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### Development of Two HD Vapor Exposure Techniques in a Rabbit Ocular Model: A Pilot Study

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## ABSTRACT

Sulfur mustard (HD)-induced ocular injury continues to remain a threat to Soldiers on the battlefield. Exposure to HD can be in the form of a liquid or vapor. The rabbit model continues to serve as an animal model to investigate HD-induced ocular injury, and until recently only a droplet method of HD exposure was used within our laboratory. This pilot study investigated the feasibility of two different vapor models to yield additional insight into HD-induced ocular injury. Eight female New Zealand white rabbits (2.0-2.4 kg) were divided into 2 groups. Rabbits were exposed to HD vapor (estimated vapor density, 1.4 gm/m<sup>3</sup>) ranging from 30 seconds to 4 minutes. Four rabbits were exposed to 10 µl neat HD instilled into a vapor cap (VC) for a corneal only exposure, while the second group received 30 µl neat HD instilled into a lavage cap (LC) for a whole eye exposure. HD-exposed eyes of all animals received Artificial Tears® three times daily for 4 weeks following exposure. Rabbit eyes were evaluated and scored weekly for 4 weeks, then at 12 and 16 weeks using Pachymetry (corneal thickness) and modified ocular severity scoring (MOSS). In both models, damage was acutely and chronically debilitating and was dependent on duration of exposure (longer duration demonstrated greater damage) and method of exposure (VC demonstrated greater damage). This pilot study demonstrated a new model for HD-induced injury that is easy, realistic, reproducible and measurable.

Artificial Tears® (polyvinyl alcohol 1.4%) Phoenix Pharmaceutical (St Joseph, MO), Inc.



## INTRODUCTION

Sulfur mustard (HD, bis-(2-chloroethyl) sulfide) continues to remain a threat for use in military conflicts such as in the Middle East or as a terrorist weapon (1, 2). Research efforts to develop effective therapies for preventing and treating HD-induced ocular injuries have not resulted in an effective postexposure treatment. Mustard has a low volatility and exists as an oily liquid under temperate conditions; however, at approximately 21°C mustard becomes a vapor hazard. Mustard has the potential of causing severe damage, and rapid penetration in either a liquid or vapor state is enhanced by moist conditions (humidity, sweat, etc.). Under field conditions (e.g., temperate climates) it would be expected that HD vapor would be the most common means of exposure, and historically the majority of mustard casualties were caused by vapor exposure (2).

In the skin, liquid HD causes a much deeper injury when compared to vapor (3, 4). Damage by vapor or aerosol is described in terms of the product of concentration and the time of exposure ( $Ct$ ). For skin, the threshold damage for erythema is about 200 mg • min/m<sup>3</sup>, while for eye damage this value is about 10-70 mg • min/m<sup>3</sup> (5-7). The eye is particularly sensitive to the damaging effects of HD, and without effective treatment(s) exposure can result in serious chronic and debilitating disorders with possible loss of vision (2). HD liquid or vapor exposures to the ocular structures for as short as 5 minutes produce severe biochemical changes that are manifested after an initial latent period (2). In addition, cutaneous HD exposure around the eye can complicate the damage even further when secondary bacterial infections invade ocular tissue (8, 9).

The rabbit eye model developed in previous investigations in this laboratory addressed neat (undiluted) HD-induced ocular injury delivered as a liquid droplet (10, 11). The droplet application of HD resulted in moderate to severe ocular damage and a surrounding skin reaction that often complicated treatment regimens. Although the droplet method is the quickest and simplest method, it may not be the most ideal. In the most recent studies conducted, a high degree of variability in injury was observed, and it was speculated to be caused partly by the concentrated direct nature of the droplet to a small surface area of the cornea (11). In addition, since HD exposure under expected battlefield conditions may also occur as vapor, an additional model that mimics this scenario would allow for a more realistic evaluation. A vapor model in addition, or as an alternative, to the current droplet application would expand investigations on candidate countermeasures, mechanisms of action, or reparative processes as related to HD exposure.

Currently there are a few investigators using a goggle HD vapor exposure to the eyes and surrounding adnexa for identification of efficacious candidate treatments for HD-induced ocular injury (12-14). In these studies this whole eye vapor model in untreated control animals demonstrated a significant degree of ocular damage that was easily scored and measured clinically. Healing was initiated 48-72 hrs following exposure with almost complete recovery by 1 week; however, secondary damage then developed that was more severe than the initial phase (13). Although this model does mimic a real life scenario of HD exposure (13) it could be considered a very profound example because the exposure to both eyes often causes near blindness in these

animals. In addition, the surrounding skin exposure, although realistic, can complicate interpretation of treatment efficacy results.

A second potential vapor model option developed by Schultz et al. (15) utilized a vacuum trephine apparatus attached to the center of the cornea, through which a toxic agent could be introduced. This model has been used successfully as a rabbit eye model to investigate candidate metalloproteinases as treatments for alkali injuries (15). A corneal only exposure would allow for an evaluation of a candidate countermeasure focusing on attenuating or healing corneal injury uncomplicated by surrounding inflammation and inherent infection of the dermal tissue superimposed on corneal tissue.

