 24 hours after "test article" application for presence of skin-injurious effects (gross and microscopic).

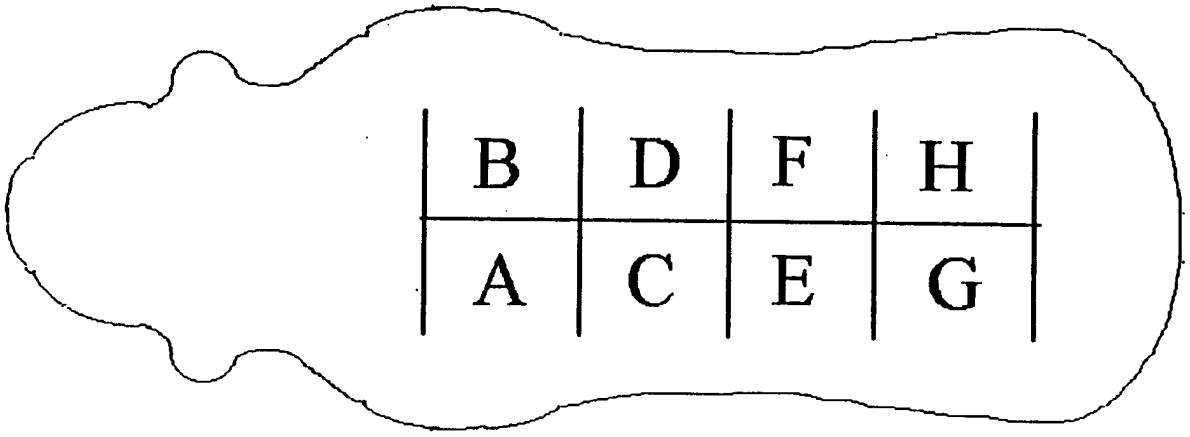
2.4.2 Care and Treatment of Animals

A total of 35 male (approximately 200-350 g and 3 to 4 weeks of age upon receipt), euthymic hairless guinea-pigs (Crl:IAF (HA)-hr BR), procured from Charles River Laboratories (Wilmington, MA; animals supplied from Portage, MI facility), were used in this study. Animals were quarantined and screened for general condition and health status, and were maintained in a program accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Ear tags were applied to maintain positive identification, and animals were maintained between approximately 64 and 79 degrees F and 40 to 70 percent relative humidity with a 12-hr diurnal light cycle. Food and water were provided ad libitum and animals were housed individually in polycarbonate cages prior to exposure to "test article". Following treatment, animals were housed individually within a chemical fume hood during the 24-hr post-exposure period. Following recovery from anesthesia, animals were given food and water.

Animal Preparation and Dosing

Initially using 6 mg xylazine hydrochloride and 35 mg ketamine hydrochloride per kg of body weight given intramuscularly and increasing this to 13 mg xylazine and 87 mg ketamine/kg following the first day of dosing, anesthetized guinea-pigs were dosed topically on both sides of the dorsal midline with "test articles" (six to eight exposure sites/animal) - see Fig 1. Table 2 presents a synopsis of treatments, application volumes, and exposure durations. Approximately 24 hours after dosing, the animals were again anesthetized, sites evaluated for erythema and edema and lesion size, and animals then sacrificed with an inhalation anesthetic (halothane) overdose. Following euthanasia skin samples were collected and processed for histopathology.

FIGURE 1. GUINEA PIG SKIN EXPOSURE SITES



2.5 Histopathologic Analysis

Following euthanasia, skin from the dosed sites was taken and placed in buffered formalin. After fixation, embedding, and sectioning, skin samples were stained with hematoxylin and eosin (H&E) and evaluated for histopathology. Histopathologic lesions (microblisters, epidermal necrosis, follicular necrosis, dermal necrosis, vascular necrosis, hemorrhage, and pustular epidermitis) were graded on a scale of 0-4, where 0 = normal, 1 = minimal, 2 = intermediate, 3 = moderate, 4 = severe. Definitions for scoring histopathology and the criteria for grading severity of lesions are summarized in Table 3. The grading of microblister formation is highlighted in Table 4.

2.6 DATA ANALYSIS

For chemistry data generated in Phase I, means and standard deviations of responses of each control standard were determined to calculate both the inter- and intra- variability of the analytical method. Calibration performance characteristics for each analyte, such as slope and standard error of the slope, R^2 (measure of fit about the regression line), method detection limits, and quantitation limits were calculated.

For Phase III data (vesicating assessment of wastestreams), statistical hypothesis tests were conducted at the 5 percent significance level to determine whether or not the neutralization process reduced the vesicating property of agents contained in CAIS. For each CAIS sample, the incidence of microblisters at sites treated with CAIS agent(s) were compared to those of contralateral sites treated with the wastestream. Although incidence of microblisters was the primary endpoint for evaluating the efficacy of each neutralization process, analyses were also conducted on other indices of skin injury (gross and microscopic). To accommodate the intra-animal correlation of multiple measurements made on the same animal, McNemar's test was used to analyze quantal data (Agresti, 1990). Analysis of variance (ANOVA) models, that include random effects for animal, were fitted to continuous data. If data were not approximately normal, ANOVA were conducted on transformed data, or nonparametric or categorical methods of analysis were performed.

TABLE 3. DEFINITIONS USED IN HISTOPATHOLOGIC EVALUATIONS AND AN EXPLANATION OF THE GRADING OF LESION SEVERITY

Microblister	Loss of epidermal basal cell attachment to the underlying basement membrane of at least two adjacent cells. The loss of attachment creates a space which may appear empty, full of proteinaceous fluid, or filled with neutrophils. One or a few isolated small areas of detachment is graded 1, minimal. Many such areas of detachment, or several larger (10 or more contiguous cells) areas of detachment is graded 2, intermediate. When half or more of the epidermis in the tissue section is detached from the dermis, it is graded 3, moderate. Such lesions typically have a much larger space between the basal cells and the dermis. When nearly all of the epidermis is separated from the dermis, it is graded 4, severe. In such situations, there are usually focal, point attachments, so the entire epidermis is not lifted along the full width of the section.
Epidermal necrosis	The epidermal cells exhibit cytoplasmic eosinophilia, nuclear loss or pyknosis, and are generally shrunken. If only individual cells are affected, it is graded 1 (these are generally isolated basal cells). If small areas are affected, with normal areas in close proximity, it is graded 2. If the epidermis exhibits cell death in a full-thickness (all layers of epidermis) pattern, and affects half or more of the skin section, it is graded 3. If the epidermis is virtually entirely necrotic, it is graded 4. Severe ulcers assume that the epidermis is necrotic.
Follicular necrosis	If isolated epithelial cells of the hair follicles exhibit eosinophilia or pyknosis, it is graded 1. If clusters of adjacent cells within follicles are dead, it is graded 2. If cells of half or more of a particular hair follicle are dead, it is graded 3. Grade 4 lesions have complete necrosis of the follicular epithelium underlying much of the epidermal lesion area. This indicates that the agent has penetrated deeply.
Dermal necrosis	Loss of collagen fiber integrity, evidenced by pale eosinophilic staining and homogeneous appearance, indicates necrosis of dermal fibers. With only isolated areas, it is graded 1. Multiple areas are graded 2. Necrosis of most of the superficial dermal collagen in the lesion area is graded 3. A grade 4 lesion requires deep (to the base of the associated adnexa) dermal necrosis.
Vascular necrosis	Loss of integrity of a medium to large blood vessel is vascular necrosis. Grading depends upon the number of vessels affected and the severity. Partial necrosis of one vessel is graded 1 to 2. Complete necrosis of a vessel is graded 3; multiple such lesions are graded 4.
Hemorrhage	Extravasated erythrocytes is hemorrhage. A few isolated foci is graded 1. Multiple, common foci is graded 2. Large pools of blood is graded 3. A grade 4 lesion requires a massive area of blood pooling and the displacement of large areas of dermal collagen.
Pustular epidermitis	Collections of neutrophils in the epidermis proper is graded by extent; one or two small foci is graded 1; three or more small foci is graded 2; one or more large foci is graded 3; a grade 4 lesion would indicate massive infiltration of the entire epidermis by neutrophils.

TABLE 4. DEFINITION OF DEGREES OF SEVERITY USED FOR HISTOPATHOLOGIC EVALUATION OF VESICATION (MICROBLISTER FORMATION[a])

Lesion Characteristic	Degree of Severity
➡ No lesion (unaffected)	0 (normal)
➡ One or a few isolated areas of detachment	1 (minimal)
➡ Many small areas of detachment or several larger areas of detachment	2 (intermediate)
➡ >50% of the epidermis in tissue section is detached from the dermis (much larger space between basal cells and dermis)	3 (moderate)
➡ Nearly all the epidermis is separated from the dermis	4 (severe)

[a] Microblister: loss of epidermal basal cell attachment to underlying basement membrane of at least two adjacent cells. Loss of attachment creates a space.

3. Results

3.1 Chemistry

Nitrogen mustard, sulfur mustard, and lewisite are components of CAIS that were chemically neutralized ("detoxified") on reaction with treatment reagent (1, 3-dichloro-5, 5-dimethylhydantoin). The selection of a particular process chemistry (designated as "Blue", "Red", or "Charcoal" process) was dependent on whether the agent was neat material (HD), in solution (agent in chloroform), or adsorbed on charcoal, The DCDMH-mediated neutralization of sulfur mustard resulted in HD concentrations below 50 ppm in "Blue" process wastestream (product solution). Chemical treatment resulted in the conversion of sulfur mustard to HD sulfoxide degradation products (Further oxidation to sulfone was also a possibility under conditions via neutralization by DCDMH). Secondary reactions (i.e. elimination/substitution) also occurred that produced chlorinated and vinyl sulfoxides. The neutralization reaction between oxidant and CAIS containing agent (HD, HN or L in chloroform - "Red" process) resulted in complex product solutions containing various products/by-products and residual amounts of unreacted agent. The process chemistry for neutralization of CAIS components containing agent (HD, HN or L) on charcoal ("Charcoal" process), also resulted in the formation of complex product solutions. Residual amounts of agent were detected. Details pertaining to the process chemistry and analyses have been reported in detail (Olson et al, 1997 and Lucas, 1997).

The "archived" wastestreams were additionally analyzed for product/by-product composition. HD sulfoxide and other degradation products resultant from secondary reactions (e.g. elimination, substitution) were detected in wastestream samples. HD sulfone and/or its vinyl containing derivatives, which are known vesicants, were not detected in the "Blue" process wastestream. Product analyses did not reveal HD sulfone or vinyl/divinyl analogs in the product solution obtained from the chemical neutralization of CAIS containing agent in chloroform ("Red" process). Product characterization of the "Charcoal" wastestream did not reveal HD sulfone; however, multichlorinated vinyl containing derivatives (non-vesicant) were present in the product solution.

3.2 Dermal Effects

3.2.1 Gross Pathologic Findings

Phase II. All skin exposures to HD and agent/chloroform solutions containing 10 percent HD, HN or L resulted in gross skin lesions consisting of well-defined areas of edema and erythema of moderate to severe intensity. In some instances, large areas of ulceration with complete loss of the covering epidermis was evident. The skin-injurant effects of HN and L were comparable to that produced by HD (refer to Table 5 and Appendix A). The skin-injurious effect of oxidant/solvent solution was minimal gross lesions (refer to Table 5 and Appendix A).

Phase III. The cutaneous injury (non-vesicant) effects after one hour exposure to HD, agent/$CHCl_3$, or CAIS wastestreams ("archived" and "fresh") were evaluated and are summarized in Table 6. Individual gross pathology data are presented in Appendix A. All agent-dosed sites demonstrated gross lesions. Wastestream-induced dermal injury resulted in mild to moderate degrees of erythema and edema.

3.2.2 Histopathologic Findings

Phase II. Two hour dermal exposures of animals to neat HD (1 μL) and to various doses (5 - 50 μL) of agent/chloroform solutions containing 10 percent HD, HN or L resulted in microblister formation of intermediate to severe intensity - refer to Table 7. Incidence of histopathologic changes are summarized in Table 8. In some animals, large areas of ulceration with loss of epidermis prevented the occurrence of microblisters. Individual animal histopathology data are presented in Appendix B. Based on the outcome of the two-hour exposure studies, other guinea pigs were dosed with 5 and 10 μL volumes of 10 percent agent in chloroform solutions and with neat HD (1 μL) at an exposure duration of one hour. Microblister formation was evident at all sites, unless occurrence was precluded by development of an ulcer, and ranged in severity from moderate to severe. The application of 5 μL of 10 percent agent/chloroform solution resulted in microblisters of at least intermediate severity. Refer to Table 7 for incidence/response summary and Appendix B for individual histopathologic findings. The oxidant/solvent system was also evaluated for skin effects. Animals treated with oxidant/solvent solution did not manifest dermal lesions other than minimal inflammatory cell infiltration - refer to Table 8 and Appendix B.

Phase III. Twenty-four animals comprising Phase III of the study were treated with "neutralized" CAIS to ascertain the vesicating potential of chemically degraded CAIS. Incidence/response data related to microvesication are summarized in Tables 9, 10, and 11. A

TABLE 5. PHASE II - SKIN REACTION (ERYTHEMA AND EDEMA) FOLLOWING EXPOSURE TO HD, AGENT/$CHCL_3$ SOLUTIONS, AND OXIDANT/SOLVENT SOLUTION

Experiment Date/ Animal ID	Test Article	Dose Volume (µL)	Time to Decontamination (hr)	No.of Animals Tested	Erythema Score, Mean	Edema Score, Mean
02/19/96 (301, 305)	10% L/$CHCl_3$	10	2	2	3.0	3.0
	10% L/$CHCl_3$	50	2	2	3.0	3.0
	10% HN/$CHCl_3$	10	2	2	2.0	2.0
	10% HN/$CHCl_3$	50	2	2	2.0	2.0
	10% HD/$CHCl_3$	10	2	2	2.0	2.0
	10% HD/$CHCl_3$	50	2	2	2.5	2.0
	Neat HD	1	2	2	2.0	2.0
02/21/96 (306, 309)	10% L/$CHCl_3$	5	2	2	2.5	3.0
	10% L/$CHCl_3$	10	2	2	2.5	3.0
	10% HN/$CHCl_3$	5	2	2	2.0	2.0
	10% HN/$CHCl_3$	10	2	2	2.0	2.5
	10% HD/$CHCl_3$	5	2	2	3.0	3.0
	10% HD/$CHCl_3$	10	2	2	2.0	2.0
	Neat HD	1	2	2	2.0	2.5
02/27/96 (312, 316)	10% L/$CHCl_3$	5	1	2	3.0	3.0
	10% L/$CHCl_3$	10	1	2	3.0	3.0
	10% HN/$CHCl_3$	5	1	2	2.0	2.5
	10% HN/$CHCl_3$	10	1	2	2.0	2.0
	10% HD/$CHCl_3$	5	1	2	3.0	2.0
	10% HD/$CHCl_3$	10	1	2	2.5	2.0
	Neat HD	1	1	2	3.0	2.5
03/05/96 (311, 313, 315, 317, 324)	10% L/$CHCl_3$	5	1	5	3.0	2.8
	10% HN/$CHCl_3$	5	1	5	1.8	2.0
	10% HD/$CHCl_3$	5	1	5	2.4	2.4
	Neutralizing Solution	20	1	5	0.0	1.0
	Neat HD	1	1	5	2.4	2.6