Conceivably either the whole eye or corneal only HD vapor exposure models could be easily adapted and/or modified to address HD-induced injuries and potential treatments used in-house to address the efficacy of candidate ocular treatments, mechanisms of action of HD or inflammatory processes. The purpose of this pilot study was to investigate two new HD exposure models that could be adapted to evaluate candidate compounds.

## **MATERIAL AND METHODS**

### **I. Vapor Models and HD Exposure**

Two models of HD vapor ocular exposure were modified from techniques used in cutaneous experiments in guinea pigs (16-18) and pigs (19, 20). The first vapor exposure, designated as a whole eye exposure, used a plastic disposable eye lavage cup (volume of 20 mls, Apothecary Products, Inc., Minneapolis, MN) that when placed over the rabbit eye covered the whole eye and an approximate 2 cm area around the eye. The lavage cup (LC) was fitted with a filter paper disk (Whatman #2) lodged into the inside top surface. The maximum amount of liquid HD that would uniformly saturate the filter paper but not run off when inverted (USAMRICD lot, 97.3% pure, estimated saturated vapor density, 1.4 g/m<sup>3</sup> at 37° C) was then determined. This volume was determined to be 30 µl. In the second model, designated as a corneal only exposure, a plastic disposable vapor cap (14 mm diameter x 9 mm deep, volume of 800 µl, Evergreen Scientific, Inc., Los Angeles, CA) was fitted with a 14-mm diameter O ring (using a methylmethacrylate adhesive). The vapor cap (VC) was fitted with a filter paper disk (Whatman #2) lodged into the inside top surface, and the maximum amount of liquid HD was determined that would uniformly saturate the filter paper but not run off when inverted. This volume was determined to be 10 µl. For both of these models a vapor concentration was generated in the cups by pipetting the neat HD onto the filter paper disks and placing them over the eye. The vapor density was estimated to be 1.4 gm/m<sup>3</sup> based on the equilibrium vapor pressure (21). The LC or VC was gently held on the eyes by hand for the required time period (see Table 1). All 8 rabbits were exposed on the same day. Laboratory temperature at the time of the study was 23.3°C with relative humidity at 23.5%. Although equilibration of vapor pressure was not confirmed, the conditions of the cap application were kept constant. As part of the study the

duration of exposure was varied as outlined in the experimental design below after this initial equilibration.

**TABLE 1: Experimental design for rabbit ocular pilot study**

ID	Treatment Group	HD Exposure Time
17	VC	30 seconds
23	VC	1 minute
19	VC	2 minutes
21	VC	4 minutes

ID	Treatment Group	HD Exposure Time
20	LC	30 seconds
22	LC	1 minute
24	LC	2 minutes
18	LC	3 minutes

VC, vapor cap filled with 10 µl HD; LC, lavage cup filled with 30 µl HD

## II. Rabbit Model

Eight female New Zealand white rabbits weighing 2.5-3.0 Kg were used in this experiment. Animals were maintained under an AAALAC accredited animal care and use program. They were quarantined and observed for evidence of disease for 7 days prior to issue and were housed singly in a stainless steel cage (52.8 cm L x 52.8 cm W x 37.4 cm H). Animals received a complete cage change weekly or were changed as needed to maintain a clean environment. During quarantine and continuing throughout the study rabbits were handled daily and gently groomed with a soft brush, particularly around the eyes, to acclimate them to the procedures. They were also acclimated to environmental enrichment that included individual daily runs in a small 4' x 4' pen, with a variety of toys, such as PVC tubing, dumbbell toys, and shaker toys with bells.

Rabbits were provided a certified commercial rabbit ration (PMI, St. Louis, MO; 125 g/day), tap water ad libitum and regular fruit and vegetable treats. Animal holding rooms were maintained at 20°C ± 2° with 45.0% ± 10% relative humidity with at least 10 complete air changes per hour of 100% conditioned fresh air. A 12-hour light/dark, full-spectrum lighting cycle with no twilight was maintained in all animal holding areas. While in the fume hood after exposure (24-hour duration) rabbits were housed in a Kennel Cab II® (A.J. Buck and Sons, Owings Mills, MD; 12" H x 22" L x 14" W). This carrier was chosen to fit within the confines of the hood and had a raised floor to keep the animal clean.

One week prior to study rabbits were lightly sedated with an i.m. injection of 7 mg/kg Ketamine HCl in combination with 3.5 mg/kg Xylazine HCl for the minor procedures of tattooing, nail clipping, and a screening ophthalmologic exam for any preexisting lesions and/or other abnormalities. Hair was clipped around the ears and back of the animal in

preparation for HD exposure and osmotic pump (buprenorphine HCl) implantation. Rabbits were tattooed using an AIMS machine (Animal Identification and Marking Systems; AIMS, Budd Lake, NJ) for placing permanent identification numbers inside the right ear.

On the day of the study rabbits were sedated with an i.m. injection of 15 mg/kg Ketamine HCl in combination with 7 mg/kg Xylazine HCl and transported to the agent dosing area for HD exposure and surgical placement of osmotic pumps (buprenorphine HCl). Pachymetry measurements for corneal thickness were taken on both eyes in triplicate followed by slit lamp (Haag-Streit Services, Mason, OH) and whole eye examinations by a board-certified ophthalmologist. A modified ocular severity score (MOSS) was recorded (10). This scoring system was developed by Babin et al. (10) and is a Draize score that was modified for the subjective assessment of ocular injury. The animal's left eye served as the control eye for comparison. Digital pictures were obtained and recorded of the eyes using an Image-Pro plus program software package (Media Cybernetics®, Silver Spring, MD).

Following the ophthalmologic examination, drug delivery pumps (Alzet osmotic pump, Palo Alto, CA, model 2ML1-10, 10  $\mu$ l/hr, 7 days) containing buprenorphine HCl (0.3 mg/ml) were aseptically implanted in the experimental animals for pain alleviation. Pumps were implanted mid scapula and the incision was closed with surgical staples. Prior to implantation, pumps were weighed before and after filling to give the net weight of the solution loaded. Once implanted, the pumps delivered continuous and constant infusion of pain control medication for up to a week, after which the pumps were removed aseptically.

### III. Experimental Design

The eight rabbits were randomly assigned to one of the two vapor exposure models (4 rabbits/LC, 4 rabbits/VC). Within the exposure model, the right eye of each rabbit (n = 1) was exposed to one of 4 HD exposure conditions (Table 1). The left eye served as the HD unexposed and untreated control. No vapor cap or lavage cup was applied to the left eye. Vapor cap exposure times were 30 seconds, 1, 2 or 4 minutes. For the 4 rabbits in the lavage cup group exposure times were 30 seconds, 1, 2 or 3 minutes (technical error resulted in a 3-minute exposure instead of the intended 4-minute exposure). All animals were treated in the right eye with 3 drops of Artificial Tears®, starting every 10 minutes for the first 30 minutes, beginning at 10 minutes postexposure. Since the purpose of the study was to address the two vapor model methods, treatments were designed as symptomatic only (no antibiotics) to allow the natural course of the HD-induced ocular injury to occur. Control (left) eyes were not required for comparing treatment effectiveness, and therefore did not receive Artificial Tears®. Treatment for exposed eyes continued every 30 minutes for 2 hours postexposure, and then started on a t.i.d. (3x/day) schedule (0800, 1200, 1700) for 4 weeks. There were no special decontamination procedures done on the eye other than the treatments and light wiping off of excess tears using gauze or surgical wicks as needed. During the study, if required, rabbits were supportively treated around the eyes

while conscious with warm water gauze and blotted dry to break up adhesions or discharges that commonly sealed the eyes closed.

Animals remained in the fume hood for 24 hours after exposure. In these carriers the animals were fed, watered, and treated by the project technicians. After 24 hours, rabbits were returned to the colony room, and t.i.d. treatments and evaluations as outlined below were initiated. Off gassing was not verified; however, an accepted standard time period (24 hours) for off gassing was conservatively used based on studies performed in rabbits by Babin et al. (10) and in pigs by Logan et al. (21). Additionally in a recent study performed by Bossone et al. (unpublished) 24 hours was determined to be a sufficient and safe duration for off gassing to have occurred (22).

#### IV. Evaluations

Eyes of rabbits were evaluated and scored weekly for 4 weeks, then at 12 and 16 weeks. During evaluations the rabbits were placed in Lomir "Bunny Snuggle" restrainers (Lomir Biomedical, Inc., Malone, NY) for approximately 10-15 minutes. One to 2 drops of tetracaine ophthalmic solution were placed on the corneas of non-anesthetized rabbits in order to perform ophthalmic examinations. The degree of injury and rate of healing were evaluated using several instrumental techniques. Measurements included corneal thickness by means of an ultrasonic pachymeter (PR, DGH200, DGH Technology, Inc., Easton, PA) and a modified ocular severity score (MOSS) as measured via slit lamp. An ultrasonic pachymeter was used to measure corneal thickness. Measurements were made in a standardized manner at the area of injury at a central, lateral, and medial location on the cornea. Using a subjective MOSS grading scheme (Table 2) via slit lamp examination, documented with photography, corneal stromal injury and scarring, neovascularization, chemosis and eyelid damage (notching) were evaluated and quantified. Tonometry was used as needed to measure intraocular pressure (Tonometer pen, Schiottz, PA).

At the conclusion of the study (16 weeks) and after a final ophthalmology exam, rabbits were humanely euthanized. Euthanasia was accomplished by first administering an i.m. injection of Ketamine:Xylazine (15.0 mg/kg:7.0 mg/kg); then while the animal was in a surgical plane of anesthesia, an intracardiac injection of Pentobarbital (65 mg/kg) was administered. Gross examinations of abnormalities of the eyes and their adnexa as well as microscopic (H & E) examination of all ocular structures were conducted. Sections were examined through the bulbar conjunctiva, the lids and adnexa, and a horizontal, vertical, or oblique axis P-O (pupil-optic nerve) section through the entire globe specifically including the cornea. Lesions in adnexal structures were rated for severity (0 = no lesion, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe), considering mucosal damage, mucosal inflammation, cleft or pustule formation, submucosal damage and submucosal inflammation. Adnexa was defined as structures accessory to the eye and included conjunctiva and eyelids. Corneal lesions were similarly rated for severity (scores from 1 to 4), considering epithelial damage, stromal damage, stromal edema, stromal vascularization and inflammation.