TABLE 6. PHASE III. SKIN REACTION (ERYTHEMA AND EDEMA) FOLLOWING EXPOSURE TO HD, AGENT/$CHCl_3$ SOLUTION OR CAIS WASTESTREAMS

Date, Source of Wastestream	Test Article	Dose Volume (µL)	No. of Animals Tested	Erythema Score		Edema Score		Lesion Area (mm^2)	
				Mean	S.D.	Mean	S.D.	Mean	S.D.
03/13/96, 03/21/96 "Archived" Wastestreams	10% L/$CHCl_3$	5	8	2.9	0.3	3.0	0.0	95.4	22.2
	10% HN/$CHCl_3$	5	8	2.0	0.9	2.0	0.5	60.1	13.6
	10% HD/$CHCl_3$	5	8	2.6	0.7	2.1	0.8	107.9	35.8
	"Red" Wastestream	25	8	1.1 [a,b,c]	0.3	1.8 [a]	0.5	237.4 [d,e,f]	71.3
	"Blue" Wastestream	25	8	1.9 [a,c]	0.8	1.6 [a,c]	0.5	236.5 [d,e,f]	72.6
	"Charcoal" Wastestream	25	8	0.4 [a,b,c]	0.2	0.4 [a,b,c]	0.2	132.9 [e]	95.9
	Neat HD	1	8	2.8	0.5	2.9	0.3	180.2	53.1
06/20/96, 06/26/96 "Fresh" Wastestreams	10% L/$CHCl_3$	5	8	3.0	0.0	3.0	0.0	156.0	67.1
	10% HN/$CHCl_3$	5	8	1.9	0.3	2.1	0.3	82.0	30.8
	10% HD/$CHCl_3$	5	8	2.4	0.5	2.3	0.5	94.9	18.4
	"Red" Wastestream	25	8	0.3 [a,b,c]	0.3	0.3 [a,b,c]	0.3	46.2 [a]	52.3
	"Blue" Wastestream	25	8	1.8 [a,c]	0.5	1.6 [a,b,c]	0.5	220.6 [d,e,f]	42.0
	Neat HD	1	8	2.5	0.5	2.4	0.5	126.8	32.6
08/13/96 "Archived" Wastestreams	10% L/$CHCl_3$	10	4	3.0	0.0	2.8	0.5	212.6	35.6
	10% HN/$CHCl_3$	10	4	2.5	0.6	2.3	0.5	155.1	21.4
	10% HD/$CHCl_3$	10	4	2.3	1.0	2.3	0.5	178.7	34.9
	"Red" Wastestream	10	4	1.1 [a,b,c]	0.6	0.8 [a,b,c]	1.0	121.1 [a,c]	41.8
	"Blue" Wastestream	10	4	1.3 [a,b,c]	0.5	1.0 [a,b,c]	0.0	142.4 [a]	42.3
	"Charcoal" Wastestream	10	4	0.4 [a,b,c]	0.2	0.0 [a,b,c]	0.0	69.3 [a,b,c]	23.0
08/29/96 "Fresh" Wastestream	10% L/$CHCl_3$	5	4	3.0	0.0	2.8	0.5	113.9	33.1
	10% HN/$CHCl_3$	5	4	1.5	0.6	2.0	0.8	91.3	19.6
	10% HD/$CHCl_3$	5	4	3.0	0.0	3.0	0.00	89.5	26.2
	"Charcoal" Wastestream	25	4	0.0 [a,b,c]	0.0	0.0 [a,b,c]	0.0	0.0 [a,b,c]	0.0

Note: All times to decontamination were 1 hr.

a Mean is significantly less than that observed on sites treated with L.
b Mean is significantly less than that observed on sites treated with HN.
c Mean is significantly less than that observed on sites treated with HD.
d Mean is significantly greater than that observed on sites treated with L.
e Mean is significantly greater than that observed on sites treated with HN.
f Mean is significantly greater than that observed on sites treated with HD.

TABLE 7. PHASE II. VESICATION (MICROBLISTER FORMATION) IN HAIRLESS GUINEA PIGS FOLLOWING DERMAL EXPOSURE TO HD, AGENT/$CHCl_3$ SOLUTIONS, OR NEUTRALIZING SOLUTION (DCDMH/$CHCl_3$/t-BuOH)[a]

Treatment[b] Group (2 hour)	Animal No.	Microblister Severity (0-4) 301	305	306	309	Response	Mean Severity
Neat HD (1 μL)		2	2	1[c]	3	4/4	2.0
10% HD/$CHCl_3$							
50 μL		2	2			2/2	2.0
10 μL		2	3	3	3	4/4	2.8
5 μL				1[c]	0[c]	**1/2**	0.5
10% HN/$CHCl_3$							
50 μL		2	2			2/2	2.0
10 μL		2	2	2[c]	4	4/4	2.5
5 μL				4	4	2/2	4.0
10% L/$CHCl_3$							
50 μL		4	3			2/2	3.5
10 μL		3	3	3	4	4/4	3.3
5 μL				4	4	2/2	4.0

Treatment[d] Group (1 hour)	Animal No.	312	316	311	313	315	317	324	Response	Mean Severity
Neat HD (1 μL)		3	3	3	2	2	2	3	7/7	2.6
10% HD/$CHCl_3$										
10 μL		3	3						2/2	3.0
5 μL		3	3	2	3	3	2	4	7/7	2.9
10% HN/$CHCl_3$										
10 μL		4	3						2/2	3.5
5 μL		3	4	3	4	2	3	4	7/7	3.3
10% L/$CHCl_3$										
10 μL		3	4						2/2	3.5
5 μL		3	4	3	4	4	2	4	7/7	3.4
DCDMH/$CHCl_3$/ t-BuOH (20 μL)				0	0	0	0	0	0/5	0

a At 24 hr after dosing, animals were evaluated for skin injury, sacrificed, and skin samples taken and prepared for histopathology.
b Exposure duration 2 hr.
c Ulceration at dosing site may have obscured evidence of microvesication
d Exposure duration 1hr.

TABLE 8. PHASE II. SUMMARY OF HISTOPATHOLOGY RESULTS

Experiment Date/ Animal ID	Test Article	Dose Volume (µL)	Time to Decon. (hr)	No.of Animals	No. of Sites	Number of Animals with Sign						
						Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
02/19/96 (301, 305)	10% L/$CHCl_3$	10	2	2	2	2	2	2	0	0	2	0
	10% L/$CHCl_3$	50	2	2	2	2	2	2	0	0	1	0
	10% HN/$CHCl_3$	10	2	2	2	2	2	2	0	0	0	0
	10% HN/$CHCl_3$	50	2	2	2	2	2	2	0	0	0	0
	10% HD/$CHCl_3$	10	2	2	2	2	2	2	0	0	1	0
	10% HD/$CHCl_3$	50	2	2	2	2	2	2	0	0	1	0
	Neat HD	1	2	2	2	2	2	2	0	0	0	0
02/21/96 (306, 309)	10% L/$CHCl_3$	5	2	2	2	2	2	2	0	1	1	0
	10% L/$CHCl_3$	10	2	2	2	2	2	2	0	1	1	0
	10% HN/$CHCl_3$	5	2	2	2	2	2	2	1	0	0	0
	10% HN/$CHCl_3$	10	2	2	2	2	2	2	1	1	0	0
	10% HD/$CHCl_3$	5	2	2	2	1	2	2	0	2	0	0
	10% HD/$CHCl_3$	10	2	2	2	2	2	2	1	1	0	0
	Neat HD	1	2	2	2	2	2	2	0	1	0	0

TABLE 8. PHASE II. SUMMARY OF HISTOPATHOLOGY RESULTS (CONT'D.)

Experiment Date/ Animal ID	Test Article	Dose Volume (µL)	Time to Decon. (Hr)	No. Of Animals	No. of Sites	Number of Animals with Sign						
						Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
02/27/96 (312, 316)	10% L/$CHCl_3$	5	1	2	2	2	2	2	0	0	2	0
	10% L/$CHCl_3$	10	1	2	2	2	2	2	0	0	2	0
	10% HN/$CHCl_3$	5	1	2	2	2	2	2	2	0	0	0
	10% HN/$CHCl_3$	10	1	2	2	2	2	2	2	0	0	0
	10% HD/$CHCl_3$	5	1	2	2	2	2	2	1	1	0	0
	10% HD/$CHCl_3$	10	1	2	2	2	2	2	1	0	1	0
	Neat HD	1	1	2	2	2	2	2	1	0	0	0
03/05/96 (311, 313, 315, 317, 324)	10% L/$CHCl_3$	5	1	5	5	5	5	5	0	1	5	0
	10% HN/$CHCl_3$	5	1	5	5	5	5	5	4	1	1	0
	10% HD/$CHCl_3$	5	1	5	5	5	5	5	1	3	2	0
	Neutralizing Solution	20	1	5	20	0	0	0	0	0	0	0
	Neat HD	1	1	5	5	5	5	5	0	0	1	0

summary of histopathologic changes, including vesication, is presented in Tables 12 and 13. Individual histopathology data appear in Appendix B. Eight animals were dosed with "archived" wastestreams, agent/chloroform solutions, and neat HD. Guinea-pigs dosed with HD and agent/chloroform solutions demonstrated at least minimal microvesication along with consistent, marked epidermal and follicular necrosis. The "Blue" process wastestream ("archived"; 25 μL application) resulted in intermediate to severe microblisters and severe epidermal necrosis at all sites dosed (refer to Tables 9 and 13 and Appendix B). The impression of the pathologist reading the slides was that lesions did not appear to be "basal cell specific", as chemical blistering agents appear to cause, nor did the lesions resulting from application of the "Blue" wastestream penetrate deeply enough to cause severe necrosis in the follicular epithelium. A photomicrograph representative of the morphologic changes observed following treatment with a vesicant is shown in Figure 2a, and one demonstrating the appearance of normal hairless guinea pig epidermis is shown in Figure 2b. The morphologic changes seen consist of ballooning degeneration and loss of epidermal basal cell attachment to the underlying basement membrane. Neither "Red" nor "Charcoal" process wastestreams ("archived"; 25 μL application) produced microblisters (Tables 9 and 12). The "Red" process wastestream produced only minimal pustular epidermitis or minimal epidermal necrosis (refer to Table 12 and Appendix B). The "Charcoal" process wastestream ("archived"; 25 μL application) killed some surface epithelial cells (minimal to intermediate epidermal necrosis) but did not penetrate to basal cells - refer to Table 12 and Appendix B. Four guinea pigs were dosed with 10 μL of "Blue", "Red", and "Charcoal" process wastestreams ("archived") and evaluated for dermal effect. The "Blue" process wastestream induced microblisters whereas the "Red" and "Charcoal" process wastestreams did not elicit microblister formation. The findings are highlighted in Table 10. Histopathology findings are summarized in Tables 12 and 13, and individual histopathology data are presented in Appendix B.

"Fresh" wastestream-induced skin effects were also evaluated. Data on microvesication are presented in Tables 11, 12 and 13, and other histopathologic skin effects data are given in Tables 12 and 13. Individual animal histopathology results are presented in Appendix B. All agent-dosed sites (neat HD and agent/chloroform solutions) and all "Blue" process wastestream sites demonstrated histopathologic lesions including microvesication. In "fresh" "Red" process wastestream-dosed animals, minimal to no lesions were seen on histopathologic examination. One "Red" process wastestream site in one animal demonstrated histopathology, including minimal microvesication; however, this lesion was incompatible with what had been noted previously. The "Charcoal" process wastestream did not produce microblisters and none of the sites demonstrated histopathology graded more than minimal.

Text continues of page 40.

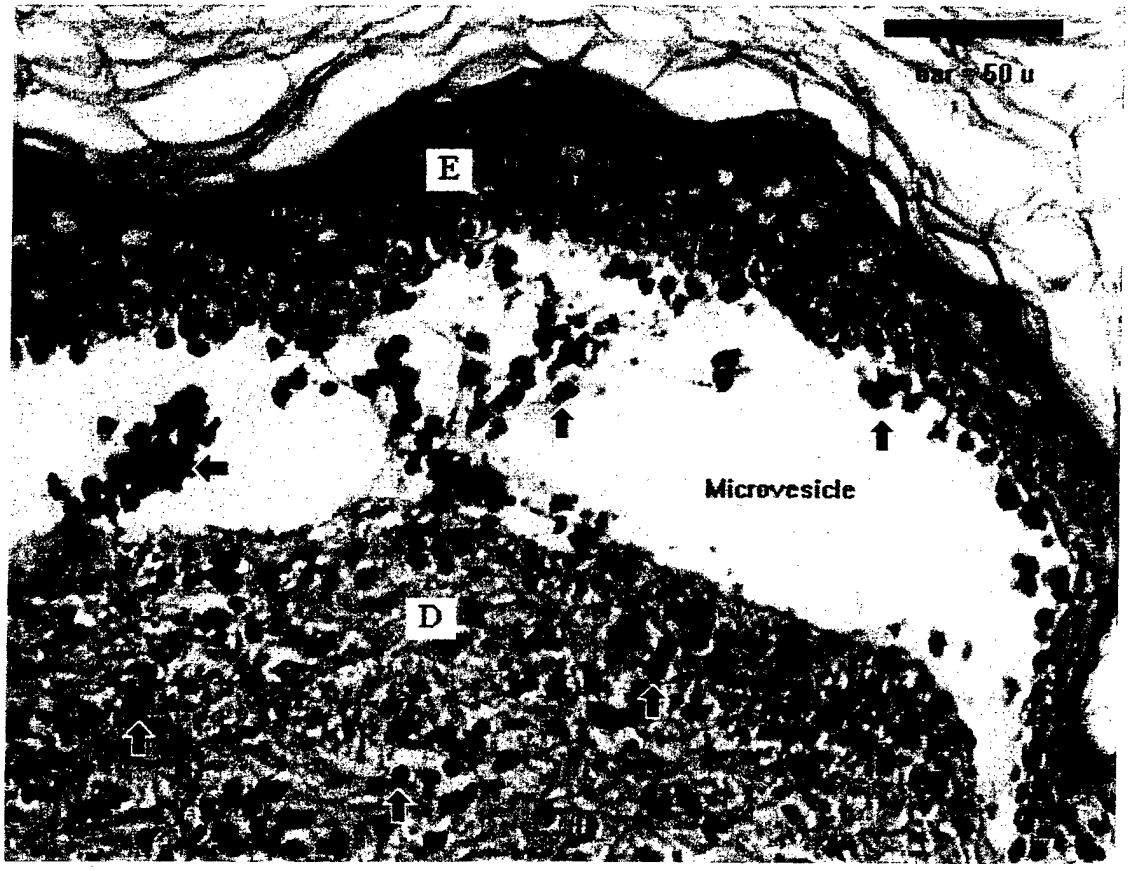


Fig. 2a. Typical microblister in a hairless guinea pig 24 hours after exposure to vesicant. Epidermis (E) is eosinophilic and shrunken due to necrotic epithelium; dermis (D) is also necrotic and contains an infiltrate of polymorphonuclear cells (arrows), as does the microblister cavity (microvesicle).

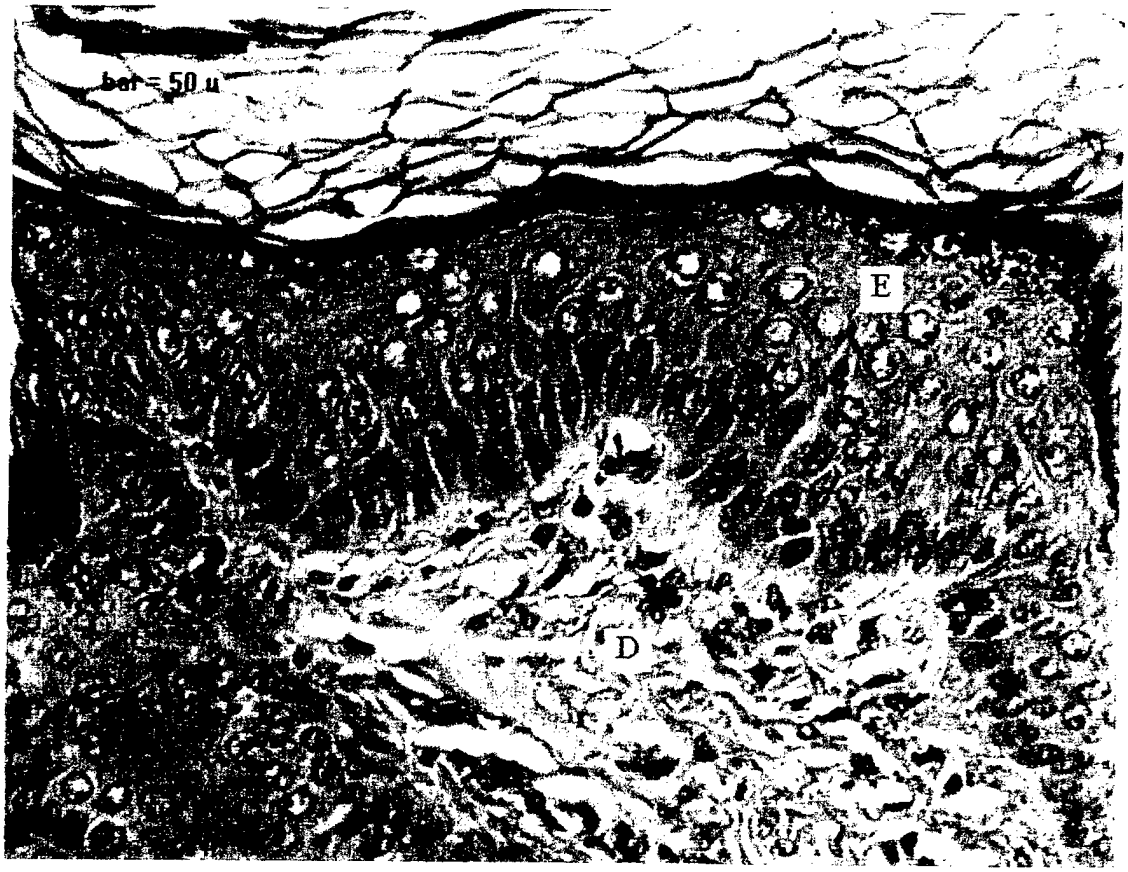


Fig. 2b. Normal skin from a hairless guinea pig. Epidermis (E) and dermis (D) are visible. Note differences in appearance from the necrotic tissue depicted in Fig. 2a. Magnification of both skin photomicrographs is the same.

TABLE 9. PHASE III. VESICATION (MICROBLISTER FORMATION) IN HAIRLESS GUINEA PIGS FOLLOWING EXPOSURE TO "ARCHIVED" RRS WASTESTREAMS, AGENT/$CHCl_3$ SOLUTIONS, OR NEAT SULFUR MUSTARD (HD) [a,b]

Treatment Group		Animal No.	Microblister Severity (0-4)									
			494	496	497	499	310	491	493	498	Response	Mean Severity Score
Neat HD (1 μL)			3	2	2	3	1	2	2	1	8/8	2.0
10% HD/$CHCl_3$	(5 μL)		1	0	2	4	2	1	1	3	7/8	1.8
10% HN/$CHCl_3$	(5 μL)		3	0	1	4	4	4	4	2	7/8	2.8
10% L/$CHCl_3$	(5 μL)		4	1	4	3	1	4	2	3	8/8	2.8
"Blue" wastestream [c]	(25 μL)		2	4	2	2	2	3	4	3	8/8	2.8
"Red" wastestream [c]	(25 μL)		0	0	0	0	0	0	0	0	0/8	0
"Charcoal" wastestream [c]	(25 μL)		0	0	0	0	0	0	0	0	0/8	0

a Each animal was dosed percutaneously (1 hr exposure) with neat HD, agent/ $CHCl_3$ solution, and "archived" wastestreams. Sites were evaluated visually at about 24 hr after dosing, and the animals then sacrificed and skin samples taken and prepared for histopathologic evaluation.

b Dosing volumes of HD and agent/$CHCl_3$ solutions, as well as the duration of exposure, were based on preliminary tests. Dosing volumes of wastestreams were based upon approximate ratio of neutralization solution volume to volume of agent treated.

c Wastestreams were generated from the reaction of DCDMH (oxidant) with neat HD ("Blue" process), with 10% HD, HN or L in $CHCl_3$ ("Red" process), or with HD, HN, or L on charcoal ("Charcoal" process).

TABLE 10. PHASE III. MICROBLISTER FORMATION IN HAIRLESS GUINEA PIGS FOLLOWING EXPOSURE TO EQUAL VOLUMES OF "ARCHIVED" RRS WASTESTREAMS OR AGENT/$CHCl_3$ SOLUTIONS [a]

Treatment Group		Animal No.	Microblister Severity (0-4) 383	385	389	400	Response	Mean Severity Score
10% HD/$CHCl_3$	(10 μL)		2	3	2	3	4/4	2.5
10% HN/$CHCl_3$	(10 μL)		3	4	3	4	4/4	3.5
10% L/$CHCl_3$	(10 μL)		3	4	2	3	4/4	3.0
"Blue" wastestream [b]	(10 μL)		0	1	2	3	3/4	1.5
"Red" wastestream [b]	(10 μL)		0	0	0	0	0/4	0
"Charcoal" wastestream [b]	(10 μL)		0	0	0	0	0/4	0

a Each animal was dosed dermally (1 hr exposure) with agent/$CHCl_3$ solutions and wastestreams. Sites were evaluated visually at about 24 hr after dosing, and the animals then sacrificed and skin samples taken and prepared for histolopathologic evaluation.

b Wastestreams (product solutions) generated from reaction of oxidant (DCDMH) with HD - "Blue"; 10% HD, HN or L in $CHCl_3$ - "Red" ; HD, HN or L on charcoal - "Charcoal".

TABLE 11. PHASE III. MICROBLISTER FORMATION IN HAIRLESS GUINEA PIGS FOLLOWING EXPOSURE TO "FRESH" RRS WASTESTREAMS, AGENT/$CHCl_3$ SOLUTIONS, OR NEAT HD [a,b]

Treatment Group		Animal No.	Microblister Severity (0-4) 339	341	342	346	340	345	351	352	Response	Mean Severity Score
Neat HD	(1 μL)		2	2	3	2	0	1	1	2	7/8	1.6
10% HD/$CHCl_3$	(5 μL)		3	2	3	2	2	2	1	1	8/8	2.0
10% HN/$CHCl_3$	(5 μL)		3	2	4	4	3	1	1	2	8/8	2.5
10% L/$CHCl_3$	(5 μL)		4	3	3	2	3	3	4	3	8/8	3.1
"Blue" wastestream [c]	(25 μL)		2.5	1	2	1	3	1.5	2	1.5	8/8	1.8
"Red" wastestream [c]	(25 μL)		0	0	0.5 [d]	0	0	0	0	0	1/8	0

Treatment Group		Animal No.	379	380	387	388	Response	Mean Severity Score
10% HD/$CHCl_3$	(5 μL)		2	3	3	4	4/4	3.0
10% HN/$CHCl_3$	(5 μL)		3	4	2	3	4/4	3.0
10% L/$CHCl_3$	(5 μL)		4	4	4	4	4/4	4.0
"Charcoal" wastestream [e]	(25 μL)		0	0	0	0	0/4	0

a Each animal was exposed dermally for 1 hr to "test article" (neat HD and/or agent/$CHCl_3$ solution, and wastestreams). At 24 hr after dosing, animals were evaluated for gross skin injury and then sacrificed and skin samples taken and prepared for histopathologic evaluation.

b Dosing volumes and duration of exposure were determined from preliminary testing. Dosing volume of wastestreams was selected on the basis of approximate neutralization solution volume to volume of agent. Wastestreams were generated via the reaction of DCDMH with neat HD - "Blue"; HD, HN, or L in $CHCl_3$ -"Red"; and HD, HN, or L on charcoal - "Charcoal".

c Mean value for the two sites dosed with each wastestream on each animal.

d Could be due to adjacent HD-treated site.

e Three sites on each animal were dosed with "Charcoal" wastestream and no sites exhibited microblisters.

TABLE 12. PHASE III. SUMMARY OF HISTOPATHOLOGY RESULTS

Date, Source of Wastestream	Agent[e] / Compound	Dose Volume (µL)	No. Of Animals	No.of Sites	Number of Animals with Sign						
					Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
03/13/96, 03/21/96 "Archived" Wastestreams	L	5	8	8	8	8	8	0	6	5	0
	HN	5	8	8	7[a]	8	8	3	4	0	0
	HD	5	8	8	7[a]	8	8	3	7	0	0
	"Red" Wastestream	25	8	8	0[b,c,d]	1[b,c,d]	0[b,c,d]	2	0[b,d]	0	0
	"Blue" Wastestream	25	8	8	8	8	1[b,c,d]	1	1[d]	0	0
	"Charcoal" Wastestream	25	8	8	0[b,c,d]	6	0[b,c,d]	2	0[b,d]	0	0
	Neat HD	1	8	8	8	8	8	0	7	1	0
06/20/96, 06/26/96 "Fresh" Wastestreams	L	5	8	8	8	8	8	1	3	5	0
	HN	5	8	8	8	8	8	2	5	2	0
	HD	5	8	8	8	8	8	2	5	5	0
	"Red" Wastestream	25	8	16	1[b,c,d]	2[b,c,d]	1[b,c,d]	1	0	0	0
	"Blue" Wastestream	25	8	16	8	8	7	2	1	0	0
	Neat HD	1	8	8	7	8	8	0	5	5	0
08/13/96 "Archived" Wastestreams	L	10	4	4	4	4	4	0	0	4	1
	HN	10	4	4	4	4	4	4	1	2	0
	HD	10	4	4	4	4	4	1	0	2	0
	"Red" Wastestream	10	4	4	0	0	0	0	0	0	0
	"Blue" Wastestream	10	4	4	3	3	2	1	0	1	0
	Charcoal Wastestream	10	4	4	0	1	0	1	0	0	0
08/29/96 "Fresh" Wastestreams	L	5	4	4	4	4	4	0	1	4	0
	HN	5	4	4	4	4	4	1	1	1	0
	HD	5	4	4	4	4	4	0	1	2	0
	"Charcoal" Wastestream	25	4	12	0	4	4	1	0	0	0

Note: All times to decontamination were 1 hr.

a Marked ulceration at the dosing site on animal number 496 obscured any evidence of microvesication.

b Incidence of sign was significantly less than that for sites dosed with L using McNemar's Test and a significance level of p=0.05.

c Incidence of sign was significantly less than that for sites dosed with HN using McNemar's Test and a significance level of p=0.05.

d Incidence of sign was significantly less than that for sites dosed with HD using McNemar's Test and a significance level of p=0.05.

e Agent (L, HN, HD) at a concentration of 10% in chloroform.

TABLE 13. PHASE III. SUMMARY OF INTERMEDIATE TO SEVERE HISTOPATHOLOGY RESULTS

Date, Source of Wastestream	Agent[e] / Compound	Dose Volume (µL)	No. Of Animals	No.of Sites	Number of Animals with Sign Rated Intermediate to Severe						
					Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
03/13/96, 03/21/96 "Archived" Wastestreams	L	5	8	8	6[a]	8	8	0	6	2	0
	HN	5	8	8	6[a]	8	8	0	3	0	0
	HD	5	8	8	4[a]	8	8	0	7	0	0
	"Red" Wastestream	25	8	8	0[b,c]	0[b,c,d]	0[b,c,d]	0	0[b,d]	0	0
	"Blue" Wastestream	25	8	8	8	8	0[b,c,d]	0	0[b,d]	0	0
	"Charcoal" Wastestream	25	8	8	0[b,c]	2[b,c,d]	0[b,c,d]	0	0[b,d]	0	0
	Neat HD	1	8	8	6[a]	8	8	0	6	0	0
06/20/96, 06/26/96 "Fresh" Wastestreams	L	5	8	8	8	8	8	0	3	2	0
	HN	5	8	8	6[a]	8	8	0	3	1	0
	HD	5	8	8	6[a]	8	8	0	5	2	0
	"Red" Wastestream	25	8	16	0[b,c,d]	1[b,c,d]	1[b,c,d]	0	0	0	0
	"Blue" Wastestream	25	8	16	7	8	2[b,c,d]	0	1	0	0
	Neat HD	1	8	8	5	8	8	0	5	1	0
08/13/96 "Archived" Wastestreams	L	10	4	4	4	4	4	0	0	4	0
	HN	10	4	4	4	4	4	0	0	0	0
	HD	10	4	4	4	4	4	0	0	1	0
	"Red" Wastestream	10	4	4	0	0	0	0	0	0	0
	"Blue" Wastestream	10	4	4	2	2	0	0	0	0	0
	"Charcoal" Wastestream	10	4	4	0	0	0	0	0	0	0
08/29/96 "Fresh" Wastestream	L	5	4	4	4	4	4	0	1	3	0
	HN	5	4	4	4	4	4	0	0	0	0
	HD	5	4	4	4	4	4	0	1	1	0
	"Charcoal" Wastestream	25	4	12	0	0	0	0	0	0	0

Note: All times to decontamination were 1 hr.

a Ulceration at some dosing sites may have obscured evidence of microvesication.

b Incidence of sign was significantly less than that for sites dosed with L using McNemar's Test and a significance level of p=0.05.

c Incidence of sign was significantly less than that for sites dosed with HN using McNemar's Test and a significance level of p=0.05.

d Incidence of sign was significantly less than that for sites dosed with HD using McNemar's Test and a significance level of p=0.05.

e Agent (L, HN, HD) at a concentration of 10% in chloroform.

3.3 Data Analysis Results

3.3.1 Gross Pathology (Erythema and Edema)

Means and standard deviations were calculated for erythema and edema scores (Phase II and III Studies) and for lesion areas (Phase III Studies). Analysis of variance was performed for inflammation scores and lesion areas. Table 6 presents means and standard deviations for erythema and edema scores. Significant decreases in average inflammation scores resulted when comparing wastestream-dosed ("archived" or "fresh" -25 μL volume application) to agent-dosed sites (HD or agent/chloroform) - refer to Table 6. Some significant increases in lesion areas were noted with wastestreams, presumably due to the larger volume dosed. For the "August 13, 1996" experiment (vesicancy assay of "archived" wastestreams), significant decreases in average inflammation scores as well as average lesion areas resulted when comparing wastestream-dosed ("archived" "Red" and "Blue" process wastestreams - 10 μL volume applications of wastestreams and agent/chloroform solutions) to agent-dosed sites. All observed inflammation scores and lesion areas from the "fresh" "Charcoal" wastestream-dosed sites were zero.