**TABLE 2. MOSS (Modified Ocular Severity Score) definitions**

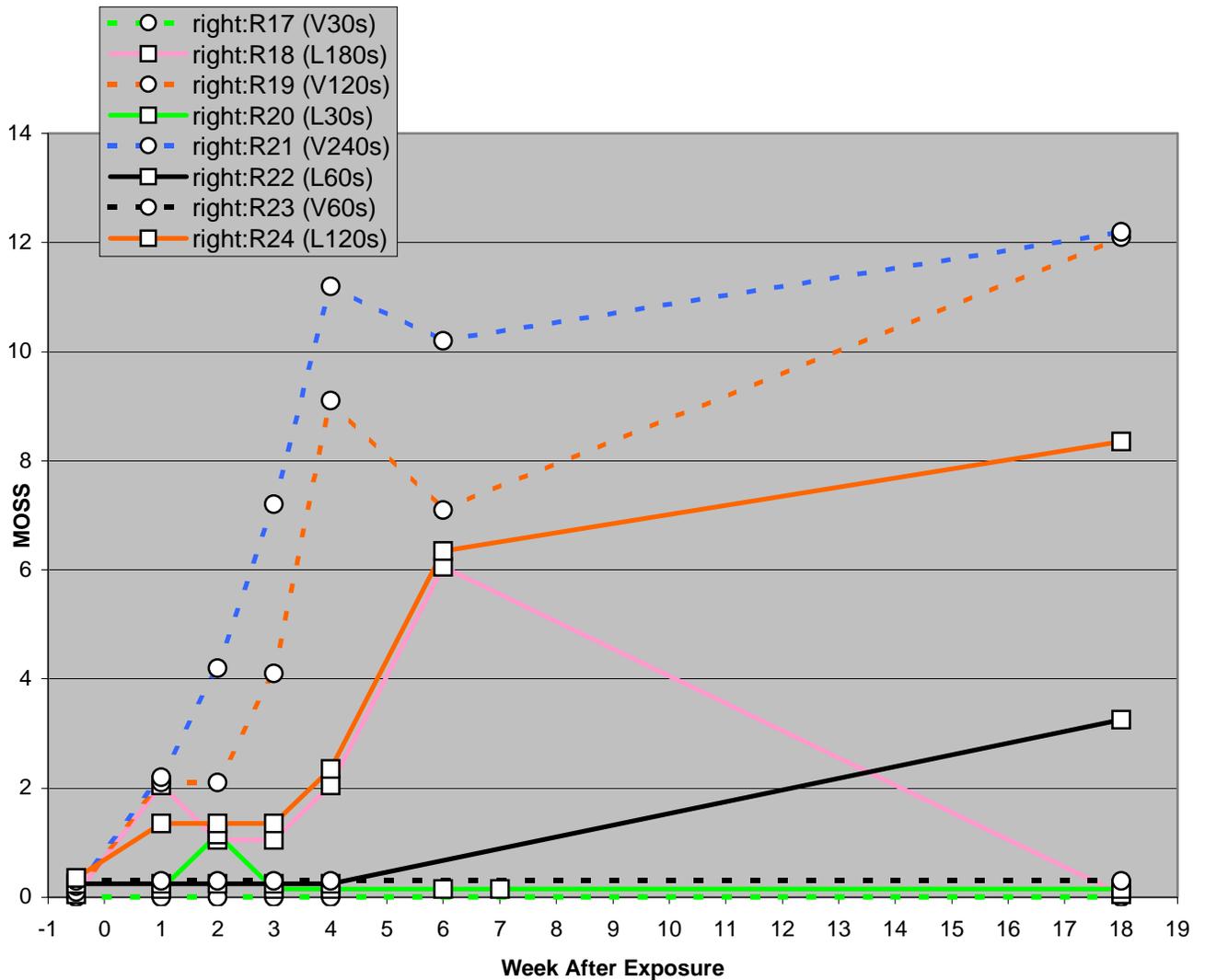
Parameter Evaluated	Parameter Score	Evaluation
Corneal Stromal Injury	0	No haze to cornea
	1	Minimal haze to cornea
	3	Moderate haze to cornea
	4	Extensive haze to cornea
NV Classification	0	No NV present
	2	One individual (twig) NV site present
	4	Two or more individual (twigs) NV sites present
	6	Diffuse NV (C or fan-shaped)
Eyelid Notching Present	1	Yes
	0	No
Chemosis Present	1	Yes
	0	No
Total MOSS Score	0 to 12	

NV = neovascularization

## RESULTS

All rabbits developed blepharospasm, photophobia, conjunctivitis and corneal edema in the right eye within 48 hours of exposure. MOSS scores on right eyes of all rabbits are illustrated in Figure 1. Rabbits exposed to HD for 30 seconds to 1 minute showed only minimal clinical symptoms of conjunctivitis, mild ulcer formation and blepharospasm at one week postexposure, and by 3 weeks the two rabbits exposed to HD for 30 seconds (rabbits #17, #20) appeared clinically normal for the remainder of the study. The 6 rabbits exposed to HD for longer than 1 minute developed mild to severe symptoms of corneal stromal injury, conjunctivitis and blepharospasm by the second week that continued to the end of the study. Over the course of the evaluations clinically both models demonstrated that injury was acutely and then chronically (except for 30-second HD-exposed rabbits) debilitating with damage appearing to be dependent on the length of HD exposure (i.e., longer time of exposure demonstrated greater damage). Additionally it appeared that the VC exposure showed higher MOSS scores than did the LC exposure. Except where noted below, left eyes remained clinically normal.

**FIGURE 1. MOSS evaluations from 8 rabbits exposed to vapor cap (VC) or lavage cup (LC) over 18 weeks.**



At approximately 2-3 weeks after HD exposure the rabbit exposed to HD for 4 minutes via VC (#21) developed glaucoma in both eyes, as assessed clinically. The glaucoma became more severe and then gradually started to resolve, but at 16 weeks both eyes were still glaucomic. Similarly, a second rabbit that was exposed to HD for 2 minutes via LC (#24) developed moderate glaucoma in the left (unexposed) eye at 2-3 weeks after HD exposure, which progressed and then became less severe towards the later 4 weeks of the study as evidenced by MOSS scoring and pachymetry. The clinical scores (MOSS) for the right eye in this rabbit became progressively worse throughout the 16 weeks. It is unclear why the unexposed eye developed a glaucoma. Tonometry readings were taken only on these rabbits when first detected; however, measurements were not reliable due to increased variability between readings and differences between readers.

Corneal stromal injury and ulcer formation were noted as early as 1 week after HD exposure in all rabbits. This appeared to heal in rabbits #17 and #20 (30-second HD exposure) and in rabbit #22 (1-minute LC); however, the remainder of the rabbits continued to show varying degrees of corneal injury. Rabbits #19 and #21 (VC; 2 and 4 minutes respectively) showed the most severe corneal injury, as noted by increasing MOSS scores. Neovascularization first appeared at 3-4 weeks and progressed throughout the full length of the study (16 weeks) in the 6 animals exposed to HD longer than 30 seconds regardless of vapor model applied.

All rabbits regardless of vapor exposure demonstrated a bacterial infection and an associated inflammation early (within 1-7 days) after HD exposure. Rabbits that were exposed to the LC also showed adnexa dermatitis (rabbits #18, #24). Symptomatic therapy often included the need to apply a warm water compress followed by dry blotting to break up adhesions that resulted in eyelids sticking. This was necessary to open the eyes so that direct eye treatments of the Artificial Tears® could be applied. In most rabbits, particularly those exposed to HD longer than 2 minutes this continued to approximately 4-6 weeks, after which these infections eventually cleared clinically.

Corneal thickness measurements over the 16 weeks of evaluations are shown in Figure 2a (lavage cup HD exposure rabbits) and Figure 2b (vapor cap HD exposure rabbits). The mean spatial average (from 3 sites taken for each eye) corneal thickness of the right eyes (n = 8) prior to HD was recorded as  $355.61 \pm 22.24 \mu\text{m}$ . Left eyes prior HD were recorded as  $353.42 \pm 20.55 \mu\text{m}$ . Left eyes of all rabbits appeared to remain consistently normal to the completion of the study except for those of rabbit #21, which demonstrated glaucoma.

Pathology scores are shown in the Appendix and are reported as outlined in the Material and Methods section. Percent lesion indicates how much of the tissue area was affected. Table 3 summarizes the total score for each rabbit. At the time of euthanasia all rabbits demonstrated no lesions or pathological abnormalities in the left eyes. Although no distinct conclusions could be made from this pilot study there was a trend towards slightly greater damage in the longer HD-exposed animals regardless of exposure condition; however, more animals would be required for confirmation.

Pathology reports on individual rabbits confirmed that the left eyes of all rabbits demonstrated no pathological lesions. Since this was a pilot study no descriptive pathology report was performed; however, scores were given as outlined in the Methods section for corneal ulceration, necrosis, neovascularization and edema. Corneal ulceration and neovascularization were more commonly found in the longer HD duration exposures (2 minutes or greater) regardless of vapor application method. Corneal epithelial necrosis was reported in only one rabbit (#21), the 4-minute VC.

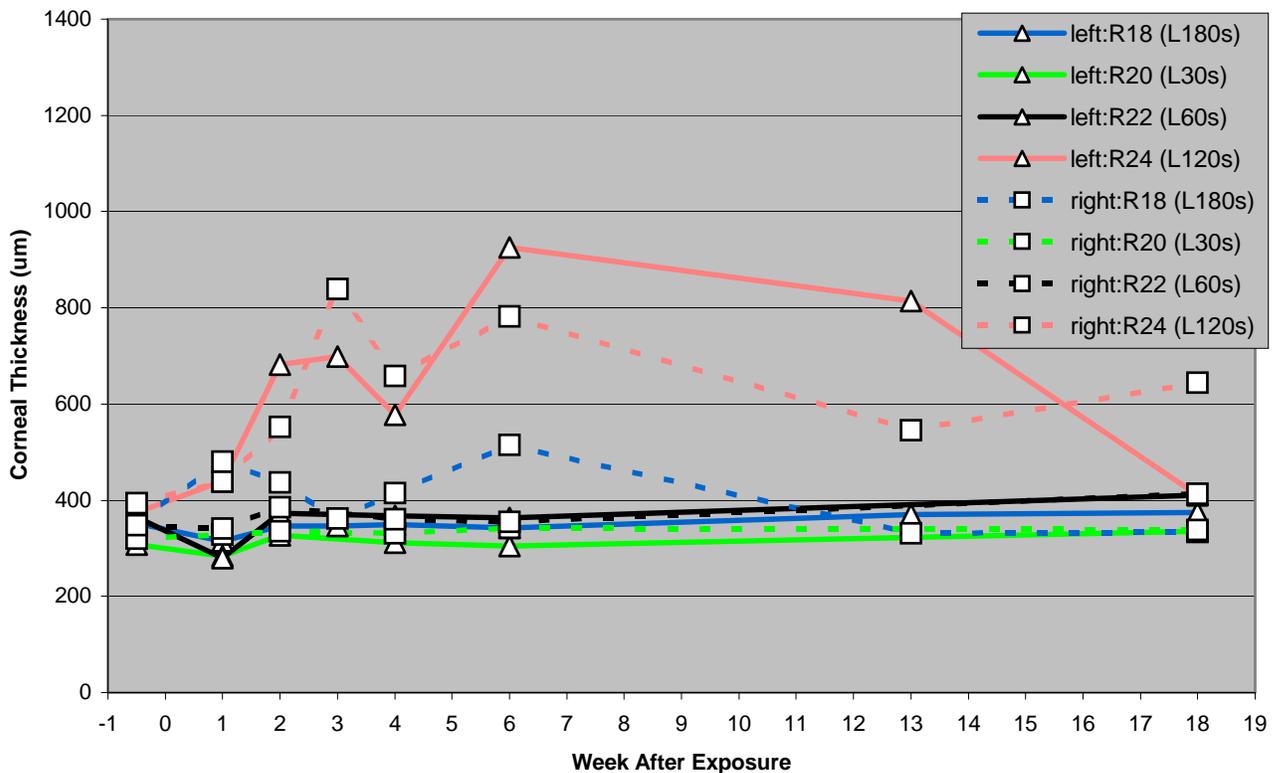
There appeared to be no corneal endothelium damage or stromal necrosis associated with either duration of HD exposure or vapor application, indicating that although damages to the eye may have been pronounced clinically the damage was not severe enough to rupture the cornea or cause debilitating irreversible damage during the 16-week time period studied in this pilot study.

**TABLE 3: Overall pathology scores for vapor cap (VC) HD-exposed and lavage cup (LC) HD-exposed eyes (n=1 for each exposure method and exposure duration). Score represents the total score for the rabbit's right eye for all structures/lesions scored in the pathology report (see Appendix).**

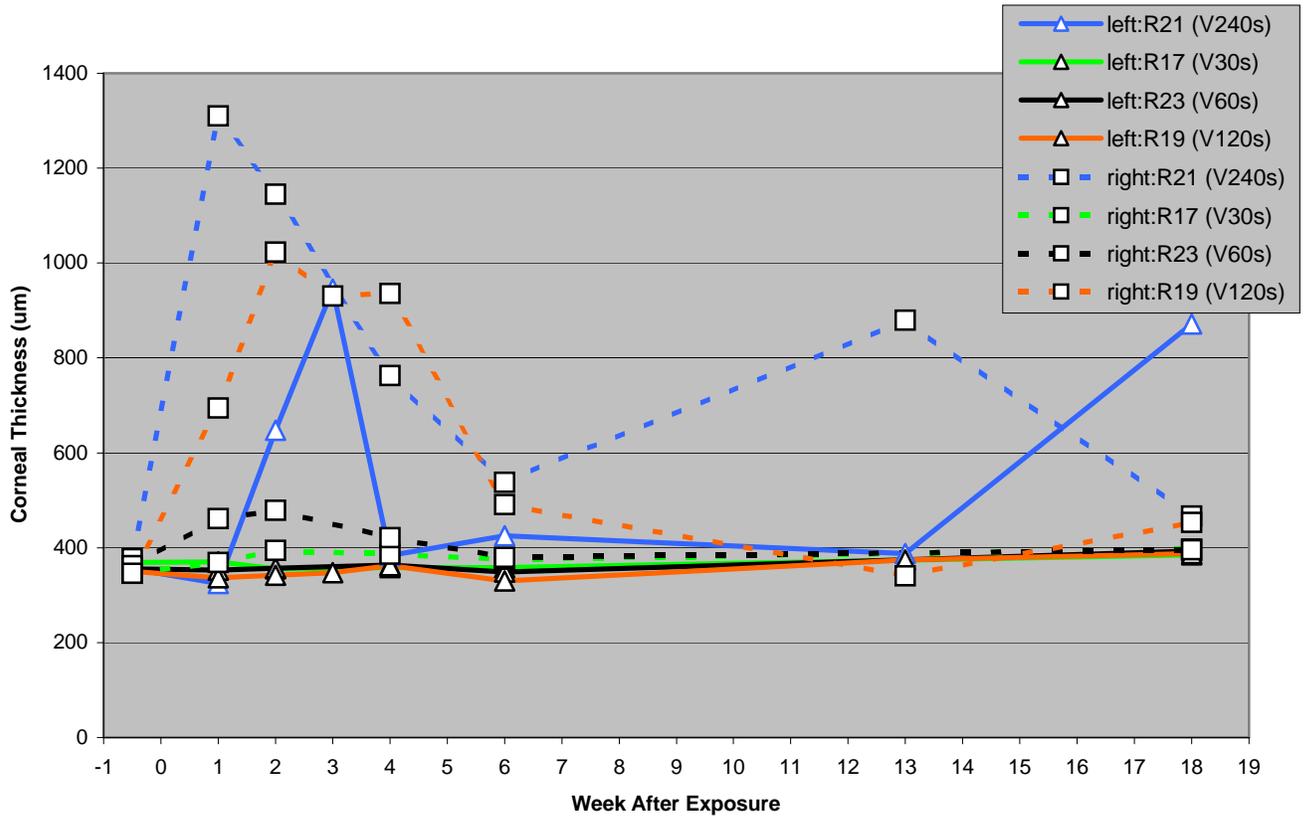
ID	Treatment Group	HD Exposure Time	Overall Score
17	VC	30 seconds	2
23	VC	1 minute	1
19	VC	2 minutes	5
21	VC	4 minutes	7

ID	Treatment Group	HD Exposure Time	Overall Score
20	LC	30 seconds	6
22	LC	1 minute	5
24	LC	2 minutes	11
18	LC	3 minutes	4

**FIGURE 2a: Pachymetry readings for lavage cup HD exposure rabbits for the left (Δ) and right eyes (□).**



**FIGURE 2b: Pachymetry readings for vapor cap HD exposure rabbits for the left (□) and right eyes (Δ).**



## DISCUSSION

The purpose of this study was to investigate the potential of two different vapor exposure procedures as options to be used in future studies for an in-house model for developing countermeasures to HD-induced ocular injury. Previous studies in this laboratory used a droplet of neat HD (0.4  $\mu$ l, 0.51 mg) that was placed on the right eye and that remained on the eye for 5 minutes (10, 11). This liquid application did not uniformly spread over the whole cornea but rather stayed concentrated in one area, which often resulted in perforations at the site of HD application (11). In addition, it is often difficult for the operator to visualize the small amount of agent as it is applied to an area of the cornea with the pipette. Pipette techniques can also result in inadvertent trauma to the cornea, further complicating measurements and analysis.

Sulfur mustard vapor exposure has been successfully used by a number of other investigators. In the studies conducted by Kadar et al. (12, 13) and Amir et al. (14) mustard was applied via goggles as a vapor to the entire eye and adnexa. These investigators exposed the eyes to HD vapor (390-420  $\mu$ g/l) for 2 minutes in a conscious animal. Their model allows for a uniform exposure of mustard to an entire surface of the eye and a portion of the skin surrounding the eye. The control-treated, HD-exposed animals in the study by Amir et al. (14) demonstrated significant corneal edema and an increase in corneal epithelial erosions early in the pathogenesis of HD injury. Although their model mimics reported HD exposure in humans and the most likely scenario, experimental treatments can be confounded by the surrounding skin responses to HD and the potential for secondary bacterial infections originating in surrounding dermal tissue (5). In the study reported here rabbits were exposed to an estimated vapor concentration of 1.4 gm/m<sup>3</sup> (30  $\mu$ l of neat liquid) HD contained in a lavage cup for 30 seconds to 3 minutes. Depending on the length of exposure to HD, a graded response (i.e., longer duration of exposure demonstrated greater ocular damage) was observed. Data from the 2- and 3-minute exposed rabbits appeared to agree with reported results for 2-minute exposure times from the studies of Kadar et al. (12, 13) and Amir et al. (14) using goggles. In our study all rabbits (except for the 30-second exposure animals) demonstrated some degree of suppurative infection around the eye, indicating bacterial involvement. A follow-on study, currently ongoing, demonstrated that ocular cultures from skin scrapings obtained from two vapor-exposed (4-minute exposure) rabbits at 6-10 weeks after HD exposure demonstrated Staphylococcal species, a common cutaneous bacteria.

In the pilot study reported here, the second model (corneal only vapor cap) was investigated as a feasible alternative to limit the confounding variable of opportunistic bacterial cutaneous infections. The healthy cornea is avascular with the Descemet's membrane normally acting as a barrier to antigens or insult. Both HD-induced injury and subsequent opportunistic bacterial infections from the surrounding ocular adnexa (e.g., conjunctiva, dermal tissue of eyelid) can potentially destroy this barrier and contribute to the delayed healing, inflammation and clinical symptoms seen in HD-exposed individuals. A corneal restricted exposure would allow for an evaluation of a candidate countermeasure, thus specifically addressing corneal healing uncomplicated by surrounding inflammation and inherent infection of the dermal tissue.

The vapor cap developed in this pilot study was modified from the vapor cap used extensively at MRICD for dermal exposures in several animal models (3, 16-20), but until reported here was never utilized in our rabbit model as a potential cornea only exposure. Schultz et al. (15) developed a corneal only exposure model in the rabbit to address treatments of alkali-injured corneas. In their studies they used a corneal block and a vacuum trephine apparatus to apply a sodium hydroxide alkali burn to the center of the cornea only. Their results allowed for a corneal injury in which they described a sharply defined circular stromal opacity at the site of exposure with no conjunctival injury. This model was used successfully to investigate candidate metalloproteinases as treatments for alkali injuries (15). We expect that a similar response could occur with the use of our vapor cap model applied to the eye, resulting in little to no adnexa involvement.

In the current study the rabbits exposed to the vapor cap, much like the lavage cup animals, demonstrated a graded response to injury depending on the duration of HD exposure. These rabbits also demonstrated a bacterial infection early in the course of healing, but clinically they did not show the more pronounced dermatitis that was seen in the LC model animals. The VC animals did result in a higher MOSS with a greater tendency to corneal ulceration compared to the LC, and the VC animals also tended to develop thicker corneas early after HD (suggesting greater corneal edema). This may potentially be attributed to the smaller volume (800  $\mu$ l) of the vapor cap when compared to the lavage cup (20 mls). Consequently because of these differences an equilibrium vapor concentration may not have been reached in the lavage cup for the duration of exposures studied (30 seconds to 3 minutes). In contrast the vapor cap with its smaller volume may have reached equilibrium sooner and therefore resulted in a more concentrated dose to the eye.

In conclusion, both models appear to be feasible alternatives or additional models to the current droplet method of applying HD to the eye. However, since this was a pilot study more animals need to be used to confirm these results. It seems reasonable to assume that an HD exposure duration of 2-4 minutes would be the ideal exposure time. This is based on the observations that none of the animals exposed less than 2 minutes showed measureable lesions either clinically or pathologically. Depending on the overall objective of the study an investigator could decide on the VC or the LC. For example, an investigator addressing the efficacy of a compound on corneal healing might consider a corneal only exposure and use the VC, whereas an investigator considering the efficacy of a combination treatment might wish to see the response of the entire eye and adnexa and therefore use the LC. These models could also be utilized in numerous other studies addressing immunological status of the eye, new diagnostic or reparative tools, or efficacy of different compounds or treatment regimens.

## **SUMMARY**

The two vapor models investigated in this pilot study were easily applied by the chemical agent operators. All animals in the study that developed lesions manifested injuries that were easily scored yet were not severe enough to cause permanent significant clinical damage. This less severe presentation of HD to the eye also resulted in less damaged eye tissue structures and only minimal adnexa involvement. These two models should be investigated further and can potentially be valuable HD exposure methods used for investigating candidate treatments for HD-induced ocular injury.



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## APPENDIX

### Pathology Results Rabbits 17-24

0 = No lesion; 1 = Minimal (1-10%); 2 = Mild (11-25%); 3 = Moderate (26-45%); 4 = Severe (>45%) NE = Not Examined NP = Not Present C = Chronic A = Acute															
Protocol	Animal #	Accession #	Eye R(right) or L(left)	Cornea Epithelium Ulceration	Cornea Epithelium Necrosis	Cornea Epithelium Attenuation	Cornea Stromal Necrosis & Loss	Cornea Stromal Edema	Cornea Stromal Neovasc	Cornea Stromal Inflamm	Cornea Stromal Deformity	Cornea Endothelium	Conjunctiva Necrosis/ Ulcer	Conjunctiva Inflamm	
A719	17	05-0611	L	0	0	0	0	0	0	0	0	0	0	0	
A719	17	05-0611	R	0	0	2	0	0	0	0	0	0	0	0	
A719	18	05-0612	L	0	0	0	0	0	0	0	0	0	0	1C	
A719	18	05-0612	R	0	0	2	0	0	1	1	0	0	2	3C	
A719	19	05-0613	L	0	0	0	0	0	0	0	0	0	0	1CA	
A719	19	05-0613	R	0	0	0	0	2	2	1	0	0	0	1A	
A719	20	05-0614	L	0	0	0	0	0	0	0	0	0	0	3CA	
A719	20	05-0614	R	1	0	1	0	1	2	1	0	0	0	2A	
A719	21	05-0615	L	0	0	0	0	0	0	0	0	0	0	2C	
A719	21	05-0615	R	1	1	1	0	1	2	1	0	0	0	3C	
A719	22	05-0616	L	0	0	0	0	0	0	0	0	0	0	1C	
A719	22	05-0616	R	0	0	2	0	0	1	0	0	0	2	2C	
A719	23	05-0617	L	0	0	0	0	0	0	0	0	0	0	0	
A719	23	05-0617	R	0	0	1	0	0	0	0	0	0	0	2C	
A719	24	05-0618	L	0	0	0	0	0	0	0	0	0	0	0	
A719	24	05-0618	R	2	0	2	0	3	2	1	0	0	1	1C	

1-5 percent indicates approximate area affected