3.3.2 Histopathology

Statistical analysis (McNemar's test) of the histopathology data was performed to ascertain the significance between treatment groups (neat HD, agent/chloroform solutions, and wastestreams) at the 0.05 significance level. Sites dosed with "Red" or "Charcoal" wastestream ("archived", 25 μL volume application) exhibited a significant decrease in incidence (incidence = 0) of microblisters compared to those sites dosed with HD or agent/chloroform solutions. Sites dosed with the wastestreams also showed a significant decrease in the incidence of follicular necrosis compared to sites dosed with any of the three agents (HD, HN, or L in chloroform; neat HD). Some significant neutralized wastestream versus agent differences also resulted with respect to incidence of epidermal and dermal necrosis.

Sites dosed with "Red" wastestream ("fresh", 25 μL volume application) showed a significant decrease in incidence of microblisters (incidence = 0), epidermal necrosis, and follicular necrosis compared to that on sites dosed with any of the three agents. Numerical reductions in some pathology from wastestream-dosed sites ("archived", 10 μL volume application) were observed, although they were not statistically significant due to the smaller number of animals tested.

Statistical analysis of incidence of intermediate to severe histopathologic signs was also performed. Sites dosed with "Red" or "Charcoal" wastestream ("archived", 25 μL volume application) demonstrated a significant decrease in incidence (incidence = 0) of microblisters compared to that on sites dosed with L/chloroform and HN/chloroform. A decrease in incidence (incidence = 0) was also observed for the "Red" or "Charcoal" wastestream compared to that on sites dosed with HD/chloroform, but were not statistically significant because only four of the

eight animals exposed to HD/chloroform had intermediate to severe microblisters. Sites dosed with "Red" or "Charcoal" wastestream ("archived", 25 μL volume application) demonstrated a significant decrease in incidence of epidermal necrosis and follicular necrosis compared to that on sites dosed with any of the three agents. Sites dosed with "Red" wastestream ("fresh", 25 μL volume application) showed a significant decrease in incidence of microblisters (incidence = 0), epidermal necrosis, and follicular necrosis compared to that on sites dosed with any of the three agents. Sites dosed with "Blue" wastestream showed a significant decrease in incidence of follicular necrosis compared to that observed on sites dosed with any of the three agents.

Sites dosed with "fresh" "Charcoal" wastestream (25 μL volume application) exhibited a numerical reduction in incidence (incidence = 0) of microblisters, although this was not statistically significant due to the smaller number of animals tested, compared to that observed on sites dosed with any of the three agents. Statistical analyses also were conducted on the pooled "Charcoal" wastestream data ("fresh" and "archived", 25 μL volume applications- see Table 14). These analyses assumed that the probability of a microblister and other histopathologic endpoints is similar for sites dosed with "archived" and "fresh" "Charcoal" wastestreams. Pooled data for sites dosed with "Charcoal" wastestream showed a significant decrease in incidence of microblisters (incidence = 0) and follicular necrosis compared to that on sites dosed with any of the three agents. Statistical analyses of incidence of intermediate to severe histopathologic signs (Table15) were also performed on the pooled "Charcoal" wastestream data ("fresh" or "archived", 25 μL volume application). Sites dosed with "Charcoal" wastestream showed a significant decrease in incidence (incidence = 0) of intermediate to severe microblisters, epidermal necrosis, and follicular necrosis compared to that observed on sites dosed with any of the three agents.

TABLE 14. PHASE III. SUMMARY OF HISTOPATHOLOGY FOLLOWING DOSING OF "CHARCOAL" WASTESTREAM [a]

Agent/ Compound	Dose Volume (µL)	No. Of Animals	No. of Sites	Number of Animals with Histopathology						
				Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
L [f]	5	12	12	12	12	12	0	7	9	0
HN [f]	5	12	12	11 [b]	12	12	4	5	1	0
HD [f]	5	12	12	11 [b]	12	12	3	8	2	0
"Charcoal" Wastestream	25	12	20	0 [c,d,e]	10	4 [c,d,e]	3	0 [c,e]	0 [c]	0

Note: All times to decontamination were 1 hr.

a Pooled data from the "Charcoal" wastestream received 1/25/96 and dosed on 3/13 and 3/21/96 and wastestream received 8/29/96 and dosed the same day. Volume of "Charcoal" wastestream dosed was 25 µL at each site.

b Marked ulceration at the dosing site on animal #496 may have obscured microvesication.

c Incidence of pathology was significantly less than that for sites dosed with L based on McNemar's Test at the 0.05 significance level.

d Incidence of pathology was significantly less than that for sites dosed with HN based on McNemar's Test at the 0.05 significance level.

e Incidence of pathology was significantly less than that for sites dosed with HD based on McNemar's Test at the 0.05 significance level.

f Agent (L, HN, HL) at a concentration of 10% in chloroform.

TABLE 15. PHASE III. SUMMARY OF INTERMEDIATE TO SEVERE HISTOPATHOLOGY FOLLOWING DOSING OF "CHARCOAL" WASTESTREAM [a]

Agent/ Compound	Dose Volume (µL)	No. of Animals	No. of Sites	Number of Animals with Histopathology Rated Intermediate to Severe						
				Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
L [e]	5	12	12	10	12	12	0	7	5	0
HN [e]	5	12	12	10	12	12	0	3	0	0
HD [e]	5	12	12	8	12	12	0	8	1	0
"Charcoal" Wastestream	25	12	20	0 [b,c,d]	2 [b,c,d]	0 [b,c,d]	0	0 [b,d]	0	0

Note: All times to decontamination were 1 hr.

a Pooled data from the "Charcoal" wastestream received 1/25/96 and dosed 3/13 and 3/21/96 and wastestream received 8/29/96 and dosed the same day.

b Incidence of pathology was significantly less than that for sites dosed with L based on McNemar's Test at the 0.05 significance level.

c Incidence of pathology was significantly less than that for sites dosed with HN based on McNemar's Test at the 0.05 significance level.

d Incidence of pathology was significantly less than that for sites dosed with HD based on McNemar's Test at the 0.05 significance level.

e Agent (L. HN, HD) at a concentration of 10% in chloroform.

4. Discussion

The intent of the process chemistries was to develop neutralization reactions that achieved destruction of CAIS agents, forming wastestreams with minimal toxic hazards. Achieving the desired objectives represented a formidable challenge since chemical reactions with the agents can result in the formation of reaction products/by-products having vesicant action and/or a high degree of systemic toxicity. Destruction of agents involves complex chemical reactions. The toxicity of the degradation products resulting from the chemical neutralization of HD, HN, or L is of concern to the toxicology, health, and regulatory communities. The current studies were undertaken to assess the vesicant properties of neutralized CAIS.

Current methods for demilitarizing CAIS are still based largely on chemical neutralization via oxidizing materials. The oxidation of sulfur mustard, as pointed out by Franke (1967), represents one of the most important decontamination reactions for HD. The oxidation of sulfur mustard via various oxidizers (e.g., hydrogen peroxide, hypochloric acid and its salts, potassium permanganate, nitric acid, DCDMH, etc.) yields various compounds whose composition depends on the nature of the oxidant used and the specific reaction conditions. Most easily formed is HD sulfoxide which on oxidation yields HD sulfone - both represent major oxidation products of sulfur mustard.

The oxidation of HD not only alters the skin-damaging properties of HD but the systemic toxicity of sulfur mustard as well. The oxidation of HD is of great interest since sulfoxide formation, on chemical neutralization of HD, can be considered a "detoxification". In contrast, the formation of mustard sulfone, a product of further oxidation, can contribute to an enhanced systemic toxicity and vesicant potential of the product solution/mixture. HD sulfone, having the $S(O)_2$ functional group, is highly poisonous and comparable in toxicity to HD[4]. Research conducted since Philips' review (Philips, 1950) on sulfur mustard pharmacology/toxicology demonstrated that HD sulfone is a highly toxic vesicant.

Certainly, based on the known toxicity characteristics of mustard sulfone, mustard sulfoxide, and their vinyl derivatives; it is crucial that the process chemistries developed for the destruction of CAIS employ oxidants that minimize the formation of HD sulfone and HD analogs having comparable biological activity (systemic toxicity and vesicancy) to that of HD.

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[4] HD is easily destroyed by all chlorinating agents (aqueous or anhydrous medium). Under appropriate conditions, the chlorination of HD can proceed to form various polychlorides. In the presence of water, chlorination of HD is altered resulting in the formation of sulfoxides (Aleksandrov, 1969). Sulfoxides may undergo further oxidation to sulfones.

The vesication potential of HD degradation products/by-products is of concern - information pertaining to sulfur mustard products/by-products is summarized in Tables 16 and 17. The reader is referred to a review on the subject matter (Olajos *et al.* 1996).

Degradation product(s) of nitrogen mustards have not been implicated as having vesicant potential. The principal degradation product of lewisite, namely L oxide, is a potent vesicant.

The vesicant potential of sulfur mustard derivatives (oxidation and chlorination products) has been investigated since the 1920's. Research has indicated that the strongest vesicant action is exerted by β-halogenated sulfides. The position and degree of chlorination influences the vesicant potential of the thioether molecule. With respect to the site of chlorination, Kirner (1928) and Dawson and Wardell (1930) concluded that compounds having the chlorine atom in the beta position were considerably more vesicant that those having chlorine in the alpha or gamma position. The degree of chlorination also influences the vesicant activity of the sulfide molecule and hence the early use of chlorination to degrade HD. Monosubstitution analogs of HD, regardless of position, are less effective vesicants than HD. As previously stated, the introduction of halogen atoms results in decreased toxicity and markedly diminished vesicant action. Research in the 1920s summarized by Bouder (1940) - indicated that the higher chlorinated derivatives (e.g., tri-, tetra-, and hexachloro derivatives) of HD (saturated or unsaturated) were non-vesicant. A summary of the vesicant potential of various chlorinated analogs of sulfur mustard are given in Table 17. Fuson *et al.* (1943) on review of the vesicant activity of sulfur compounds concluded that compounds containing the S(0) group were non-vesicant. Mustard sulfone, containing the S $(0)_2$, functional group is a known vesicant (vesicancy potential 1/7 to 1/5 of HD; Bergmann *et al.*, 1945). The formation of HD sulfone can contribute to an enhanced vesicant potential of the product solution/mixture (wastestream).

The lack of vesicancy following treatment with "Red" and "Charcoal" process wastestreams is indicative of the effectiveness of the neutralization chemistries in destruction of chemical agent concomitant with the minimization of potentially vesicant-inducing products/by-products. The composite agent (HD, HN and L) levels in "archived" and "fresh" "Red" wastestreams and in "archived" and "fresh" "Charcoal" wastestreams did not elicit vesication in the volumes dosed. Treatment with "Blue" process wastestreams ("archived" and "fresh") resulted in a vesicant response. The bioassay results were unexpected since the agent (HD) residual level was below 50 ppm, a level not expected to elicit a vesicant response. The most plausible explanation is the presence of vesicating product(s)/byproduct(s).

TABLE 16.
SYNOPSIS OF DERMAL TOXICITY DATA FOR CAIS AGENTS, AGENT DEGRADATION PRODUCTS, RRS OXIDANT, AND SOLVENTS[a]

Compound	Dermal Toxicity[b] (LD_{50}/LDLo/TDLo)	References	Skin Effects (Irritation, Vesication)[b]	References
AGENTS				
HD [bis(2-chloroethyl)sulfide]	LD_{50} (40-100 mg/kg)	Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Marshall & Williams (1921); Gates & Moore (1946); Renshaw (1946)
L [dichloro(2-chlorovinyl)arsine]	LD_{50} (5-6 mg/kg)	Cameron et al. (1946); Gates *et al.* (1946)	Severe irritant/escharotic, severe vesicant	Gates *et al.* (1946)
HN-1 [bis(2-chloroethyl)ethylamine]	LD_{50} (15-20 mg/kg)	Smith (1943a); Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Cope *et al.* (1946); Renshaw (1946)
HN-3 [tris(2-chloroethyl)amine]	LD_{50} (5-20 mg/kg)	Smith (1943d); Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Cope *et al.* (1946); Renshaw (1946);
OXIDIZED DERIVATIVES				
HD sulfoxide	(-)[c]	(-)[c]	Irritant, non-vesicant	Marshall & Williams (1921); Lawson & Dawson (1927); Young *et al.* (1944)
Sulfoxide, 2-chloroethyl vinyl	(-)[d]	(-)[d]	Irritant, non-vesicant	Thomson *et al.* (1945)
Divinyl sulfoxide	(-)[e]	(-)[e]	Irritant, non-vesicant	Fuson *et al.* (1943); Young *et al.* (1944); Thomson *et al.* (1945)
HD sulfone	(-)[f]	(-)[f]	Irritant/escharotic, vesicant	Marshall & Williams 1921); Young *et al.* (1944)
Sulfone, 2-chloroethyl vinyl	(-)[g]	(-)[g]	Irritant/escharotic, vesicant	Young *et al.* (1944); Thomson *et al.* (1945)
Divinyl sulfone	LD_{50} (~ 20 mg/kg)	Smyth *et al.* (1962)	Irritant/escharotic, vesicant	Young *et al.* (1944); Thomson *et al.* (1945)
HN-1 oxide	(-)[h]	(-)[h]	(-)[n]	(-)[n]

TABLE 16. (Continued)

Compound	Dermal Toxicity[a] (LD_{50}/LDLo/TDLo)	References	Skin Effects (Irritation, Vesication)[b]	References
OXIDIZED DERIVATIVES (Cont.)				
HN-3 oxide	(-)[i]	(-)[i]	(-)[n]	(-)[n]
Lewisite oxide	(-)[j]	(-)[j]	Irritant/escharotic, vesicant	Young *et al.* (1944); Thomson *et al.* (1945)
2-chlorovinylarsonic acid	(-)[k]	(-)[k]	Irritant, non-vesicant	Young *et al.* (1944); Thomson et al (1945)
2-chlorovinylarsonous acid	(-)[l]	(-)[l]	Irritant, non-vesicant	Cameron *et al.* (1946)
OXIDIZERS				
DCDMH	LD_{50} (>20 g/kg)	EPA 8EHQ0281-0382; EPA 88-8100-228	Severe irritant	EPA 8EHQ0281-0382; EPA #88-8100-173 (cited in RTECS)
SOLVENTS				
Chloroform	LD_{50}(>20 g/kg)	NTIS AD-A062-138	Mild irritant (cited in RTECS)	Guido and Martins (1988)
t-butyl alcohol	(-)[m]	(-)[m]	Mild irritant	Oettel (1936)

[a] Table modified from that originally compiled by Olajos *et al.*, 1996.
[b] Rabbit as animal model unless otherwise indicated. Tests for irritancy based on animal and/or human studies. Test for vesicant action of agents conducted on human subjects.
[c] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow and Houck (1946)].
[d] Rat oral (100 mg/kg, mortality 1/1) [Young *et al.*, 1944]
[e] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow and Houck (1946)].
[f] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow and Houck (1946)].
[g] Acute toxicity undetermined.
[h] Mouse i.p. LD_{50} (50-100 mg/kg) [Bergmann and Fruton (1943); Stahmann and Bergmann (1946a)].
[i] Mouse i.p. LD_{50} (2-5 mg/kg) [Bergmann and Fruton (1943); Stahmann and Bergmann (1946a)].
[j] Mouse s.c. [mortalities: 2 mg/kg (0/5); 5 mg/kg (5/5); 10 mg/kg (5/5)] Young *et al.* (1944).
[k] Mouse i.p. [mortalities: (1000 mg/kg 10/10; 500 mg/kg 0/10] (Young *et al.*, 1944).
[l] Reported as highly toxic, details not given (Cameron *et al.*, 1946).
[m] Rabbit oral LDLo (4.5 g/kg) [RTECS].
[n] Young *et al.*, (1944) reported HN2 oxide as non-vesicant; no data for HN1, HN3.

TABLE 17. VESICATION POTENTIAL OF VARIOUS ANALOGS/ DERIVATIVES OF SULFUR MUSTARD[a]

Analogs/Derivatives (Saturated and Unsaturated)	Vesicant Activity	References[b]
OXIDIZED DERIVATIVES		
Mustard Sulfone (sulfone, bis(2-chloroethyl)	(POS)	Marshall & Williams (1921), Young *et al.* (1944)
Sulfone, 2-chloroethyl vinyl	(POS)	Young *et al.* (1944)
Divinyl Sulfone	(POS)	Young *et al.* (1944), Thomson *et al.* (1945)
Mustard Sulfoxide (sulfoxide, bis(2-chloroethyl)	(NEG)	Marshall & Williams (1921) Lawson & Dawson (1927) Fuson *et al.* (1943) Bergmann *et al.* (1945)
Divinyl Sulfoxide	(NEG)	Young *et al.* (1944) Thompson *et al.* (1945) Bergmann *et al.* (1945)
β-chloroethyl vinyl sulfoxide	(NEG)	Young *et al.* (1944)
α, β′ -trichlorodiethyl sulfoxide	(NEG)	Young *et al.* (1944)
CHLORINATED DERIVATIVES		
bis(α-chloroethyl) sulfide	(NEG)	Peters and Walker 1923) Baldwin *et al.* (1924) Kirner (1928) Dawson & Wardell (1930)
α, β, β′ -trichlorodiethyl sulfide	(NEG)	Mann & Pope (1922) Lawson & Dawson (1927)
α, β, β, β′ tetrachlorodiethyl sulfide	(NEG)	Mann & Pope (1922) Lawson & Dawson (1927)
α, α', β, β' tetrachlorodiethyl sulfide	(NEG)	Lawson & Dawson (1927)
α, α β, β, β, β′ hexachlorodiethyl sulfide	(NEG)	Mann & Pope (1922) Lawson & Dawson (1926) Dawson & Wardell (1930)
β-chloroethyl α, β dichlorovinyl sulfide	(NEG)	Lawson & Dawson (1926) Kirner (1928) Dawson & Wardell (1930)
β-chloroethyl α, β, β′ trichlorovinyl sulfide	(NEG)	Lawson & Dawson (1926) Kirner (1928) Dawson & Wardell (1930)
β-chloroethyl chlorovinyl sulfide (α and β isomers)	(POS)	Lawson & Dawson (1926) Dawson & Wardell (1930) Fuson *et al.* (1943)

[a] Table from Olajos et al., 1996

[b] Citations are primary and/or secondary

5. Conclusions

Based on the findings of these studies the following conclusions can be made.

- The vesicating properties of the "Blue" wastestream (product solution from neutralized neat HD) were not significantly reduced from that of the untreated CAIS (neat HD) prior to treatment with neutralization solution.

- The vesicating properties of both "Red" and "Charcoal" wastestreams (product solutions from neutralized agent/$CHCl_3$ and agent/charcoal, respectively), in the volumes dosed, were significantly lower than the untreated CAIS agent solutions.

- The microvesicancy test results on the "archived" wastestreams and "fresh" wastestreams suggest that storage had not altered the vesicancy potential of the product solutions (wastestreams).

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APPENDIX A

Gross Lesion Appearance (24-hr)

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LESION SIZE DETERMINATION SHEET

Project #: G1555-38A

Date: 2-20-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: CTO

Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
301	15/10	15/8	16/14	13/14	22/23	19/22	13/20	readings taken in mm
	E-2 R-2	R-2 E-2	E-3 R-3	R-2 E-2	R-3 E-2	R-2 E-2	R-3 E-3	
Mean Average								

CDW 2-20-96 EMM

All Measurements in Millimeters.
N/A = Not applicable
Nil = None required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10ul 10% HD in $CHCl_3$

Site B 50ul 10% HD in $CHCl_3$

Site C 10ul 10% HN in $CHCl_3$

Site D 50ul 10% HN in $CHCl_3$

Site E 10ul 10% L in $CHCl_3$

Site F 50ul 10% L in $CHCl_3$

Site G 1ul neat HD

Reviewed By: CT Olson

Date: 2/23/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 2-20-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: CO Lesions Recorded By: RMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
305	10/8	12/12	9/16	14/13	19/20	22/21	21/25	readings taken in mm
	R-2 E-2	R-3 E-3	R-2 E-2	R-2 E-2	R-2 E-2	R-3 E-3	R-2 E-2	
Mean Average								

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10ul 10% HN in $CHCl_3$

Site B 50ul 10% HN in $CHCl_3$

Site C 10ul 10% L in $CHCl_3$

Site D 50ul 10% L in $CHCl_3$

Site E 10ul 10% HD in $CHCl_3$

Site F 50ul 10% HD in $CHCl_3$

Site G 1ul neat HD

Reviewed By: CO Date: 2/23/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A

Date: 2-22-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: BK

Lesions Recorded By: RMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
306	15/8	7/8	7/8	16/17	16/13	12/14	12/10	readings taken in mm
	R-2 E-3	R-3 E-3	R-2 E-2	R-2 E-3	R-2 E-3	R-2 E-2	R-2 E-3	
Mean Average								

@IE 2-22-96 RMM

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 5% 10% L in $CHCl_3$

Site B 10% 10% L in $CHCl_3$

Site C 5% 10% HD in $CHCl_3$

Site D 10% 10% HD in $CHCl_3$

Site E 5% 10% HN in $CHCl_3$

Site F 10% 10% HN in $CHCl_3$

Site G 1% not HD

Reviewed By: C T Olson

Date: 2/23/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 2-22-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: [illegible]

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
309	16/10	12/13	15/14	14/10	7/9	9/7	7/10	readings taken in mm
	R-2 E-2	R-2 E-2	R-3 E-3	R-2 E-2	R-3 E-3	R-2 E-2	R-3 E-3	
Mean Average								

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10 ul 10% HD in $CHCl_3$

Site B 5 ul 10% HD in $CHCl_3$

Site C 10 ul 10% HN in $CHCl_3$

Site D 5 ul 10% HN in $CHCl_3$

Site E 10 ul 10% L in $CHCl_3$

Site F 5 ul 10% L in $CHCl_3$

Site G 1 ul neat HD

Reviewed By: [illegible] Date: 2/23/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A

Date: 2-28-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: CT

Lesions Recorded By: [illegible]

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
312	15/12	14/10	14/12	20/15	9/12	9/11	12/8	Readings taken in mm
	R-3 E-3	R-2 E-1	R-2 E-2	R-3 E-3	R-3 E-3	R-3 E-2	R-2 E-2	
Mean Average								

DWN 10-23-96 DMM

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10ul 10% L in CHCl3

Site B 5ul 10% L in CHCl3

Site C 10ul 10% HD in CHCl3

Site D 5ul 10% HD in CHCl3

Site E 10ul 10% HN in CHCl3

Site F 5ul 10% HN in CHCl3

Site G 1ul neat HD

Reviewed By: CT Olson

Date: 2/25/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 2-25-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: [illegible]

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
316	12/9	15/13	21/14	22/14	11/9	15/9	14/14	Readings taken in mm
	R-2 E-2	R-3 E-3	R-3 E-2	R-3 E-2	R-2 E-3	R-3 E-3	R-3 E-2	
Mean Average								

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10ul 10% Histamine CHCl3

Site B 5ul 10% Histamine CHCl3

Site C 10ul 10% Lim CHCl3

Site D 5ul 10% Lim CHCl3

Site E 10ul 10% Histamine CHCl3

Site F 5ul 10% Histamine CHCl3

Site G Lab grade HCl

Reviewed By: CT Olson Date: 2/25/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-9001 @ 33A Date: 3-6-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: CT Lesions Recorded By: JNH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #						④			
313	10/13	12/9	12/14	14/16	N/A / N/A	N/A / N/A	N/A / N/A	N/A / N/A	reading taken in mm
	R-2 E-1	R-2 E-3	R-3 E-3	R-3 E-3	~~R-0 E-0~~ E-0 E-1	R-0 E-1 ③ JNH	E-0 E-1	E-0 E-1	

@WW 3-6-96 DHW

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = Not apparent

④AC NA is equivalent to 0 on this form through out the study 10-24-96 DHW

Site A 5ul 10% HD in $CHCl_3$

Site B 20ul neutralizing solution

Site C 5ul 10% HN-1 in $CHCl_3$

Site D 20ul neutralizing solution

Site E 5ul 10% L in $CHCl_3$

Site F 20ul neutralizing solution

Site G 1ul neat HD

Site H 20ul neutralizing solution

Form No. MREF-LESION.SIZ-07

①Ery/Edema 3-6-96 JNH
③ E.E 3-6-96 JNH

Reviewed by C T Olson 3/7/96

LESION SIZE DETERMINATION SHEET

Project #: D384 G1555-~~9001~~ Date: 3-6-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: JMH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #					(2)				
315	15 / 9	12 / 13	11 / 9	18 / 12	N/A / N/A	N/A / N/A	N/A / N/A	N/A / N/A	reading taken mm
	R-1 E-1	R-3 E-3	R-2 E-3	R-2 E-3	R-0 E-1	R-0 E-1	R-0 E-1	R-0 E-1	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = Not at apparent OWN 3-6-96 DM

(2) AC NA is equivalent to C on this from through the study 10-24-96 DM

Site A 5ul 10% HD in CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% L in CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% HD in CHCl3

Site F 20ul neutralizing solution

Site G 1ul neat HD

Site H 20ul neutralizing solution

Form No. MREF-LESION.SIZ-07

Reviewed by C T Olson 3/7/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-~~9001~~ 0.33A Date: 3-6-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: JmH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
317	9/15	8/8	12/12	15/10	0/0	0/0	0/0	0/0	reading taken HM
	R-3 E-3	R-2 E-3	R-2 E-2	R-3 E-2	R-0 E-1	R-0 E-1	R=0 E-1	R-0 E-1	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = Not Apparent ①WN 3-6-96 DMA
②IE 3-6-96 DMA

Site A 5ul 10% L in CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% HD in CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% HN in CHCl3

Site F 20ul neutralizing solution

Site G 1ul neat HD

Site H 20 ul ~~neutralizing~~ ② neutralizing solution

Form No. MREF-LESION.SIZ-07

Reviewed by CT Olson 7/7/96

LESION SIZE DETERMINATION SHEET

Project #: 0354 G1555-~~9001~~ Date: 3-6-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: JMH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
324	9/12	10/11	12/14	14/9	0/0	0/0	0/0	0/0	readings taken in mm
	R-3 E-3	R-2 E-2	R-3 E-3	R-2 E-3	R-0 E-0	R-0 E-1	R-0 E-1	R-0 E-1	

DWV 3-6-96 Done

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = Not Apparent

Site A 5ul 10% HD in CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% HD in CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% L in CHCl3

Site F 20ul neutralizing solution

Site G 1ul neat HD

Site H 20ul neutralizing solution

Form No. MREF-LESION.SIZ-07

Reviewed by C T Olson 3/7/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-~~9001~~ 38.10 Date: 3-6-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: JmcH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
311	13/11	14/9	12/9	15/10	0/0	0/0	0/0	0/0	readings taken in mm
	R-2 E-2	R-3 E-3	R-3 E-2	R-2 E-2	R-0 E-1	R-0 E-1	R-0 E-1	R-0 E-1	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = Not apparent @ WN 3-6-96 DMN @ WN 3-6-96 DMN

Site A 5ul 10% HNin CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% Lin CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% HDin CHCl3

Site F 20ul neutralizing solution

Site G @ 5ul neat HD

Site H 20ul neutralizing solution

Form No. MREF-LESION.SIZ-07

Reviewed by CT Olson 3/7/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: AB Lesions Recorded By: LO mm

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #	(6)							
310	A 12/15	9/8	12/10	9/10	17/22	11/17	15/16	readings taken in mm
	R-1 E-2	R-1 E-2	R-3 E-3	R-3 E-3	R-1 E-2	R-1 E-2	R-00 (2) E-05	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0 = Not apparent
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① EE 3-14-96 Emm

② AC The lesion readings were last determined at times between ③ 0 and 1 and was therefore designated as 0.5. 3-14-96 Emm

③ EE 10-16-96 Emm

Site A 5ul 10% HD in $CHCl_3$

Site B 25ul Red Wastestream

Site C 5ul 10% HN in $CHCl_3$

Site D 25ul Blue Wastestream

Site E 5ul 10% Lin $CHCl_3$

Site F 25ul Charcoal Wastestream

Site G 1ul neat HD

Form No. MREF-LESION.SIZ-07

Reviewed by CT Olson 3/15/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CO Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
491	12/9	11/15	8/10	13/19	7/12	15/17	9/26	readings taken in mm
	R-3 E-3	R-3 E-2	R-1 E-2	R-2 E-3	R-0.5-④ E-0.5	R-1 E-2	R-2① E-2	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0 = Not apparent ① WN 3-14-96 DMM
R = Erythema ② AC 3-14-96 DMM
E = Edema ③ SE 3-14-96 DMM
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% L in CHCl3

Site B 25ul Charcoal Wastestream

Site C 5ul 10% HD in CHCl3

Site D 25ul Red Wastestream

Site E 5ul 10% HN in CHCl3

Site F 25ul Blue Wastestream

Site G 1ul neat HD

② Site A on day 2 appears to have a piece of skin pulled back across the lesion attached at one point. 3-14-96 DMM Could be due to trauma trauma induced over night by the animal Elizabethan Collars were not used on any animals on this study day 3-14-96 DMM

④ AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5. 3-14-96 DMM

Form No. MREF-LESION.SIZ-07

Reviewed by CT Olson 3/15/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: AB Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
493	9/7	11/7	10/9	14/15	15/20	19/19	15/20	Readings taken in MM
	R-3 E-1	R-1 E-2	R-3 E-3	R-3 E-3	R-1 E-1	R-1 E-2	R-0.5 ① E-0.5	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0=not apparent ① AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 3-14-96 DMM

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% HD in CHCl3

Site B 25ul Red Wastestream

Site C 5ul 10% HN in CHCl3

Site D 25ul Blue Wastestream

Site E 5ul 10% Lin CHCl3

Site F 25ul Charcoal Wastestream

Site G 1ul neat HD

Reviewed by CT Olson 3/18/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: [illegible]

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
498	7/9	12/11	9/10	17/15	17/23	11/20	17/19	Readings taken in mm
	R-3 E-2	R-3 E-3	R-3 E-3	R-2 E-3	R-3 E-1	R-0 E-0.5	R-1 E-2	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

O=not apparent ① AC
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 3 14-96 DMV

Site A 5ul 10% HN in CHCl3

Site B 25ul Blue Wastestream

Site C 5ul 10% L in CHCl3

Site D 25ul Charcoal Wastestream

Site E 5ul 10% HD in CHCl3

Site F 25ul Red Wastestream

Site G 1 ul neat HD

Form No. MREF-LESION.SIZ-07

Reviewed by CT Dix 3/15/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-22-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: AB Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
499	7/8	10/10	11/11	12/6	13/15	15/16	9/16	
	R-2 E-2	R-2 E-3	R-3 E-1	R-3 E-2	R-2 E-1	R-0.5 E-0.5①	R-1 E-2	
		U=4②	U=6②	U=4②				

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0 = not apparent
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC The lesion readings were best determinded at levels between 0.0 and 1 and was therefore designated as 0.5. 3-22-96 DMM

② AC ulceration of dose sites were not perivously record 3-28-96 DMM

U = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5 ul 10% HD in CHCl3

Site B 25 ul Blue Wastestream

Site C 5 ul 10% L in CHCl3

Site D 25 ul Charcoal Wastream

Site E 5 ul 10% HD in CHCl3

Site F 25 ul Red Wastestream

Site G 1 ul neat HD

Reviewed by C. T Ellis
3/28/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-22-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: Dmm

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
494	14/13	10/8	10/11	17/15	0/0	21/19	13/20	
	R-3 E-3	R-2 E-2	R-2 E-2	R-3 E-3	R-0 E-0	R-1 E-1	R-2 E-1	
			U=4 (1)	U=4 (1)				

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = not apparent
① AC ulceration of dose sites was not previously recorded 3-28-96 Dmm
U = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% LinCHCl3

Site B 25ul Charcoal Wastestream

Site C 5ul 10% HDinCHCl3

Site D 25ul Red Wastestream

Site E 5ul 10% HWinCHCl3

Site F 25ul Blue Wastestream

Site G 1ul neat HD

Reviewed by CT Olson
3/25/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-22-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: AG Lesions Recorded By: Anm

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
496	12/15	10/9	13/10	17/16	19/21	19/19	0/0	
	R-3 E-3	R-3 E-1	R-3 E-3	R-3 E-3	R-1 E-2	R-3 E-2	R-0 E-0	
	u=6 ①	u=6 ①	u=6 ①	u=4				

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = not apparent
① AC ulceration of dose sites not previously recorded 3-28-96 Anm

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% HD in CHCl3

Site B 25ul Red Wastestream

Site C 5ul 10% HW in CHCl3

Site D 25ul Blue Wastestream

Site E 5ul 10% L in CHCl3

Site F 25ul Charcoal Wastestream

Site G 1ul neat HD

Form No. MREF-LESION.SIZ-07

Reviewed by C. J. Olson 3/25/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-22-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
497	8/8	11/10	10/12	15/21	21/20	18/15	14/11	
	R-3 E-3	R-3 E-3	R-3 E-3	R-3 E-3	R-2 E-2	R-0.5 E-0.5	R-2 E-2	
	u=6 ③		u=6 ③	u=6 ③				

OIE 3-22-96 DMM

All measurements in millimeters
N/A = Not applicable
N/R = Not required

D=not apparent ② AC
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

The lesion readings were best determined at levels between 0.0 and 1 and were therefore designated as 0.5 3-22-96 DMM

③ AC ulceration of dose sites were not previously recorded. 3-28-96 DMM

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5 ul 10% HN in CHCl3

Site B 25 ul Blue Wastestream

Site C 5 ul 10% L in CHCl3

Site D 25 ul Charcoal Wastestream

Site E 5 ul 10% HD in CHCl3

Site F 25 ul Red Wastestream

Site G 1 ul neat HD

Form No. MREF-LESION.SIZ-07

Reviewed by C. T. Olson 3/28/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-21-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
346	28/12	12/8	10/9	11/14	0/0	16/21	0/0	19/20	
	R-3 E-3	R-2 E-2	R-2 E-2	R-2 E-2	R-0 E-0	R-1 E-1	R-0 E-0	R-1 E-1	

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5ml 10% L in $CHCl_3$

Site B 25ml red motsturam

Site C 5ml 10% HD in $CHCl_3$

Site D 25ml blue motsturam

Site E 5ml 10% HD in $CHCl_3$

Site F 25ml red motsturam

Site G 1ml neat HD

Site H 25ml blue motsturam

Reviewed by
LT QA 6/24/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-21-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BL Lesions Recorded By: Dmm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
341	11/12	11/8	14/13	15/9	24/13	0/0	11/12	0/0	
	R-2 E-2	R-2 E-2	R-3 E-3	R-3 E-3	R-2 E-2	R-0 E-0	R-3 E-2	R-0 E-0	
	u=5 ①	u=4 ①	u=6 ①				u=6 ①		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC ulceration of dose sites were not previously recorded 6-21-96 Dmm

① WD 6-21-96 Dmm ②

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5 ul 10% HD in CHCl3

Site B 25 ul blue nontoxic

Site C 5 ul 10% HD in CHCl3

Site D 25 ul red nontoxic

Site E 5 ul 10% L in CHCl3

Site F 25 ul blue nontoxic

Site G 1 ul neat HD

Site H 25 ul red nontoxic

Form No. MREF-LESION.SIZ-07

Reviewed by CT Olson 6/24/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-21-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
339	10/9	18/13	12/12	9/10	0/0	19/14	0/0	28/13	
	R=2 E=1	R=3 E=3	R=3 E=3	R=3 E=2	R=0 E=0	R=2 E=2	R=0 E=0	R=2 E=2	
		u=4 ①	u=4 ①						

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC ulceration of dose sites was not previously recorded is DMM

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% HNinCHCl3

Site B 25ul red ~~mototram~~

Site C 5ul 10% LininCHCl3

Site D 25ul blue ~~mototram~~

Site E 5ul 10% HDinCHCl3

Site F 25ul red ~~mototram~~

Site G 1ul neat HD

Site H 25ul blue ~~mototram~~

Reviewed by CT Olson 6/24/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-21-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
342	22/14	11/8	9/10	16/9	23/15	0/0	11/22	0/0	
	R-3 E-3	R-2 E-2	R-2 E-2	R-2 E-2	R-2 E-2	R-0 E-0	R-2 E-2	R-0 E-0	

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5 µl 10% Lin $CHCl_3$

Site B 25 µl blue material

Site C 5 µl 10% HD in $CHCl_3$

Site D 25 µl red material

Site E 5 µl 10% HN in $CHCl_3$

Site F 25 µl blue material

Site G 1 µl neat HD

Site H 25 µl red material

Reviewed by
C T Olson 6/24/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-27-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: AH Lesions Recorded By: Emm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
340	12/11	8/8	11/14	11/14	19/12	19/14	22/16	0/0	
	R-2 E-2	R-2 E-2	R-3 E-3	R-2 E-2	R-2 E-1	R-1 E-1	R-2 E-1	R-0 E-0	
	u=4m			u=6①					

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC ulceration of dose sites were not previously record 6-27-96 Emm

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% HD in CHCl3

Site B 25 ul Blue Wastestream

Site C 5ul 10% HD in CHCl3

Site D 25 ul red Wastestream

Site E 5 ul 10% HD in CHCl3

Site F 25 ul Blue Wastestream

Site G 1 ul neat HD

Site H 25 ul red Wastestream

Reviewed by C T Olson 6/25/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-27-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: DMH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
345	9/12	9/14	11/10	14/15	0/0	19/15	21/15	20/16	
	R-3 E-3	R-3 E-3	R-2 E-3	R-2 E-3	R-0 E-0	R-2 E-1	R-1 E-1	R-2 E-1	
	u=6 ①	u=6 ①	u=6 ①	u=6 ①				u=4	

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC ulceration of dose sites were not previously recorded. 6-27-96

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% L in CHCl3

Site B 25 ul red Wastestream

Site C 5ul 10% HD in CHCl3

Site D 25 ul blue Wastestream

Site E 5ul 10% HN in CHCl3

Site F 25 ul red Wastestream

Site G 1ul neat HD

Site H 25 ul blue Wastestream

Reviewed by CT Olson 6/25/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-27-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BK Lesions Recorded By: Spm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
351	13/15	15/9	10/15	15/14	16/19	15/12	21/12	0/0	
	R-2 E-2	R-3 E-3	R-3 E-2	R-3 E-2	R-1 E-2	R-1 E-1	R-1 E-2	R-0 E-0	
		u-4 ①	u-2 ①	u-5 ①					

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC ulceration of dose sites were not previously recorded 6-27-96 DPm

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% HN in CHCl3

Site B 25ul blue Wastestream

Site C 5ul 10% L in CHCl3

Site D 25ul red Wastestream

Site E 5ul 10% HD in CHCl3

Site F 25ul blue Wastestream

Site G 1ul neat HD

Site H 25ul red Wastestream

Reviewed by CT Olson 6/25/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-27-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BL Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
352	11/9	12/9	11/12	13/15	0/0	16/15	15/12	15/9	
	R-2 E-2	R-1 E-2	R-3 E-3	R-3 E-3	R-0 E-0	R-1 E-2	R-1 E-1	R-2 E-1	
	U-5 ①	U-4 ②		U-6 ③					

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① WN 6-27-96 DMM

② AC ulceration of dose sites were not previously recorded 6-27-96 DMM

U = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% HD in CHC13

Site B 25ul red Wastestream

Site C 5ul 10% HN in CHC13

Site D 25ul blue Wastestream

Site E 5ul 10% L in CHC13

Site F 25ul red Wastestream

Site G 1ul neat HD

Site H 25ul blue Wastestream

Reviewed by CT DMM 6/25/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: DB Lesions Recorded By: DM/m

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
383	15/20	11/18	12/18	①	6/21	10/11	16/14	①	
	R-3 E-3	R-2 E-2	R-2 E-2		R-2 E-1	R-1.5 ② E-0	R-1 E-1		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 10ul 10% L in CHCl3

Site B 10ul blue wastestream

Site C 10ul 10% HD in CHCl3

Site D 10ul Charcoal wastestream

Site E 10ul 10% H in CHCl3

Site F 10ul red wastestream

Reviewed by CT Dix 9/14/96

① ~~Site G~~

② ~~Site H~~

① AC J-free sites were not read on ~~this day~~ 8-13-96 ③ AM.

② AC The lesion readings were best determined at Times between 0.0 and 1 and was therefore designated as 0.5 8-14-96 AM

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: RDmm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
385	12/15	12/13	12/16	①	12/12	15/17	11/9	②	
	R-2 E-2	R-3 E-2	R-1 E-2		R-2 E-2	R-1 E-1	R-1.5③ E-0		

0 = ② not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 10ul 10% HA in CHCl3

Site B 10ul red Wastestream

Site C 10ul 10% L in CHCl3

Site D 10ul blue Wastestream

Site E 10ul 10% HD in CHCl3

Site F 10ul charcoal wastestream.

Reviewed by CTDS 8/14/96.

① ~~Site G~~ _____

① ~~Site H~~ _____

④ ~~1st day~~ 8-13-96 RDmm

① AC These sites were not used on

② IE 8-14-96 RDmm.

③ AC The lesion readings were best determined at levels between [illegible] 0.5 8-14-96 RDmm

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CS Lesions Recorded By: KGMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
400	17/17	15/15	15/21	①	5-9	11-14	12-14	①	
	R-3 E-3	R-3 E-3	R-3 E-3		R-1.5 ② E-0	R-1 E-0	R-1 E-1		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 10ul 10% HD in C.HC/3

Site B 10ul Charcoal Wastestream

Site C 10ul 10% HN in C.HC/3

Site D 10ul red wastestream

Site E 10ul 10% L in C.HC/3

Site F 10ul blue wastestream

~~① Site G~~

~~① Site H~~

Reviewed by C.T. Olson 5/14/12

① AC These sites were not scored on ③ ~~this day~~ 8-13-96 KMM

② AC The lesion readings were best determined at 1 and 2 between 1 and 2 and were therefore designated as 0.5 8-14-96 KMM

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CT Lesions Recorded By: Emm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
389	13/13	12/21	11/21	①	5/19	11/16	11/9	①	
	R-3 E-2	R-3 E-3	R-3 E-3		R-.5 ② E-0	R-1 E-1	R-0 E-0		

C = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 10ul 10% HD in CHCl3

Site B 10ul red wastestream

Site C 10ul 10% L in CHCl3

Site D 10ul blue wastestream

Site E 10ul 10% HD in CHCl3

Site F 10ul charcoal wastestream

Reviewed by CT Olson 8/14/96

① ~~Site G~~

① ~~Site H~~

③ ~~Study~~ 8-17-96 Emm

① AC Three sites were not scored on

② AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 8-14-96 Em

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-30-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CO Lesions Recorded By: RC

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
379	14/9	13/10	10/14	①	0/0	0/0	0/0	①	
	R-3 E-3	R-3 E-3	R-1 E-1		R-0 E-0	R-0 E-0	R-0 E-0		

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = not apparent

Site A 5ul 10% Lin CHCl3

Site B 25ul charcoal Wastestream

Site C 5ul 10% HDin CHCl3

Site D 25ul charcoal wastestream

Site E 5ul 10% H.V in CHCl3

Site F 25ul charcoal Wastestream.

Reviewed by
C T Olson
8/30/96

① ~~Site G~~

① ~~Site H~~

① AC These sites were not used on 8-29-96. RC

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-30-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CO Lesions Recorded By: RM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
380	10/10	12/10	8/8	①	0/0	0/0	0/0	①	
	R-1 E-2	R-3 E-2 ②	R-3 E-3		R-0 E-0	R-0 E-0	R-0 E-0		

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

② RM 1x2mm ulceration 8-30-96

0 = not apparent

Site A 5ul 10% HN in CHCl3

Site B 25ul charcoal wastestream

Site C 5ul 10% L in CHCl3

Site D 25ul charcoal wastestream

Site E 5ul 10% HD in CHCl3

Site F 25ul charcoal wastestream

Reviewed by C T Olson 8/30/96

① ~~Site G~~

① ~~Site H~~

① AC These sites were not used on 8-29-96. RM

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-30-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: Rec

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
387	11/12	10/9	16/13	①	0/0	0/0	0/0	①	
	R-3 E-3	R-2 E-2	R-3+ E-3+		R-0 E-0	R-0 E-0	R-0 E-0		
				Rec				Rec	

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% HD in CHCl3

Site B 25ul charcoal wastestream

Site C 5ul 10% HD in CHCl3

Site D 25ul charcoal wastestream

Site E 5ul 10% L in CHCl3

Site F 25ul charcoal wastestream

Reviewed by CT Dls. 8/30/96

① ~~Site G~~

① ~~Site H~~

CAC These sites were not used on 8-29-96. Rec

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-30-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: Pen

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
388	14/9	13/10	15/9	①	0/0	0/0	0/0	①	
	R-3 E-3	R-3 E-3	R-2 E-3		R-0 E-0	R-0 E-0	R-0 E-0		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% L in CHCl3

Site B 25 ul Charcoal wastestream

Site C 5ul 10% HD in CHCl3

Site D 25 ul Charcoal wastestream

Site E 5ul 10% H in CHCl3

Site F 25 ul Charcoal wastestream

Reviewed by CT Olson 8/30/96 CTO

① ~~Site G~~

① ~~Site H~~

① AC. These sites were not used on 8-29-96. Pen

LD CTO 8/30/96

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APPENDIX B

Dosage Site Code and Histopathology

Definitions Used in Histopathologic Evaluations
and an Explanation of the Grading of Lesion Severity

Microblister: Loss of epidermal basal cell attachment to the underlying basement membrane of at least two adjacent cells. The loss of attachment creates a space which may appear empty, full of proteinaceous fluid, or filled with neutrophils. One or a few isolated small areas of detachment is graded 1, minimal. Many such areas of detachment, or several larger (10 or more contiguous cells) areas of detachment is graded 2, mild. When half or more of the epidermis in the tissue section is detached from the dermis, it is graded 3, moderate. Such lesions typically have a much larger space between the basal cells and the dermis. When nearly all of the epidermis is separated from the dermis, it is graded 4, marked. In such situations, there are usually focal, point attachments, so the entire epidermis is not lifted along the full width of the section.

Epidermal necrosis: The epidermal cells exhibit cytoplasmic eosinophilia, nuclear loss or pyknosis, and are generally shrunken. If only individual cells are affected, it is graded 1 (these are generally isolated basal cells). If small areas are affected, with normal areas in close proximity, it is graded 2. If the epidermis exhibits cell death in a full-thickness (all layers of epidermis) pattern, and affects half or more of the skin section, it is graded 3. If the epidermis is virtually entirely necrotic, it is graded 4. Severe ulcers assume that the epidermis is necrotic.

Follicular necrosis: If isolated epithelial cells of the hair follicles exhibit eosinophilia or pyknosis, it is graded 1. If clusters of adjacent cells within follicles are dead, it is graded 2. If cells of half or more of a particular hair follicle are dead, it is graded 3. Grade 4 lesions have complete necrosis of the follicular epithelium underlying much of the epidermal lesion area. This indicates that the agent has penetrated deeply.

Dermal necrosis: Loss of collagen fiber integrity, evidenced by pale eosinophilic staining and homogeneous appearance, indicates necrosis of dermal fibers. With only isolated areas, it is graded 1. Multiple areas are graded 2. Necrosis of most of the superficial dermal collagen in the lesion area is graded 3. A grade four lesion requires deep (to the base of the associated adnexa) dermal necrosis.

Hemorrhage: Extravasated erythrocytes is hemorrhage. A few isolated foci is graded 1. Multiple, common foci is graded 2. Large pools of blood is graded 3. A grade four lesion requires a massive area of blood pooling with displacement of large areas of dermal collagen.

Vascular necrosis: Loss of integrity of a medium to large blood vessel is vascular necrosis. Grading depends upon the number of vessels affected and the severity. Partial necrosis of one vessel is graded 1 to 2. Complete necrosis of a vessel is graded 3; multiple such lesions are graded 4.

Pustular epidermitis: Collections of neutrophils in the epidermis proper is graded by extent; one or two small foci is graded 1; three or more small foci is graded 2; one or more large foci is graded 3; a grade four lesion would indicate massive infiltration of the entire epidermis by neutrophils.

Task 95-38, Phase 2a, Day 1

Key for HGPs #301 and 305 dosed 2/19/1996. Exposure duration - 2 hr.

Animal # 301

Site	Treatment
A	10 μL of 10% HD in $CHCl_3$
B	50 μL of 10% HD in $CHCl_3$
C	10 μL of 10% HN in $CHCl_3$
D	50 μL of 10% HN in $CHCl_3$
E	10 μL of 10% L in $CHCl_3$
F	50 μL of 10% L in $CHCl_3$
G	1 μL of neat HD
H	

Animal #305

Site	Treatment
A	10 μL of 10% HN in $CHCl_3$
B	50 μL of 10% HN in $CHCl_3$
C	10 μL of 10% L in $CHCl_3$
D	50 μL of 10% L in $CHCl_3$
E	10 μL of 10% HD in $CHCl_3$
F	50 μL of 10% HD in $CHCl_3$
G	1 μL of neat HD
H	

Dosing Date: 2/19/96

MREF Task 95-38
G1555-38A

Animal # 301	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2	2	2	2	3	4	2
Epidermal Necrosis		2	4	4	3	3	4	3
Follicular Necrosis		3	4	4	4	2	4	4
Dermal Necrosis		0	0	0	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	2	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: all lesions are centrally located; some normal skin present on all			mild dermal inflam	min dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	min dermal inflam

Animal # 305	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2	2	3	3	3	2	2
Epidermal Necrosis		4	4	4	4	4	4	4
Follicular Necrosis		3	4	4	4	4	4	4
Dermal Necrosis		0	0	0	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	1	1	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: all lesions are centrally located; some normal skin present on all		mild dermal inflam	mild dermal inflam	mild dermal inflam		mild dermal inflam	min dermal inflam	mild dermal inflam

Degree of Severity Grading Scale:
0 = Normal, 1 = Minimal, 2 = Intermediate, 3 = Moderate, 4 = Severe

A. W. Singer DVM DACVP

Task 95-38, Phase 2a, Day 2

Key for HGPs #306 and 309 dosed 2/21/1996. Exposure duration - 2 hr.

Animal # 306

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	10 µL of 10% L in $CHCl_3$
C	5 µL of 10% HD in $CHCl_3$
D	10 µL of 10% HD in $CHCl_3$
E	5 µL of 10% HN in $CHCl_3$
F	10 µL of 10% HN in $CHCl_3$
G	1 µL of neat HD
H	

Animal #309

Site	Treatment
A	10 µL of 10% HD in $CHCl_3$
B	5 µL of 10% HD in $CHCl_3$
C	10 µL of 10% HN in $CHCl_3$
D	5 µL of 10% HN in $CHCl_3$
E	10 µL of 10% L in $CHCl_3$
F	5 µL of 10% L in $CHCl_3$
G	1 µL of neat HD
H	

Animal # 306	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		4	3	1***	3	4	2***	1***
Epidermal Necrosis		4	4*	4***	4	4	4***	4***
Follicular Necrosis		4	4	4	4	4	4	4
Dermal Necrosis		1	1**	2	0	0	2	2
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		1	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *focal ulceration **deep dermal edema ***large ulcer precludes much blister potential		mod dermal inflam	mild dermal inflam	mild dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam

Animal # 309	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		3	0*	4	4	4	4	3
Epidermal Necrosis		3	4*	4	4	4	4	4
Follicular Necrosis		4	4	3	2	4	3	4
Dermal Necrosis		1	2	0	0	0**	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	0	0
Pustular Epidermitis		1	0	1	1	0	0	0
Notes: *large ulceration precludes blister potential **deep dermal edema		mild dermal inflam	mild dermal inflam	mod dermal inflam	mod derm infla m	mod dermal inflam	mod dermal inflam	mild derm infla m

Note: Some normal skin is present on all sections, both animals; lesions are centrally located in trimmed area.

Degree of Severity Grading Scale:
0 = Normal; 1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Severe Allen W. Singer, D.V.M.

Task 95-38, Phase 2a, Day 3

Key for HGPs #312 and 316 dosed 2/27/1996. Exposure duration - 1 hr.

Animal # 312

Site	Treatment
A	10 µL of 10% L in $CHCl_3$
B	5 µL of 10% L in $CHCl_3$
C	10 µL of 10% HD in $CHCl_3$
D	5 µL of 10% HD in $CHCl_3$
E	10 µL of 10% HN in $CHCl_3$
F	5 µL of 10% HN in $CHCl_3$
G	1 µL of neat HD
H	

Animal #316

Site:	Treatment
A	10 µL of 10% HN in $CHCl_3$
B	5 µL of 10% HN in $CHCl_3$
C	10 µL of 10% L in $CHCl_3$
D	5 µL of 10% L in $CHCl_3$
E	10 µL of 10% HD in $CHCl_3$
F	5 µL of 10% HD in $CHCl_3$
G	1 µL of neat HD
H	

Animal # 312	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		3	3	3	3	4	3	3
Epidermal Necrosis		4	4	4	4	4	4	4
Follicular Necrosis		4	4	4	4	4	3	4
Dermal Necrosis		0*	0*	0	0**	0	0	0*
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		1	2	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	1	2	0
Notes: *mod dermal edema **minimal dermal edema		mild dermal inflam	mod dermal inflam	mild dermal inflam	mild derm infla m	mild dermal inflam	mod derm inflam	mild derm infla m

Animal # 316	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		3	4	4	4	3	3	3
Epidermal Necrosis		4	4	4	4	4	4	4
Follicular Necrosis		4	3	4	4	4	4	4
Dermal Necrosis		0*	0	0**	0**	0	1	0**
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	2	2	1	0	0
Pustular Epidermitis		1	1	0	0	1	1	2
Notes: *minimal dermal edema **moderate dermal edema		mod dermal inflam	mod dermal inflam	mod dermal inflam	sever e derm infla m	mild dermal inflam	mod dermal inflam	mod derm infla m

Note: All sections (312 and 316) have normal, unaffected skin at one or both margins of the section.

Degree of Severity Grading Scale:
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

Allen W. Singer, D.V.M.

Task 95-38, Phase 2b, Day 1

Key for HGPs #311, 313, 315, 317, and 324 dosed 3/5/1996. Exposure duration - 1 hr.

Animal # 311

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	20 µL of neutralization solution
C	5 µL of 10% L in $CHCl_3$
D	20 µL of neutralization solution
E	5 µL of 10% HD in $CHCl_3$
F	20 µL of neutralization solution
G	1 µL of neat HD
H	20 µL of neutralization solution

Animal # 313

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	20 µL of neutralization solution
C	5 µL of 10% HN in $CHCl_3$
D	20 µL of neutralization solution
E	5 µL of 10% L in $CHCl_3$
F	20 µL of neutralization solution
G	1 µL of neat HD
H	20 µL of neutralization solution

Animal # 315

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	20 µL of neutralization solution
C	5 µL of 10% L in $CHCl_3$
D	20 µL of neutralization solution
E	5 µL of 10% HD in $CHCl_3$
F	20 µL of neutralization solution
G	1 µL of neat HD
H	20 µL of neutralization solution

Animal # 317

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	20 µL of neutralization solution
C	5 µL of 10% HD in $CHCl_3$
D	20 µL of neutralization solution
E	5 µL of 10% HN in $CHCl_3$
F	20 µL of neutralization solution
G	1 µL of neat HD
H	20 µL of neutralization solution

Animal # 324

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	20 µL of neutralization solution
C	5 µL of 10% HN in $CHCl_3$
D	20 µL of neutralization solution
E	5 µL of 10% L in $CHCl_3$
F	20 µL of neutralization solution
G	1 µL of neat HD
H	20 µL of neutralization solution

Animal # 311	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3	0	3	0	2	0	3	0
Epidermal Necrosis		4	0	4	0	4	0	4	0
Follicular Necrosis		2	0	4	0	4	0	4	0
Dermal Necrosis		0	0	0	0	0	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	3	0	0	0	1	0
Pustular Epidermitis		2	0	0	0	1	0	0	0
Note: *moderate deep dermal edema		mod dermal inflam		mod dermal inflam		mod dermal inflam		mod derm infla m	

Animal # 313	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3	0	4	0	4	0	2	0
Epidermal Necrosis		4	0	4	0	4	0	4	0
Follicular Necrosis		4	0	4	0	3	0	4	0
Dermal Necrosis		0	0	1	0	0	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Note: *moderate deep dermal edema		mild derm inflam		mod dermal inflam	min dermal inflam	mod dermal inflam		mild dermal inflam	

Animal # 315	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2	0	4	0	3	0	2	0
Epidermal Necrosis		3	0	4	0	4	0	4	0
Follicular Necrosis		2	0	4	0	4	0	4	0
Dermal Necrosis		0	0	1	0	1	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	2	0	0	0
Pustular Epidermitis		1	0	0	0	0	0	0	0
Note: *moderal dermal edema		mod dermal inflam		marke d dermal inflam		mod dermal inflam		mild dermal inflam	

Animal # 317	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2	0	2	0	3	0	2	0
Epidermal Necrosis		4	0	4	0	4	0	4	0
Follicular Necrosis		4	0	4	0	3	0	4	0
Dermal Necrosis		0*	0	2**	0	0	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	1	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	2	0	0	0
Notes: *mild dermal edema **focal ulceration(s)		mild dermal inflam	min dermal inflam	mod dermal inflam		mod dermal inflam		mild dermal inflam	

Animal # 324	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		4	0	4	0	4	0	3	0
Epidermal Necrosis		4	0	4	0	4	0	4	0
Follicular Necrosis		4	0	2	0	4	0	4	0
Dermal Necrosis		1	0	0	0	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	0	0	0
Pustular Epidermitis		0	0	1	0	0	0	0	0
Notes:		mod dermal inflam	min dermal inflam	mod dermal inflam		mod dermal inflam		min dermal inflam	

Note: Normal (unaffected) skin present laterally on all sections where lesions were observed.

Histopathological Markers
Degree of Severity Grading Scale

3/7/96
Allen W. Singer, DVM

0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

Task 95-38, Phase 3, Day 1

Key for HGPs #310, 491, 493, and 498 dosed 3/13/1996. Exposure duration - 1 hr.

Animal # 310

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	25 µL of Red waste stream
C	5 µL of 10% HN in $CHCl_3$
D	25 µL of Blue waste stream
E	5 µL of 10% L in $CHCl_3$
F	25 µL of Charcoal waste stream
G	1 µL of neat HD
H	

Animal # 491

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	25 µL of Charcoal waste stream
C	5 µL of 10% HD in $CHCl_3$
D	25 µL of Red waste stream
E	5 µL of 10% HN in $CHCl_3$
F	25 µL of Blue waste stream
G	1 µL of neat HD
H	

Animal # 493

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	25 µL of Red waste stream
C	5 µL of 10% HN in $CHCl_3$
D	25 µL of Blue waste stream
E	5 µL of 10% L in $CHCl_3$
F	25 µL of Charcoal waste stream
G	1 µL of neat HD
H	

Animal # 498

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	25 µL of Blue waste stream
C	5 µL of 10% L in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HD in $CHCl_3$
F	25 µL of Red waste stream
G	1 µL of neat HD
H	

Animal # 310	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2	0	4	2	1	0	1
Epidermal Necrosis		4	1	4	4	4*	2	4*
Follicular Necrosis		4	0	4	1	4	0	4
Dermal Necrosis		0	0	1	0	3	0	3**
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	1
Pustular Epidermitis		0	1	0	1	0	1	0
Notes: *marked ulceration **moderate dermal edema		mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mild dermal inflam	mod dermal inflam

Animal # 491	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		4	0	1	0	4	3	2
Epidermal Necrosis		4*	1	4**	0	4	4	4
Follicular Necrosis		4	0	4	0	3	0	4
Dermal Necrosis		3	0	3	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		2	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *mild ulceration **marked ulceration		mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam

Animal # 493	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		1*	0	4	4	2	0	2*
Epidermal Necrosis		4**	0	4	4	4**	1	4**
Follicular Necrosis		4	0	3	0	4	0	4
Dermal Necrosis		3	0	0	0	3	0	3
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	0	0
Pustular Epidermitis		0	1	0	0	0	0	0
Notes: *at edge of ulcer **marked ulceration		mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam

Animal # 498	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2*	3	3	0	3	0	1
Epidermal Necrosis		4**	4***	4***	0	4**	0	4***
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		3	1	2	0	3	0	2
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	0	0	0
Pustular Epidermitis		1	0	0	1	1	0	0
Notes: *at edge of ulcer **marked ulceration ***minimal ulceration		mod dermal inflam	mild dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mild dermal inflam	mild dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale 3/18/96
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM

Task 95-38, Phase 3, Day 2

Key for HGPs #494, 496, 497, and 499 dosed 3/21/1996. Exposure duration - 1 hr.

Animal # 494

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	25 µL of Charcoal waste stream
C	5 µL of 10% HD in $CHCl_3$
D	25 µL of Red waste stream
E	5 µL of 10% HN in $CHCl_3$
F	25 µL of Blue waste stream
G	1 µL of neat HD
H	

Animal # 496

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	25 µL of Red waste stream
C	5 µL of 10% HN in $CHCl_3$
D	25 µL of Blue waste stream
E	5 µL of 10% L in $CHCl_3$
F	25 µL of Charcoal waste stream
G	1 µL of neat HD
H	

Animal # 497

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	25 µL of Blue waste stream
C	5 µL of 10% L in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HD in $CHCl_3$
F	25 µL of Red waste stream
G	1 µL of neat HD
H	

Animal # 499

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	25 µL of Blue waste stream
C	5 µL of 10% L in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HD in $CHCl_3$
F	25 µL of Red waste stream
G	1 µL of neat HD
H	

Animal # 494	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		4	0	1	0	3	2	3
Epidermal Necrosis		4	0	4**	0	4	2	4***
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		0*	0	3	0	0	0	2
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		3	0	0	0	0	0	0
Pustular Epidermitis		0	0	1	0	1	0	0

Animal # 496	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		0	0	0	4	1	0	2
Epidermal Necrosis		4*	0	4*	3	4*	1	4*
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		3	0	3	0	4	0	3**
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *marked ulcer precludes potential blister **mild dermal edema		mod dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam

Animal # 497	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		1	2	4	0	2	0	2
Epidermal Necrosis		4*	4	4	1***	4*	0	4*
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		3	0	0**	0	2	0	2**
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *marked ulceration **moderate dermal edema ***mild epithelial cell edema		mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam	mod dermal inflam

Animal # 499	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		4	2	3	0	4	0	3
Epidermal Necrosis		4	3	4	2	4	0	4
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		0	0	2*	0	2	0	1*
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	0	0	0
Pustular Epidermitis		1	0	0	0	1	0	0
Note: *mild dermal edema		mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mild dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale 3/25/96
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM

Task 95-38, Phase 3, Day 3

"Fresh" Blue and Red waste streams received 6/19/1996

Key for HGPs #339, 341, 342, and 346 dosed 6/20/1996. Exposure duration - 1 hr.

Animal # 339

Site	Treatment
A	5 μL of 10% HN in $CHCl_3$
B	25 μL of Red waste stream
C	5 μL of 10% L in $CHCl_3$
D	25 μL of Blue waste stream
E	5 μL of 10% HD in $CHCl_3$
F	25 μL of Red waste stream
G	1 μL of neat HD
H	25 μL of Blue waste stream

Animal # 341

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	25 μL of Blue waste stream
C	5 μL of 10% HN in $CHCl_3$
D	25 μL of Red waste stream
E	5 μL of 10% L in $CHCl_3$
F	25 μL of Blue waste stream
G	1 μL of neat HD
H	25 μL of Red waste stream

Animal # 342

Site	Treatment
A	5 μL of 10% L in $CHCl_3$
B	25 μL of Blue waste stream
C	5 μL of 10% HD in $CHCl_3$
D	25 μL of Red waste stream
E	5 μL of 10% HN in $CHCl_3$
F	25 μL of Blue waste stream
G	1 μL of neat HD
H	25 μL of Red waste stream

Animal # 346

Site	Treatment
A	5 μL of 10% L in $CHCl_3$
B	25 μL of Red waste stream
C	5 μL of 10% HD in $CHCl_3$
D	25 μL of Blue waste stream
E	5 μL of 10% HN in $CHCl_3$
F	25 μL of Red waste stream
G	1 μL of neat HD
H	25 μL of Blue waste stream

Animal # 339	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3	0	4	3	3	0	2	2
Epidermal Necrosis		4	0	4	4	4**	0	4	2
Follicular Necrosis		4	0	4	0	4	0	4	0
Dermal Necrosis		0	0	2*	0	2	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0	0
Pustular Epidermitis		1	0	1	1	1	0	0	0
Notes: *moderate dermal edema **focal ulceration(s)		mod dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mild dermal inflam	min dermal inflam

Animal # 341	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2	2	2	0	3	0	2	0
Epidermal Necrosis		4*	4	4*	0	4*	4*	4*	0
Follicular Necrosis		4	1	4	0	4	2	2	0
Dermal Necrosis		3	1	2	0	3**	3	3**	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		1	0	0	0	1	0	1	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Notes: *focal ulceration(s); **moderate dermal edema		mild dermal inflam	mild dermal inflam	mild dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam	min dermal inflam

Animal # 342	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		3	1	3	0	4	3	3
Epidermal Necrosis		4	4	4	0	4	4	4
Follicular Necrosis		4	0	4	0	4	1	4
Dermal Necrosis		0*	0	0*	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0
Pustular Epidermitis		0	0	1	0	0	0	0
Notes: *mild to moderate dermal edema		mild dermal inflam	min dermal inflam	mild dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mild dermal inflam

Animal # 346	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2	0	2	1	4	0	2
Epidermal Necrosis		4	0	4	4	4	0	4
Follicular Necrosis		4	0	4	1	4	0	4
Dermal Necrosis		0*	0	0	0	2	0	0*
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *moderate dermal edema; **most of surface epithelium artifactually stripped away		mild dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam	mild dermal inflam		mild dermal inflam

Note: Normal (unaffected) skin presented laterally on all skin sections with lesions.
Histopathological Markers 6/25/96
Degree of Severity Grading Scale Allen W. Singer, DVM
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

Task 95-38, Phase 3, Day 4

"Fresh" Blue and Red waste streams received 6/19/1996

Key for HGPs #340, 345, 351, and 352 dosed 6/26/1996. Exposure duration - 1 hr.

Animal # 340

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	25 μL of Blue waste stream
C	5 μL of 10% HN in $CHCl_3$
D	25 μL of Red waste stream
E	5 μL of 10% L in $CHCl_3$
F	25 μL of Blue waste stream
G	1 μL of neat HD
H	25 μL of Red waste stream

Animal # 345

Site	Treatement
A	5 μL of 10% L in $CHCl_3$
B	25 μL of Red waste stream
C	5 μL of 10% HD in $CHCl_3$
D	25 μL of Blue waste stream
E	5 μL of 10% HN in $CHCl_3$
F	25 μL of Red waste stream
G	1 μL of neat HD
H	25 μL of Blue waste stream

Animal # 351

Site	Treatment
A	5 μL of 10% HN in $CHCl_3$
B	25 μL of Blue waste stream
C	5 μL of 10% L in $CHCl_3$
D	25 μL of Red waste stream
E	5 μL of 10% HD in $CHCl_3$
F	25 μL of Blue waste stream
G	1 μL of neat HD
H	25 μL of Red waste stream

Animal # 352

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	25 μL of Red waste stream
C	5 μL of 10% HN in $CHCl_3$
D	25 μL of Blue waste stream
E	5 μL of 10% L in $CHCl_3$
F	25 μL of Red waste stream
G	1 μL of neat HD
H	25 μL of Blue waste stream

Animal # 340	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2*	3	3	0	3	3	0	0
Epidermalal Necrosis		4**	4	4	0	4	4	4	1
Follicular Necrosis		4	2	4	0	4	0	4	0
Dermal Necrosis		2	0	1	0	0***	0	3***	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	0	0	2	0	2	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Notes: *at edge of ulcer **mild ulceration ***mild dermal edema		mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	min dermal inflam	min dermal inflam

Animal # 345	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3*	0	2	1	1	0	1	2
Epidermal Necrosis		4**	0	4	4	4**	0	4	4
Follicular Necrosis		3	0	4	1	4	0	4	1
Dermal Necrosis		3	0	0	0	3	0	2***	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	1	0	2	0	1	0
Pustular Epidermitis		0	1	0	0	0	0	0	0
Notes: *at one edge of ulcer **marked ulceration present ***mild dermal edema		mod dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	mod dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam

Animal # 351	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		1	2	4	0	1*	2	1	0
Epidermal Necrosis		4	4	4	0	4**	4	4	0
Follicular Necrosis		4	1	3	0	4	1	4	0
Dermal Necrosis		0	0	0	0	3	0	3	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	1	0	1	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Notes: *at one edge of ulcer **marked ulceration present		mild dermal inflam	min dermal inflam	mod dermal inflam		mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam

Animal # 352	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		1*	0	2	1	3	0	2	2
Epidermal Necrosis		4**	0	4**	4	4	0	4**	4
Follicular Necrosis		4	0	3	0	4	0	4	1
Dermal Necrosis		2	0	1	0	0***	0	3	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	1	0	1	0	1	0
Pustular Epidermitis		0	0	1	1	0	0	0	0
Notes: *at edge of ulcer **moderate ulceration ***mild dermal edema		mod dermal inflam	min dermal inflam	mod dermal inflam	mod dermal inflam	mild dermal inflam		mod dermal inflam	mild dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale 7/1/96
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM

Blue and Red waste streams received 11/28/1995; Charcoal waste stream received 1/25/96.

Equal volumes of waste streams and 10% HD, HN and L solutions - 10 μL

Key for HGPs #383, 385, 389, and 400 dosed 8/13/1996. Exposure duration - 1 hr.

Animal # 383

Site	Treatment
A	10 μL of 10% L in $CHCl_3$
B	10 μL of Blue waste stream
C	10 μL of 10% HD in $CHCl_3$
D	10 μL of Charcoal waste stream
E	10 μL of 10% HN in $CHCl_3$
F	10 μL of Red waste stream

Animal # 385

Site	Treatment
A	10 μL of 10% HN in $CHCl_3$
B	10 μL of Red waste stream
C	10 μL of 10% L in $CHCl_3$
D	10 μL of Blue waste stream
E	10 μL of 10% HD in $CHCl_3$
F	10 μL of Charcoal waste stream

Animal # 389

Site	Treatment
A	10 μL of 10% HN in $CHCl_3$
B	10 μL of Red waste stream
C	10 μL of 10% L in $CHCl_3$
D	10 μL of Blue waste stream
E	10 μL of 10% HD in $CHCl_3$
F	10 μL of Charcoal waste stream

Animal # 400

Site	Treatment
A	10 μL of 10% HD in $CHCl_3$
B	10 μL of Charcoal waste stream
C	10 μL of 10% HN in $CHCl_3$
D	10 μL of Red waste stream
E	10 μL of 10% L in $CHCl_3$
F	10 μL of Blue waste stream

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Animal # 383	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		3	0	2	0	3	0
Epidermal Necrosis		4	0	4	0	4	0
Follicular Necrosis		4	0	4	0	3	0
Dermal Necrosis		0*	0	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		2	0	1	0	0	0
Pustular Epidermitis		0	1	0	0	1	0
Notes: *moderate dermal edema		mod dermal inflam		mild dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam

Animal # 385	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		4	0	4	1	3	0
Epidermal . Necrosis		4	0	4	1**	4	0
Follicular Necrosis		4	0	4	0	4	0
Dermal Necrosis		1	0	0*	0	0*	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		0	0	2	0	0	0
Pustular Epidermitis		1	0	0	0	0	0
Notes: *mod dermal edema **vacuolar degeneration of epith cells leading to intra- and subepithelial microblister		marked dermal inflam	min dermal inflam	mod dermal inflam		mild dermal inflam	min dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

8/19/96
Allen W. Singer, DVM

Animal # 389	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		3	0	2	2	2	0
Epidermal Necrosis		4	0	4	2	4	1
Follicular Necrosis		2	0	4	1	4	0
Dermalal Necrosis		0	0	0*	0	0*	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		1	0	3	0	2	0
Pustular Epidermitis		1	0	0	0	0	1
Notes: *severe dermalal edema		mod dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam

Animal # 400	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		3	0	4	0	3	3
Epidermal Necrosis		4	0	4	0	4	2
Follicular Necrosis		4	0	2	0	4	1
Dermal Necrosis		0*	0	0*	0	0**	0
Vascular Necrosis		0	0	0	0	1	0
Hemorrhage		0	0	1	0	3	1
Pustular Epidermitis		1	0	1	0	0	0
Notes: *mild dermal edema **severe dermal edema		mod dermal inflam		mod dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

8/19/96
Allen W. Singer, DVM

Task 95-38, Phase 3, Day 6

"Fresh" Charcoal waste stream received 8/29/96.

25 µL of freshly prepared Charcoal waste stream and 5 µL of 10% HD, HN and L solutions

Key for HGPs #379, 380, 387, and 388 dosed 8/29/1996. Exposure duration - 1 hr.

Animal # 379

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	25 µL of Charcoal waste stream
C	5 µL of 10% HD in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HN in $CHCl_3$
F	25 µL of Charcoal waste stream

Animal # 380

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	25 µL of Charcoal waste stream
C	5 µL of 10% L in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HD in $CHCl_3$
F	25 µL of Charcoal waste stream

Animal # 387

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	25 µL of Charcoal waste stream
C	5 µL of 10% HN in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% L in $CHCl_3$
F	25 µL of Charcoal waste stream

Animal # 388

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	25 µL of Charcoal waste stream
C	5 µL of 10% HD in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HN in $CHCl_3$
F	25 µL of Charcoal waste stream

MREF Task 95-38
G1555-38A

Animal # 379	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		4	0	2	0	3	0
Epidermal Necrosis		4	1**	4	1**	4	1**
Follicular Necrosis		4	1**	4	1**	4	1**
Dermalal Necrosis		0	0	0*	0	0	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		2	0	2	0	1	0
Pustular Epidermitis		0	0	0	0	0	0
Notes: *moderate dermal edema; **random single-cell necrosis noted		mod dermal inflam	min dermal inflam	mild dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam

Animal # 380	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		4	0	4	0	3	0
Epidermal Necrosis		4	1*	4	1*	4	0
Follicular Necrosis		4	1*	4	1*	4	1*
Dermal Necrosis		1	0	2**	0	3**	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		0	0	1	0	0	0
Pustular Epidermitis		0	0	0	0	0	0
Notes: *random single cell necrosis **mod dermal edema; focal ulcer in area of necrosis		mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.
Histopathological Markers: Degree of Severity Grading Scale 9/9/96
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM

Animal # 387	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		3	0	2	0	4	0
Epidermal Necrosis		4	1**	4	1**	4	1**
Follicular Necrosis		4	1**	3	1**	4	1**
Dermal Necrosis		0	0	0	0	0*	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		0	0	0	0	2	0
Pustular Epidermitis		0	0	0	0	0	1
Notes: *moderate dermal edema; **random single-cell necrosis noted		mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam

Animal # 388	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		4	0	4	0	3	0
Epidermal Necrosis		4	1*	4	1*	4	1*
Follicular Necrosis		4	1*	4	1*	2	1*
Dermal Necrosis		0**	0	0**	0	0	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		3	0	1	0	0	0
Pustular Epidermitis		0	0	0	0	1	0
Notes: *random single cell necrosis **mod dermal edema		mild dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale 9/9/96

0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM