CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-084

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Key Words: Topical Skin Protectant

JAN 28 2000

Reviewer: Lynnda Reid, Ph.D.
Division: Dermatologic and Dental Drug Products, HFD-540
Date: January 28, 2000

NDA No: 21-084
Dates: August 19, 1999 (N000) and October 18, 1999 (BZ)

Information to Sponsor: Yes ( ) No (x)

Sponsor: Office of the Surgeon General
Department of the Army
Commander, U.S. Army Medical Research and Material Command
Attn: MCMR-RCQ
Fort Detrick, Frederick, MD 21702-5012
(301) 619-2165

Drug: Topical Skin Protectant (ICD 2289)

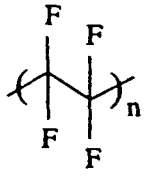
1) PTFE (50%): Polymist® F5A

Chemical Name(s): Polytetrafluoroethylene; Polytef; Ethene, tetrafluoro, homopolymer
CAS Number: CAS-9002-84-0
Molecular Formula: $(CF_2CF_2)_n$ - Polymer of recurring tetrafluoroethylene units
Description: Fine White Particulate

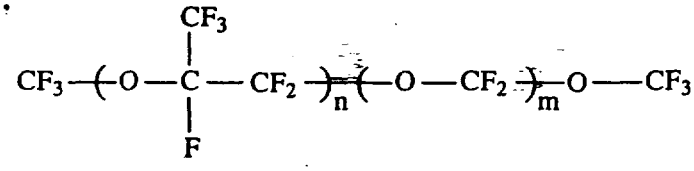
2) PFPE (50%): Fomblin Y 25® or Fomblin HC/25

Chemical Name(s): Perfluoroalkylpolyether; Trifluoromethyl-poly [oxy-2-(3-trifluoromethyl)-trifluoro-ethylene]-poly(oxy-difluoromethylene)-trifluoromethoxy; propene 1,1,2,3,3,3-hexafluoro, oxidized, polymerized; perfluorinated polyether; perfluoropolyether; polyoxyperfluoroalkane; perfluoropolymethylisopropyl ether.
CAS Number: CAS 69991-67-9
Molecular Formula: C_xF_{2x+1}-(O-CF(CF_3)-CF_2)$_n$-(O-CF_2)$_m$-O-C_yF_{2y+1} where x, y = 1, 2 or 3 and n/m>40.
Average Molecular Weight: 3200;
Average Viscosity: 250 cSt
Description: Colorless, Odorless Oil

Chemical Structure(s):



PTFE



PFPE

Clinical Formulation and Route of Administration: ICD-2289 is composed of polytetrafluoroethylene and perfluoroalkylpolyether and (PTEE/ PFPE 1:1). Physically, the substance is a heavy white cream, greasy to the touch. It is intended for topical administration and will be supplied in a single use 84 g/unit pouch.

Drug Class: Topical Barrier Cream

Indication: For the prevention of percutaneous penetration and subsequent toxicity of chemical warfare agents.

Related Documents: IND ——

Directions for Use: Topical Skin Protectant (TSP) is indicated for protection of the skin from contact with chemical warfare agents (CWA). It is to be used in conjunction with appropriate chemical protective clothing and applied prior to exposure to CWA. Military personnel will be instructed in the use of TSP during training. Before applying the chemical protective overgarment, sweat, insect repellent, sand or dirt should be wiped from the skin with a dry towel. Approximately 1/3 of the packet should be rubbed evenly around the wrists, neck and boot tops of lower legs forming a barely noticeable white film. The remaining 2/3 should be rubbed evenly onto armpits, groin area and waistline. To remove TSP, scrub sites with a dry towel or cloth using soap and water.

Previous Clinical Experience: The combination of PTEE/ PFPE has not previously been marketed for human use. However, the individual components of TSP have been marketed widely. Polymist® F5A has been used previously in surgical devices and implants, and is biologically inert. Fomblin® Y 25 has been used in cosmetics and is extremely stable

Disclaimer: Note some material may be taken directly from Sponsor's submission.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase	LAR	Lesion Area Ratio (%)
CR/CS	Riot Control Agents	PEG	Polyethylene Glycol 540
CWA	Chemical Warfare Agents	sem	Standard Error of the Mean
GD	Soman	TGD	Thickened Soman
HD	Sulfur Mustard	TSP	Topical Skin Protectant *

INTRODUCTION

Topical Skin Protectant, ICD 2289, is being investigated for use as a protective barrier against chemical warfare agents, to reduce the numbers of casualties and the seriousness of injuries from these agents. This material would be applied by the soldier in the field to act as a physical barrier to protect the skin and prevent penetration of toxic agents. Sites targeted for application would include around the closure sites of protective overgarments (e.g. neck, wrist and boot tops), as well as in areas such as the arm pits, waistline and groin area known to be particularly sensitive to blister agents (e.g. mustard gas). It is intended to be used as an adjuvant for single application use on an as-needed basis and is being developed to insure effectivity for at least 5 hours after application.

Nonclinical efficacy tests include the following:

- *in vitro* paper penetration test,
- *in vivo* measurements of lesion area following applications of liquid sulfur mustard in rabbits,
- *in vivo* measurements of lethality and erythrocyte cholinesterase activity following applications of soman, thickened soman and VX nerve agents in rabbits,
- *in vivo* measurement of erythema and edema following applications of T-2 mycotoxin in rabbits.

Background Material

TSP ICD 2289 was selected for further evaluation after having been identified as relatively efficacious against all challenges with various chemical warfare agents (CWA). Only pivotal study results pertaining to TSP ICD 2289 plus any reference preparations used as controls will be presented in this review.

Physical Properties of ICD 2289 Components:

1) Polymist® F5A (PTFE): Polymist F5A, also known as Teflon, is a free-flowing white powder ~~consisting of insoluble particles averaging in size. It is not~~ absorbed by intact skin and does not appear to have any local or systemic toxicity. However, when burned, it releases a toxic vapor resulting in a syndrome in humans known as ~~Polymer~~ Fume Fever.

Polymer Fume Fever: Polymer fume fever is a debilitating, delayed syndrome caused by the inhalation of the pyrolysis products ~~of PTFE. This syndrome is known to occur~~ in individuals who smoke tobacco products contaminated with PTFE. The amount of PTFE contamination on cigarettes that appears to be sufficient to cause illness is approximately 400 μg, either as a single dose or divided among as many as 10 cigarettes. Symptoms generally consist of tightness of chest, shortness of breath, cough, malaise, headache, chills, leukocytosis, and fever. ~~Acute~~ syndrome typically clears within 24 to 48 hours and most persons report no long-term debilitating effects.

Fluoropolymer inhalation toxicology has been studied in a variety of experimental animals including rats, rabbits, guinea pigs and mice. The toxicity of PTFE pyrolysis products varies with the exact conditions of exposure, i.e., pyrolitic temperature, species, and duration of exposure. Higher pyrolysis temperatures were associated with greater lethality per unit weight of starting material. Toxicity was found to be greatest with the submicron (0.03-0.15 μm) fraction of particles and was more pronounced with exposure to fresh (as opposed to aged) fumes. (Shusterman DJ, 1993, Occ Med: State of the Art Reviews 8(3):519-531)

Pathologic lesions in exposed animals included focal pulmonary hemorrhage and edema, with focal emphysema and interstitial fibrosis among survivors; most fatalities occur within the first 24 hours. Ultrastructural studies demonstrated necrosis and sloughing of the bronchial epithelium, as well as damage to type I pneumocytes and endothelial cells. In additions to the pathological structural changes observed with polymer fume inhalation, an acute pulmonary inflammatory response was observed resulting in neutrophil infiltration and the release of specific pro-inflammatory cytokines. In rats exposed to polymer fumes there were abundant dose-related increases in the antioxidants manganese super oxide dismutase (MnSOD) and metallothionein (MT), the interleukins (IL-1α, IL-1ß, IL-6) and cytokines inducible nitric oxide synthetase (iNOS), macrophage inflammatory protein-2 (MIP-2), and tumor necrosis factor alpha (TNFα).

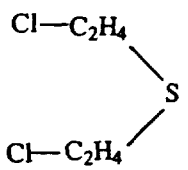
2) Fomblin Y 25® or Fomblin HC/25 (PFPE): Characteristics of perfluoropolyethers which make them good candidates to function as topical skin protective agents include the following:

- a high film forming power, so that it is possible to obtain thin and non-occlusive film on the skin
- insoluble either in polar solvents (alcohols and hydrosolutions) and in apolar solvents
- both hydrophobic and lipophobic
- free of free fluorine and inorganic fluorides
- nongreasy liquids
- nonpenetrating
- do not absorb in the UVA or UVB range

Fomblin HC has previously been used at concentrations up to 1% in skin and hair care products including moisturizing creams, barrier creams, sunscreen products, make-up, shampoos, anti-perspirants, body powders, soaps and baby care preparations. [Ref. 5.4.9, vol. 2.15, page 347]

Chemical Warfare Agents (CWA): The following chemical warfare agents were selected to test the efficacy of TSP: sulfur mustard, T-2 mycotoxin, soman and VX.

2,2'-dichloroethyl sulfide (HD): Sulfur Mustard is commonly referred to as 'mustard gas' and classed as a vesicant gas. However, at ambient temperatures, HD can also exist as a heavy oily liquid (equilibrium = 1.6 mm Hg or 1.4 mg/L @ 30°C).



In army reported field experience, only about 1% of all HD skin related casualties resulted from direct liquid contact from a burst (splash), and no more than 22% of the casualties were attributed to indirect liquid pickup. The remaining HD induced skin injuries are ascribed to mustard vapor or a combination of aerosolized liquid and vapor.

HD is lethal in concentrations varying from 0.006 to 0.2 mg/L of air, according to the length of exposure (0.07 mg/L is lethal in 30 minutes). On a hot day, it is theoretically possible to be exposed to a concentration as high as 3.66 mg/L. When HD is dispersed in the form of a fine spray or mist, even higher concentrations are theoretically possible. HD is capable of penetrating ordinary clothing and affects all parts of the body with which it comes into contact. It is persistent and its action is delayed, acting first as a cell irritant and finally as a cell poison. Direct contact may result in symptoms within 1 hour of exposure, however, in most cases the first symptoms of HD poisoning appear between 4 to 6 hours and may not appear for up to 24 hours. Symptoms include the following: conjunctivitis; erythema, blistering and excoriation or ulceration of exposed skin; inflammation of nose, throat, trachea, and bronchi and possibly necrosis and sloughing of the mucous membranes following inhalation; and inflammation of the mucous lining of the GI tract resulting in nausea if swallowed. Other symptoms include nervous system effects, i.e., profound apathy and depression, effects on circulation, and disordered metabolism associated with anorexia.

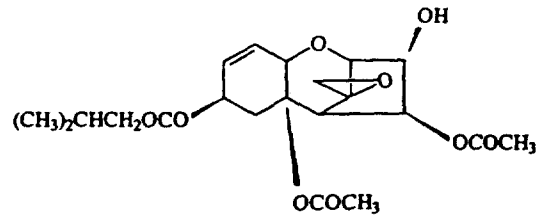
HD reacts irreversibly with DNA, causing a cascade of cellular and extracellular events that lead to cell death within 18-24 hours and may eventually lead to malignancies [Ref. 5.4.4, vol. 2.13, page 232]. The cutaneous syndrome that follows the latency period (4-8 hours) begins with erythema. This is followed by the formation of vesicles and blisters usually occurring between 12-48 hours and continuing for several days before reaching a maximum. In severe cases necrotic lesions may form, penetrating into the epidermis and dermis. These lesions generally form after blister disruption, but also may occur without previous blister development. The most sensitive areas are the face, armpits, genitals, neck, skin between fingers, and nailbeds. [Ref. 5.4.1, Vol. 2.13, page 118]

Estimated deposition area coverage following field attacks with HD [Ref. 5.4.12, Vol. 2.15, p 463] are as follows.

Distance from Target (m)	Deposition from Shells[1] (mg/m^2)	Deposition from Bombs[2] (mg/m^2)	Deposition from Bombs[3] (mg/m^2)
0 - 100	1000 - 5000	3000 - 10,000	200 - 4000
100 - 200	400 - 1000	2000 - 3000	1 - 200
200 - 300	20 - 400	1000 - 3000	<1
300 - 400	1 - 200	600 - 1000	<1

1) 152 mm Shell, 256 rounds, 1200 x 400 m target area
2) 250 kg explosive bomb, 72 bombs (4 aircraft), 1200 x 400 m target area
3) 250 kg release bomb, 72 bombs (4 aircraft), 1200 x 400 m target area

T-2 mycotoxin (T-2): Mycotoxins are by-products of fungal metabolism. The trichothecenes are a large family of chemically related mycotoxins which contain a trichothecene ring and an epoxide group.



The trichothecene mycotoxins are cytotoxic to most eukaryotic cells by inhibiting protein synthesis. They are considered to be primarily blister agents that, at lower exposure concentrations, can cause severe skin and eye irritation, and at larger doses can produce considerable incapacitation and death within minutes to hours. Multiorgan effects including emesis and diarrhea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostatic derangements, skin toxicity, decreased reproductive capacity, and bone marrow damage.

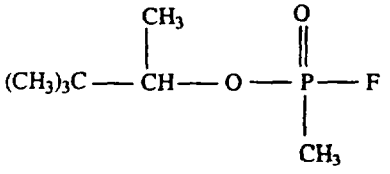
T-2 has been reported to be approximately 400 fold more potent (50 ng vs 20 μg) than sulfur mustard in producing skin injury. Following dermal exposures, the LD_{50} for T-2 ranges between 2-12 mg/kg depending on species (Table 1).

Table 1: Comparative LD_{50} values following Dermal exposure to T-2 (mg/kg in DMSO).

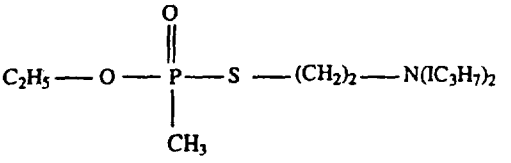
Mouse	Rat	Guinea Pig	Rabbit	Monkey
6.6	4.3	2.2	10	>8

T-2 from air-to-surface rockets caused symptoms within minutes in both humans and animals, including severe nausea, vomiting, burning superficial skin discomfort, lethargy, weakness, dizziness and loss of coordination. Exposed cutaneous areas had a multitude of symptoms including erythema, tenderness, swelling, pain, and/or pruritus. Severe cases developed vesicles and bullae, petechiae, ecchymoses and necrotic lesions. [Ref 5.4.2, Vol. 2.13, page 168]

Soman (GD), Thickened Soman (TGD) and VX: Collectively, these agents are referred to as nerve gases or agents, and are classed as organophosphates.



Soman (GD)



VX

Nerve agents function by inhibiting acetylcholinesterase activity by preventing the hydrolysis of acetylcholine. This results in massive stimulation of cholinergic receptors resulting in continual stimulation of electrical activity and a cholinergic crisis which can occur within minutes after exposure. At >70% inhibition of blood AChE activity, progression of symptoms to maximum effect is rapid, minutes to hours depending on the degree of inhibition. Adverse effects result from over stimulation of the muscarinic receptors of the parasympathetic autonomic nervous system and over stimulation and subsequent blockage of nicotinic receptors. The signs of toxicity associated with over stimulation of the muscarinic receptors of the parasympathetic autonomic nervous system include increased secretions, bronchoconstriction, miosis, gastrointestinal cramps, diarrhea, urination and bradycardia. Signs of toxicity associated with over stimulation and subsequent blockage of nicotinic receptors, including the ganglia of the sympathetic and parasympathetic division of the autonomic nervous system as well as the junction between nerves and muscles, include tachycardia, hypertension, muscle fasciculations, tremors, muscle weakness, and/or flaccid paralysis. Generalized signs of toxicity resulting from effects on the central nervous system include restlessness, emotional

lability, ataxia, lethargy, mental confusion, loss of memory, generalized weakness, convulsions, cyanosis, and coma. Effects which may persist for several months following exposure include neurobehavioral, cognitive and neuromuscular functions.

Nerve agents are liquids at moderate temperature and humidity, however the low vapor pressure of the "G" agents, i.e., tabun, sarin and soman, makes them significant inhalation hazards. In humans, the lethal dose of inhaled GD is approximately 1 mg. Percutaneous absorption of G agents is much less rapid and complete than following inhalation. The percutaneous LD_{50}s have been estimated to be 100 mg for tabun, 1700 mg for sarin, 100 mg for soman, and 10 mg for VS.
VX is an oily liquid that may remain in place for weeks or longer after dispersion. This nerve agent does not pose a major inhalation hazard, but it is well absorbed through the skin.

[Ref. 5.4.5, Vol. 2.13, pages 291 and 309; DJ Echobichon, Toxic Effects of Pesticides in *Toxicology: The Basic Science of Poisons*, Casarett and Doull, Eds., 5th Ed. 1996, pp 655-665]

RBC and Plasma Acetylcholinesterase Activity as a Surrogate Biological Endpoint for Organophosphase Poisoning: The end point for all for nerve agents was the timed (30, 60, and 120 min) measurement of erythrocyte AChE activity expressed at a percentage of pre-exposed baseline values. Nerve agent doses were selected to produce a 70 to 90% decrease in AChE relative activity in the 120-min blood samples from rabbits treated with PEG.

AChE is a ubiquitous tissue enzyme which catalyzes the hydrolysis of the neurotransmitter acetylcholine to prevent prolonged postsynaptic action potentials. Adverse neuro/muscular effects and lethality are associated with prolonged postsynaptic action potentials.

Cholinesterases are also found in the red blood cells (RBC AChE) and plasma (pseudo AChE). The activity of these cholinesterases can be easily assayed in the laboratory and has routinely been used to diagnose and monitor patients exposed to organophosphate pesticides. Normal activity can vary between individuals by ~11% in men and 16% in women. In addition, mild to moderate exposures to organophosphates preferentially inhibit one or the other enzyme. In humans, VX has a much higher affinity for RBC AChE compared to plasma AChE, demonstrating ≥70% inhibition of RBC AChE compared to only 20% plasma AChE for the same dose. Similar results are observed with the nerve agent sarin where RBC AChE is inhibited by 80-100% compared to inhibition of only 30-50% of the plasma AChE. RBC and whole blood AChE appears to act as a scavenger for organophosphates, thereby reducing the effects of these toxicants on physiologically critical targets such as the brain.

Following percutaneous exposure to doses at or greater than the LD_{50}, enzyme inhibition and onset of effects occur within 1 to 30 minutes, with the time inversely related to the amount of agent. In general, systemic effects in humans occur when the RBC AChE is inhibited by >70%. In a study of percutaneous application of VX, 30 subjects were asymptomatic with RBC AChE levels as low as 40% of control activity. In cases of severe percutaneous exposure to nerve agents, subjects were asymptomatic for a period of 10 to 30 minutes followed by precipitated onset of loss of consciousness, convulsive activity, and cessation of respiration minutes later. [Ref. 5.4.5, Vol. 2.13, pages 313]

INDEX OF NONCLINICAL STUDIES AND PUBLICATIONS

PHARMACOLOGY AND TOXICOLOGY REVIEW

SECTION I: PHARMACOLOGY STUDIES

Development of a topical skin protectant is to provide an integrated approach to improving overall effectiveness in the field. Optimally, this agent should enhance the soldier's ability to both survive and operate in hazardous environments.

Casualty simulation models developed by the Army to assess the numbers of casualties with and without TSP under varying field conditions, e.g., munitions parameters, meteorological parameters, and troop dress and preparedness, predict that use of TSP will significantly reduce the morbidity following short-term exposures to chemical warfare agents such as sulfur mustard (HD), T-2 Toxin, soman (GD) and VX.

Mechanism of Action: TSP acts by providing a physical barrier to prevent dermal contact from a wide variety of chemical warfare agents having very different chemical properties. TSP has no other known pharmacologic action other than to serve as an antipenetrant barrier at the surface of the skin where it is applied.

Physicochemical testing revealed that TSP is comprised of a stable suspension of particles in oil, and that it has a low miscibility/solubility with nearly all liquid materials, including CWAs. The high contact angle and low absorptivity with CWAs inhibits wetting and penetration by most solvents, while the high concentration of PTFE particulates serves as a barrier to diffusion, reducing the mean free path of small molecules within the TSP.

[redacted]

SECTION III: PHARMACOKINETIC STUDIES

***Study 2.1* - Analysis of the Dermal Penetration of Perfluoropolyethers through Human Skin.**
Study No.: ?
Volume and Page No.: Vol. 2.19, page 055 [Ref. 5.4.40]
Conducting Laboratory and Location: Department of Chemistry, United States Naval Academy, 121 Blake Road, Annapolis, MD,
Date of Study Initiation:
GLP Compliance: Yes
QA Report: Yes ( ) No ( )
Methods and Dosing: The purpose of this study was to determine if the components of ICD-2289 were capable of penetrating human skin and if so, the degree of absorption. Fluorad fluorochemical surfactants (FC-99, -120, -129 and -135), compounds consisting of a fluorocarbon insoluble tail and either a hydrophilic head for use in aqueous solvents or an organophilic solubilizing group for used in nonaqueous systems, were used as the positive controls for skin penetration. Human skin samples were predominantly breast tissue from mammoplasties and mastectomies, although abdominal and big toe sections were also received. Skin samples were stored at 4°C and used within 24 hours of receipt. To analyze for dermal penetration, 25 µl of fluorochemical surfactant or 200 mg of ICD-2289 were placed on the outer surface of full thickness skin samples from which the fat layer had been removed. Tissue samples treated with 25 µl PBS served as the negative control. To degrade the components of the stratum corneum barrier, a second set of skin samples was treated with either acetone or DMSO for one hour prior to application of ICD-2289.

Fluorine analysis was performed using _____ nuclear magnetic resonance (NMR) spectrometer. All surfactant work was performed using _____ Tissues were evaluated using a scanning electron microscope (SEM) using _____

Results: Analysis of samples following application of the ICD-2289 for 1 hour indicated no transmission of fluorinated material past the stratum corneum. Preliminary tests conducted with longer contact residence times (2 to 24 hours) demonstrate similar results, with no evidence of dermal penetration. In studies where skin samples were first treated with either acetone or DMSO, ICD-2289 was able to permeate the skin surface, suggesting that intact stratum corneum acts as an effective barrier against penetration.

Review Note: Analysis of tissue samples for fluorines using NMR and SEM proved to be only a marginally acceptable method for detecting ICD 2289 in skin. These assays were supplemented with _____ confirming the presence or absence of both the PTFE and PFPE.

Study 2.2* - Skin Absorption of Topical Skin Protectant ICD-2289.
Study No.: ?
Volume and Page No.: Vol. 2.19, page 090 [Ref. 5.4.42]
Conducting Laboratory and Location: Drug Assessment Division, United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Grounds, MD
Methods and Dosing: This study was designed to assess the absorption of TSP (ICD #2289) into male hairless guinea pig skin and the extent to which TSP is removed from the skin following washing. was used to detect TSP (ICD #2289, Batch 02193A) residues on the cleansed skin of guinea pigs 24 hours after application. Prior to application of TSP, the guinea pigs were washed with soap and water and patted dry using a white paper towel. ICD 2289 was applied using a foam tipped swab until the surface of the skin was no longer visible. An attempt was made to occlude the application sites with gauze and surgical tape, however, once recaged, the animals were able to quickly free the dressings and no attempt was made to replace the dressings. After 24 hours, TSP was removed from the skin by wiping vigorously with either a foam tipped swab or a more abrasive wash cloth, soap and water.
Species/Strain: Euthymic Hairless Guinea Pig [Crl:IAF(HA)-hrBR(Outbred)]
#/sex/group or time point: 6 males
Age: ?
Weight: 433-680 g
Drug Lot #, Radiolabel, and % Purity: ——Batch 02193A
Formulation/Vehicle: Neat

Results: Spectra acquired of guinea pig skin after washing with a foam tipped swab (n=8) or a wash cloth, soap and water (n=5) demonstrated residual amounts of ICD 2289 left on the skin following both methods of removal, however, there was less TSP left on the skin after washing with the more abrasive cloth. Only minimal amounts, e.g., generally lower than the lowest standard of 400 μg, were detected in the first layer of stratum corneum removed by tape stripping. The average detectable depth for concentrations of ICD 2289 was 3.3 tape strips. At no point during the tape stripping did the exposed skin surface have the glistening, dry, erythematous appearance that would indicated complete removal of the stratum corneum.

Key Pharmacokinetic Findings: Intact stratum corneum appears to act as an effective barrier against penetration and subsequent absorption of TSP.

SECTION III: EFFICACY - *In Vitro* and *In Vivo* Efficacy Studies in Animal Models

1. *In Vitro* Penetration Models

The following *in vitro* studies demonstrate the ability of TSP to perform as a protective barrier. M8 Chemical Agent Detector Paper is designed to detect chemical warfare agents by changing color upon contact with a CWA.

In vitro Protocol MREF V-001-01 or 1-11-89-000-A-535: In each assay, A TSP application rate of 0.015 ml/cm^2, equivalent to a thickness of 0.15 mm, was adopted for these tests. An 8-cm strip of white ——— labeling tape, 0.15 mm thick, was placed on a cutting board and perforated with three, 2-cm diameter holes. The perforated tape was then placed on a strip of M8 Chemical Detection Paper, and the resulting wells were filled with 100 µl of either PEG 540 or TSP. A small spatula was used to spread TSP into all regions of the well and one end of a glass microscope slide was dragged across the top of the well to make the TSP surface smooth and flush with the top of the tape. Dosing commenced 1 hour after test site preparation. An 8 µl volume of neat CWA was applied to the center of each test site, which was immediately occluded with a 2 cm inside diameter cap and the bottom of the M8 paper was observed continuously (through a glass observation table on which the test was performed) for color changes for the first hour and every 30 minutes thereafter for 5 hours. Total test time was 6 hours (360 minutes) and nominal sample sizes were 12-15 penetration cells per TSP. Results are presented as the arithmetic mean ± the standard error of the mean.

Study 3.1 - **Letter Report (Attachment C): Raw Data for TSPs Containing Either Polymist F5A or Fomblin Y25 and Challenged with TGD on M-8 Chemical Detection Paper.**
Task No: Task 90-18
Volume and Page: Vol. 2.16, page 089 [Ref. 5.4.18]
Conducting Laboratory and Location: Battelle, 505 King Ave., Columbus, OH [Contract No. DAMD 17-89-C-9050]
Date of Study Initiation: July 12, 1994
GLP Compliance: ?
QA Report: Yes ( ) No (x)
Methods: Per *in vitro* Protocol MREF V-001-01

Results: Applications of TGD resulted in color changes in the chemical detection paper beginning at approximately 30 minutes when applied following pretreatment with PEG 540. When TGD was applied following pretreatment with ——— ICD 2289, there were no incidences of breakthrough observed over the 6 hour observation period (Table 3.1.1).

Table 3.1.1: TGD breakthrough times as detected by M-8 Chemical Detection Paper treated with PEG 540, ——— and ICD 2289.

Agent	Breakthrough Times (min)	Mean (min)*
PEG 540	33 - 125	67.4 ± 20.2
———	>360	-
ICD 2289	>360	-

* arithmetic mean ± sem of 4 separate tests each performed in triplicate (n=36)

Study 3.2 - **TSP/rTSP M-8 Paper Test – Pilot Patches.**
Task No: N/A
Volume and Page: Vol. 2.16/018 [Ref. 5.4.14]
Conducting Laboratory and Location: U.S. Army Medical Research Institute of Chemical Defense (USAMRICD)
Date of Study Initiation: June 14, 1994
GLP Compliance: ?
QA Report: Yes ( ) No (x)
Method: M-8 paper was prepared as described above (Protocol 1-11-89-000-A-535). 8 µl HD was applied/well and examined over a 4 hour period for color changes indicative of breakthrough.
Drug Lot #, Radiolabel, and % Purity: ICD 2289 Lot WRAST02 P28-1

Results: There were no signs of breakthrough in 24/24 wells pretreated with ICD 2289 and challenged with HD over a 4-hour exposure/observation period.

Study 3.3 - **TSP/rTSP M-8 Paper Test – Clinical Batches.**
Task No: N/A
Volume and Page: Vol. 2.16/022 [Ref. 5.4.15]
Conducting Laboratory and Location: U.S. Army Medical Research Institute of Chemical Defense (USAMRICD)
Date of Study Initiation: August 11, 1994
GLP Compliance: ?
QA Report: Yes (x) No ( )
Method: M-8 paper was prepared as described above (Protocol 1-11-89-000-A-535). 8 µl HD was applied/well and examined over a 4 hour period for color changes indicative of breakthrough.
Drug Lot #, Radiolabel, and % Purity: ICD 2289 Lot 305 – 794

Results: There were no signs of breakthrough in 36/36 wells pretreated with ICD 2289 and challenged with HD over a 4-hour exposure/observation period.

Study 3.4 - **Letter Report: M-8 Paper Test Results with Sunscreens and TSP**
Task No: Task 95-42
Volume and Page: Vol. 2.19, page 001 [Ref. 5.4.37]

Conducting Laboratory and Location: [redacted]

Date of Study Initiation: July 9, 1997
GLP Compliance: ?
QA Report: Yes ( ) No (x)
Methods: In this study, two sunscreens (ICD 2946 and ICD 2947) were tested with and without TSP [redacted] ICD 2289) pretreatment against liquid HD challenge. Both sunscreens and ICD 2289 (0.015 ml/sq. cm) were tested alone and in combination using the M-8 paper model.

Results: The breakthrough times for each agent challenged alone and in combination are presented in Table 3.4.1. As can be seen, pretreatment with the sunscreens had no protective effects against HD. When pretreated with only ICD 2289, 2/9 cells demonstrated breakthrough. When ICD 2289 pretreatments were combined with sunscreen only 1/18 cells demonstrated breakthrough. Based on these results, there does not appear to be any significant evidence that either of the army issue sunscreens tested, ICD 2946 and ICD 2947, will interfere with the performance of ICD 2289 in inhibiting *in vitro* penetration of HD.

Table 3.4.1: M-8 Paper breakthrough times (minutes) for 9 replicate HD challenge tests following pretreatment alone and in combinations of ICD 2289 and sunscreens 2946 and 2947.

Treatment	1	2	3	4	5	6	7	8	9
[redacted]	>360	>360	>360	>360	>360	>360	>360	>360	>360
ICD 2289	0.5	41	>360	>360	>360	>360	>360	>360	>360
SC 2946	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SC 2947	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SC 2946 + ICD 2289	17	>360	>360	>360	>360	>360	>360	>360	>360
SC 2947 + ICD 2289	>360	>360	>360	>360	>360	>360	>360	>360	>360

Study 3.5 - **Letter Report: Evaluation of Data from Test Results on Samples of TSP Using the M8 Paper Test.**

Task No: [redacted]
Volume and Page: Vol. 2.19, page 397 [Ref. 5.4.47]
Conducting Laboratory and Location: [redacted]

Date of Study Initiation: March 5, 1998, May 18, 1998, and July 17, 1998
GLP Compliance: ?
QA Report: Yes ( ) No (x)
Methods: This study was performed in compliance with *in vitro* protocol MREF V-001-03. Its purpose was to evaluate the antipenetrant effect of scale-up batches of ICD 2289 alone, in combination with permethrin, and following wear-time following challenge with HD. In the interaction tests, 25 μl aliquots of a solution of 19.6 or 196 mg of permethrin in 25 ml of isopropanol were applied onto the TSP and allowed to dry before the sulfur mustard (8 μl) was applied. The target permethrin application rates were 6.25 or 62.5 $\mu g/cm^2$. Permethrin alone had no effect on the color of the M-8 paper.

For the wear-time study, the TSP was applied and allowed to dry for 5, 15, 30 and 60 minutes, and 24 hours prior to challenge with HD.

Results: Evaluation of the *in vitro* breakthrough times following HD challenge were indicative of the following:

1) Scale-up production did not appear to affect the *in vitro* antipenetrant properties of ICD 2289.

2) At concentrations of 6.25 $\mu g/cm^2$ permethrin did not appear to interfere *in vitro* with the antipenetration effects of ICD 2289 against sulfur mustard. However, at 62.5 $\mu g/cm^2$, there were two (2/9) breakthroughs within the first hour after dosing indicative that permethrin may have altered the antipenetrant effects of ICD 2289 (Table 3.5.1).

3) Although 2 breakthroughs were noted, 1/8 at 15 minutes and 1/9 at 30 minutes, there was no consistent pattern to indicate that "wear-time" (interval between TSP application and challenge) had any adverse effect on the performance of ICD 2289 against sulfur mustard *in vitro* (Table 3.5.2).

Table 3.5.1: M-8 Paper breakthrough times (minutes) for 9 replicate HD challenge tests following pretreatment alone, in combination with permethrin.

Treatment	1	2	3	4	5	6	7	8	9
~~	>360	>360	>360	>360	>360	>360	>360	>360	>360
ICD 2289	>360	>360	>360	>360	>360	>360	>360	>360	>360
+ 6.25 µg/cm2 Permethrin	>360	>360	>360	>360	>360	>360	>360	>360	>360
+ 62.5 µg/cm2 Permethrin	>360	>360	**42**	>360	>360	>360	>360	**25**	>360

Table 3.5.2: Evaluation of the 'wear time' effect on M-8 Paper breakthrough times (minutes) for 9 replicate HD challenge tests following pretreatment with ICD 2289 and challenge with HD.

Application Time Intervals	1	2	3	4	5	6	7	8	9
5 minutes	>360	>360	>360	>360	>360	>360	>360	>360	>360
15 minutes	>360	>360	**1**	>360	>360	>360	>360	ND	>360
30 minutes	ND	>360	>360	>360	>360	>360	>360	**35**	>360
24 hours	>360	>360	>360	>360	>360	>360	>360	>360	>360

Note: results at 60 minutes were not included in the report.

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2. *In Vivo* Efficacy in Animals Models

Multiple biological endpoints were used to evaluate the effectiveness of TSP following topical exposures of HD, GD, TGD, VX and T-2 in rabbits. Endpoints evaluated included measurements of RBC and plasma AChE activity, lethality and dermal irritation. In each assay, animals were pretreated with a TSP candidate prior to topical applications of the CWA.

[

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In vivo Protocol MREF II-012-00: Each animal had 6 to 8 exposure sites (2.5 x 5 cm) with sites located contralateral to each other on both sides of the dorsal midline. Treatment sites were randomized to eliminate bias based on treatment site relative to body site. A 0.1 mm thick layer of TSP was applied to the clipped dorsa of anesthetized animals followed 1 hour later by a fixed dose of either GD (1.35 mg/kg), TGD (3.35 mg/kg), or VX (0.5 mg/kg); or in the case of HD, 1.0 μl applied at multiple test sites. Unless otherwise stated, *in vivo* challenges were conducted for 4 hours, following which both CWA and TSP were removed and animals were observed for an additional 20 hours.

The end point in all screens with HD challenges was the dermal lesion area ratio (LAR), the lesion area at challenged site divided by the lesion area at an unprotected site times 100 (expressed at %) on the same rabbit at 1, 2 and 4 hours post-exposure. End points following challenge with nerve agents consisted of measurement of either absolute and relative RBC or whole blood AChE at 1, 2, 3 and 4 hour exposure points, and lethality at 4 and 24 hours following initiation of challenge doses. All results are presented in terms of the arithmetic group mean ± the standard error of the mean.

Review Note: The Sponsor used relative activity (RA) values which were averaged across the after-dose sampling times to form an individual score parameter for each animal. These scores were then averaged across rabbits within each treatment group to obtain a general index of treatment group performance. Optimal TSP efficacy against CWAs would be a score of 1.0. These values are not reported in this review since it is judged that efficacy evaluations should be based both on time-to-onset as well as the degree of morbidity, and the number of individual animals adversely affected. Therefore, all results in this review are based on the numbers of animals affected and the group mean values at each time point evaluated.

An asterisk will be used to identify studies considered to be pivotal to the evaluation of efficacy in animals.

Study 3.6 - **Letter Report: Raw Data for TSPs Containing Either Polymist F5A or Fomblin Y25 and Challenged with HD, TGD and VX on Rabbit Backs.** (Also see Efficacy and Functional Stability Testing of Candidate Topical Skin Protectants. Ref. 5.4.6, Vol. 2.13, page 353)

Task No: Task 90-18

Volume and Page: Vol. 2.16, page 089 [Ref. 5.4.18]

Conducting Laboratory and Location: Battelle, 505 King Ave., Columbus, OH [Contract No. DAMD 17-89-C-9050]

Date of Study Initiation: July 12, 1994

GLP Compliance: ?
QA Report: Yes ( ) No (x)
Methods: Per *in vivo* Protocol MREF II-012-00. These studies were performed to evaluate the protective effects of pretreatment with numerous ICD formulations containing either Polymist F5A or Fomblin Y25 against challenge with HD, TGD and VX on rabbit backs. Only the results of the control substances and ICD 2289 will be given in this report.
Species/Strain: New Zealand White Rabbits
#/group or time point: 8 males/replicate group, three replicate groups per agent
Age, sex, weight: 2-4 kg

Results:

1) Dermal Effects (Attachment D): Applications of ICD 2289 prior to challenge with HD significantly reduced both the size of the lesions and the mean LAR when compared to pretreatment with PEG. Mean lesion sizes were 3.5 fold smaller and the mean LAR was reduced by almost 75%. The range of arithmetic mean lesion sizes (square mm) and lesion area ratios (%) compared with unprotected treatment sites are presented in Table 3.6.1. Geometric means were not calculated.

2) Retention (%) of AChE Activity (Attachments E and F): The absolute RBC AChE activity level (units/ml)/time point and the relative (%) AChE activity, calculated as the absolute RBC AChE activity at the sample time divided by the mean baseline RBC AChE activity level, are presented in Tables 3.6.2 and 3.6.3 (mean values are in parenthesis). The number of animals with RBC AChE activity reduced by >50%, >90% and 100% of their initial activity is presented in Table 3.6.4.

Table 3.6.1: Lesion areas and lesion area ratios (LAR) following HD challenge to rabbit skin pretreated with PEG 540, [redacted] or ICD 2289.

Agent	(n)	Area (square mm)	Mean (square mm)*	LAR (%)*
PEG 540	71	44.0 to 377.0	176.7 ± 85.8	64.8 ± 29.3
[redacted]	71	3.1 to 113.1	31.1 ± 20.2	11.2 ± 6.6
ICD 2289	48	7.1 to 141.4	50.6 ± 28.6	17.3 ± 8.1

* Arithmetic Mean ± sem

Table 3.6.2: Absolute AChE levels and relative (%) AChE activity in rabbits following topical exposures to TGD.

Time	PEG 540		[redacted]		ICD 2289	
(h)	AChE	%	AChE	%	AChE	%
0	[redacted] (1.7)*	100	[redacted] (1.8)	100	[redacted] (1.7)	100
0.5	[redacted] (1.7)	[redacted] (88)	[redacted] (1.7)	[redacted] (96)	[redacted] (1.6)	[redacted] (98)
1.0	[redacted] (1.2)	[redacted] (70)	[redacted] (1.6)	[redacted] (92)	[redacted] (1.5)	[redacted] (90)
3.0	[redacted] (0.9)	[redacted] (50)	[redacted] (1.5)	[redacted] (84)	[redacted] (1.4)	[redacted] (83)
24.0	[redacted] (0.9)	[redacted] (47)	[redacted] (1.5)	[redacted] (81)	[redacted] (1.3)	[redacted] (75)

* Arithmetic Mean

Table 3.6.3: Absolute AChE levels and relative (%) AChE activity in rabbits following topical exposures to VX.

Time (h)	PEG 540		[redacted]		ICD 2289	
	AChE	%	AChE	%	AChE	%
0	[redacted] (2.0)*	100	[redacted] (1.9)	100	[redacted] (1.8)	100
0.5	[redacted] (1.7)	[redacted] (83)	[redacted] (1.8)	[redacted] (93)	[redacted] (1.7)	[redacted] (95)
1.0	[redacted] (1.4)	[redacted] (69)	[redacted] (1.8)	[redacted] (91)	[redacted] (1.5)	[redacted] (85)
3.0	[redacted] (0.8)	[redacted] (39)	[redacted] (1.5)	[redacted] (75)	[redacted] (1.2)	[redacted] (66)
24.0	[redacted] (0.9)	[redacted] (20)	[redacted] (1.0)	[redacted] (49)	[redacted] (1.3)	[redacted] (70)

* Arithmetic Mean

When comparing the mean absolute and relative AChE levels following challenge with TGD, it can be seen that levels in rabbits protected with PEG 540 begin to significantly decrease after 1 hour, whereas the majority of rabbits pretreated with [redacted] or ICD 2289 retained 75% to 80% of their RBC AChE activity at 24 hours. Similar results were observed following pretreatment with ICD 2289 and challenge with VX, where animals pretreated with PEG 540 and [redacted] had retained less than 20% and 49% of their RBC AChE activity, respectively, at 24 hours compared to retention of greater than 70% RBC AChE activity in animals pretreated with ICD 2289.

Table 3.6.4: Number of treated animals which demonstrated decreases in AChE activity at each time point of ≥50%.

Time (h)	PEG 540		[redacted]		ICD 2289	
	TGD	VX	TGD	VX	TGD	VX
0.5	2/64	6/64	0/24	0/24	0/24	2/24
1.0	13/64	17/64	0/24	1/24	1/24	3/24
3.0	28/64	37/64	0/24	5/24	1/24	6/24
24.0*	11/24	24/24	0/9	4/9	1/9	2/6

* Only a predetermined subset of animals were examined at 24 hours (24 for controls and 9 for TSP treated animals). Mortality data were not included in the report however, it is assumed that only 6/9 of the preselected animals treated with ICD 2289 and VX survived for the 24-hour analysis.

*Study 3.7** - **Investigation of Efficacy of Perfluorinated Polyether Based TSPs Against HD Vapor in Hairless Guinea Pigs.**

Study No: ?

Volume and Page No.: Vol. 2.16, page 048 [Ref. 5.4.17] and Vol. 2.15, page 374 [Ref. 5.4.10]

Conducting Laboratory and Location: Drug Assessment Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland

Date of Study Initiation: ?
GLP Compliance: ?
QA Report: Yes (x) No ( )
Methods and Dosing: [redacted] ICD 2289 and [redacted] were compared against unprotected exposed sites on the backs of anesthetized guinea pigs. Each animal had 8 exposure sites with 4 sites contralateral to each other on both sides of the dorsal midline. Two contralateral sites served as positive control sites. The remaining 6 sites (3/ICD) were protected by the candidate TSPs at a thickness of 0.2 mm. Exposures were initiated 15 minutes following TSP application. HD vapor at its equilibrium concentration of 1.4 mg/L @ 30°C was applied for 4 to 20 minutes using 12 mm diameter, plastic, vial caps inverted onto each site. A 4-minute exposure was previously shown to yield an erythema score at the top portion of the linear part of the dose-response curve. At 3 hours post-exposure to HD, each site was cleaned with a cotton-tipped swab saturated with water to remove any visible signs of the TSP coating which might interfere with the reflectance color meter readings taken at 4 hours post-exposure.
Species/Strain: Crl:IAF/HA(hr/hr)BR VAF/Plus euthymic, hairless guinea pigs
#/sex/group or time point: 9 males
Age: ?
Weight: 250-400 g

Observations and Times: Four replicate readings were taken prior to exposure. Skin erythema resulting from exposures was quantified by chromaticity readings from a chromameter 4 hours post-exposure. Scores were determined on a site by site basis, the difference between the average before and after was calculated to determine the net increase in erythema. The average increase in erythema for candidate TSPs was then compared to that of the unprotected sites exposed for only 4-5 minutes.

Results:

1) In the initial part of the study, consisting of 4-minute exposures to HD vapor, ICD 2289 reduced erythema by 48%, exhibiting efficacy that was statistically better than unprotected controls. In contrast, [redacted] reduced erythema by an average of only 12% compared to the unprotected control sites.

2) In the second part of the study, ICD 2289 was shown to significantly reduce dermal irritation in guinea pigs following either a 5 or 10 minute exposure to HD vapor but provided no significant protection at 15 minutes, and at 20 minutes, actually resulted in greater irritation in relationship to controls challenged without TSP pretreatments.

TSP	5 min	10 min	15 min	20 min
ICD 2289	↓ 86%	↓ 55 %	↓ 8.6 %	↑ 9.7 %

3) In addition, this study demonstrated that efficacy against HD vapor could only be attained by using formulations, i.e., ICD 2289, containing perfluorinated base oils possessing high viscosity. Fomblin Y25 (PFPE) used in ICD 2289 had a viscosity approximately 7 times greater than the [redacted] used in [redacted] Similarly, it was shown in preliminary tests that reducing the concentration of Fomblin Y25 [redacted]) resulted in approximately 25% reduction in erythema compared to untreated control sites.

***Study 3.8* - Letter Report Number 1: Validation of Topical Skin Protectant (TSP) Scale-up and Production Samples.**

Task No: Task 93-34

Volume and Page No.: Vol. 2.16, page 029 [Ref. 5.4.16]

Conducting Laboratory and Location: Medical Research and Evaluation Facility, Battelle, Columbus, OH (Contract DAMD 17-89-C-9050)

Date of Study Initiation: 7/15/94

GLP Compliance: ?

QA Report: Yes ( ) No (x)

Methods and Dosing: Eight 2.5 x 5 cm sites on the dorsum of each rabbit were marked for treatment. Four sites were pretreated with TSP_1, TSP_2, TSP_3 or ——— A fifth test site was selected as a no-pretreatment control site and the remaining 3 sites served as untreated control sites. Treatments sites were randomly rotated from one animal to another to prevent confounding due to site location. One hour after TSP application, a 1μl topical challenge dose of HD was applied at each of the five pretreated sites. Following a 4-hour exposure period, sites were decontaminated and animals were allowed to recover. Each rabbit was normalized for its sensitivity to HD by comparing the lesion area at the unprotected site with an historical data base for statistical analysis. Univariate statistics were tabulated by TSP, and comparisons were made between each pair. Two multiple comparisons tests were conducted using the general linear models: the least squares means and Tukey-Kramer methods.

Species/Strain: New Zealand White Rabbits

#/group or time point: 24

Age, sex, weight: ?

Route, Form, Volume and/or Infusion Rate: topical application of 0.01 ml/cm^2

Drug Lot #, Radiolabel, and % Purity: ?

Results: Mean LAR scores for unprotected site lesions and ——— protected site lesions were within previously experimentally derived limits. ICD 2289 (lots 1-3) demonstrated significant suppression (~80%) of lesion area following 4-hour topical exposures to HD (Table 3.8.1).

Table 3.8.1: Absolute lesion area and lesion area ratios in rabbits following 4-hour topical exposures to HD with and without pretreatment with TSP.

Formulation	n	Lesion Area Ranges (square mm)	Mean Area*	LAR (%)*
Untreated	22	165 to 684	321.2 ± 111.5	100
———	21	15 to 144	56.3 ± 28.7	17.5 ± 6.7
ICD 2289_1	22	9 to 169	62.6 ± 26.3	20.7 ± 7.8
ICD 2289_2	23		65.4 ± 30.3	20.8 ± 8.2
ICD 2289_3	22		71.2 ± 30.2	21.4 ± 7.0

* Arithmetic mean ± sem

Although pretreatment with ——— led to smaller lesion sizes than pretreatment with ICD 2289, the differences in LAR were not statistical significant.

Study 3.9 - **Letter Report Number 2:** ***Protection by TSPs Against HD Using the Lesion Area Ratio (LAR) Test in Rabbits.***

Task No: 93-34

Volume and Page No.: Vol. 2.19, page 285 [Ref. 5.4.44]

Conducting Laboratory and Location: Medical Research and Evaluation Facility, Battelle, Columbus, OH (Contract DAMD 17-89-C-9050)

Date of Study Initiation: November 16, 1994

GLP Compliance: Yes

QA Report: Yes ( ) No (x)

Methods and Dosing: Ten 2.5 x 5 cm sites on the dorsum of each sedated rabbit were marked for treatment.

A	C	E	G
B	D	F	H

Sites (A-F) were randomly pretreated with one of the four ICD 2289 test lots or ——— , and one site was left untreated. Site G received no TSP and site H was pretreated with ~ on all animals. Sites G and H were compared with an historical database for process quality control and were not included in the comparisons with results from ICD 2289 treated sites. TSPs were applied at a depth of 0.1 mm (0.01 ml/cm^2). One hour after application of TSP, 1 μl HD was applied to each site. After 4 hours, all test sites except site G were decontaminated and rabbits were placed in stainless steel stanchions for the remainder of the 24 hour experimental period. At twenty-four hours after treatment, exposure sites were measured and LAR calculated.

Species/Strain: New Zealand White Rabbits (VAF/Plus®)

#/group or time point: 8 animals/day x 3 days

Weight: 2.0-2.7 kg

Supplier: ———

Drug Lot #, Radiolabel, and % Purity: ICD 2289 manufactured by ——— – Lot No. ——— No.): 305 —794 (—198-94), 306 —794 —199-94), 307 —794 —200-94), and 329 —794 (— 201-94)

Observations and Times: The endpoint reported for these *in vivo* tests was the lesion area ratio (LAR) at approximately 24 hours following HD exposure.

Results: As can be seen in Table 3.9.1, pretreatment with ICD 2289 prior to challenge with HD effectively reduced mean lesion area ratios by approximately 75% compared with unprotected sites challenged by HD applications, and mean lesion areas were approximate 5-6 fold smaller than unprotected sites.

Table 3.9.1: Effects of TSP pretreatment on skin lesions challenged with HD.

Parameter	Unprotected		———		ICD 2899 (Sites A-F)			
	Site G	A-F	Site H	A-F	UN201-94	UN198-94	UN200-94	UN199-94
Area (sq. mm)*	329 ± 165	240 ± 109	50 ± 30	51 ± 32	58 ± 28	58 ± 33	58 ± 42	58 ± 33
LAR (%)*	100 ± 31		21 ± 10		24 ± 13	24 ± 11	25 ± 13	25 ± 12

*Arithmetic mean ± sem

Study 3.10 - **Letter Report No. 3:** ***Protection by TSP Against Dermal Exposure to TGD: Reduction in Inhibition of Erythrocyte Acetylcholinesterase (AChE) Activity in Rabbits.***

Task No: Task 93-34

Volume and Page: Vol. 2.18, page 077 [Ref. 5.4.32]

Conducting Laboratory and Location: Medical Research Evaluation Facility, Battelle, 505 King Ave., Columbus, OH [Contract DAMD 17-89-C-9030, Study No. SC94009]

Date of Study Initiation: August 17, 1994

GLP Compliance: Yes

QA Report: Yes ( ) No (x)

Methods: Per *in vivo* Protocol MREF II-012-00: A 2 hour challenge dose of TGD (3.35 mg/kg) was applied on clipped rabbit backs that had been pretreated with three separate lots of ICD 2289 or the standard ———. The endpoint was RBC AChE activity. The TGD dose represents the historical 24-hr LD_{50}.

Species/Strain: New Zealand White Rabbits

#/group or time point: 3 animals/replicate group, eight replicate groups

Age, sex, weight: 2-4 kg

Drug Lot #, Radiolabel, and % Purity: Lot Nos. I-1, I-2 and I-3

Observations and Times: Blood samples were collected from the medial ear artery of the anesthetized rabbits prior to TSP application (-65, -35 and -5 minutes) and at 0.5, 1 and 2 hours after TGD application.

The Tukey-Kramer method, which allows for unequal sample sizes (since some animals died prior to the 2 hour sampling time) was used for statistical analysis.

Results (Tables 3.10.1, 3.10.2 and 3.10.3): Except for the single animal that died in treatment group ICD 2289(I-3), there were no statistically significant differences in the protection rendered between any of the ICD 2289 lots or between ICD 2289 and ——— Topical applications of TGD following pretreatment with ICD 2289 resulted in 14% (10/72) failure to protect, as defined by greater than a 50% reduction in RBC AChE activity following 2 hours of exposure.

Table 3.10.1: Absolute AChE levels in rabbits following topical exposures to 3.35 mg/kg TGD.

Time (h)	———	ICD 2289 I-1	ICD 2289 I-2	ICD 2289 I-3
0	2.1 ± 0.4	2.1 ± 0.4	2.2 ± 0.3	2.0 ± 0.4
0.5	2.0 ± 0.4	2.0 ± 0.5	2.2 ± 0.4	1.8 ± 0.5
1.0	1.9 ± 0.4	1.9 ± 0.5	1.8 ± 0.4	1.7 ± 0.6
2.0	1.7 ± 0.4	1.8 ± 0.5	1.7 ± 0.4	1.5 ± 0.7 *

* One animal found dead at 2 hours. An AChE level of 0.0 was assigned to this animal for the 2 hr reading.

Table 3.10.2: Relative AChE levels in rabbits following topical exposures to 3.35 mg/kg TGD.

Time (h)	———	ICD 2289 I-1	ICD 2289 I-2	ICD 2289 I-3
0.5	94 ± 13	98 ± 20	96 ± 14	89 ± 24
1.0	87 ± 14	90 ± 22	82 ± 17	86 ± 29
2.0	79 ± 14	86 ± 25	75 ± 19	76 ± 34

Table 3.10.3: Number of animals with <50%, <10% and 0% AChE activity following 0.5 to 2-hour exposures to TGD.

AChE Activity*	———	ICD 2289 I-1	ICD 2289 I-2	ICD 2289 I-3
<50%	1/72 (1 %)	1/24 (4 %)	3/24 (13%)	6/24 (25%)
<10%	0/72 (0 %)	0/24 (0 %)	0/24 (0 %)	1/24 (4 %)
0%	0/72 (0 %)	0/24 (0 %)	0/24 (0 %)	1/24 (4 %)

* <50% and <10% include all animals below these levels at any time during the 2 hour evaluation period, including those animals with 0% activity. The single animal treated with ICD 2289_{I-3} and found dead at the 2-hour time point is incorporated into all three categories.

Study 3.11 - **Letter Report Number 4**: ***Protection by TSP Against Dermal Exposure to TGD: "Reduction in Inhibition of Erythrocyte Acetylcholinesterase (AChE) Activity in Rabbits".***
Task No: Task 93-34
Volume and Page: Vol. 2.17, page 245 [Ref. 5.4.30]
Conducting Laboratory and Location: Battelle, 505 King Ave., Columbus, OH [Contract No. DAMD 17-89-C-9050]
Date of Study Initiation: June 27, 1995
GLP Compliance: Yes
QA Report: Yes ( ) No (x)
Methods: Per *in vivo* Protocol MREF II-012-00: A 3.35 mg/kg challenge dose of TGD was applied to rabbit backs pretreated with ICD 2899 or ———
Species/Strain: New Zealand White Rabbits
#/group or time point: 8 males/replicate group, three replicate groups per agent
Age, sex, weight: 2-4 kg
Drug Lot #, Radiolabel, and % Purity: Lot No. 305 — 794

Observations and Times: Blood samples were collected from the medial ear artery of the anesthetized rabbits prior to TSP application and at 0.5, 1 and 3 hours after TGD application.

The mean relative activity of each of the after-dose times and the overall score for each animal was compared to that for untreated groups using Dunnett's pairwise t-test and Tukey's Studentized Range multiple comparison test performed at the p = 0.05 level. The ANOVA and pairwise comparisons were performed using SAS.

Results: Statistically significant retention of RBC AChE levels was achieved with both ——— and ICD 2289 compared to unprotected animals at each time point following exposures to TGD

(Table 3.11.1). Furthermore, at no time was the AChE activity of any individual animal pretreated with ICD 2289 reduced by greater than 50% of its pre-dosing level.

Table 3.11.1: Absolute and relative AChE levels in rabbits following topical exposures to 3.35 mg/kg TGD.

Time (h)	Unprotected		[redacted]		ICD 2289	
	AChE	%	AChE	%	AChE	%
0	2.0 ± 0.4	-	2.0 ± 0.4	-	2.1 ± 0.4	-
0.5	1.2 ± 0.5	60 ± 30	2.0 ± 0.4	90 ± 10	1.9 ± 0.3	90 ± 10
1.0	0.8 ± 0.5	40 ± 20	1.7 ± 0.3	90 ± 10	1.7 ± 0.3	80 ± 10
3.0	0.6 ± 0.4	30 ± 20	1.5 ± 0.3	70 ± 10	1.6 ± 0.3	80 ± 20

Arithmetic mean ± sem

Study 3.12* - **Letter Report Number 7:** ***Evaluation of Candidate Topical Skin Protectants (ICD 2289 [redacted] Using in vivo Models to Determine Their Relative Effectiveness Against Thickened GD and HD.***

Task No: Task 93-34

Volume and Page: Vol. 2.18, page 001 [Ref. 5.4.31]

Conducting Laboratory and Location: Medical Research Evaluation Facility, Battelle, 505 King Ave., Columbus, OH [Contract DAMD 17-89-C-9050, Study No. SC94009]

Date of Study Initiation: March 12, 1996

GLP Compliance: Yes

QA Report: Yes ( ) No (x)

Methods: Per *in vivo* Protocol MREF II-012-00: A 4-hour challenge dose of either VX (0.50 mg/kg) or TGD (3.35 mg/kg) was applied on clipped rabbit backs that had been pretreated with either ICD 2289 or [redacted] The endpoints were RBC AChE activity and lethality. The dose of VX represents 10 times the 24-hr LD_{50} dose for unprotected rabbits (0.05 mg/kg), while the TGD dose represents the historical 24-hr LD_{50}.

Species/Strain: New Zealand White Rabbits

#/group or time point: 8 males/replicate group, three replicate groups per agent

Age, sex, weight: 2-4 kg

Drug Lot #, Radiolabel, and % Purity: Lot No. [redacted]198-94

Observations and Times: Blood samples were collected from the medial ear artery of the anesthetized rabbits prior to TSP application (-65, -35 and -5 minutes) and at 1, 2, 3 and 4 hours after CWA application.

The mean relative activity of each of the after-dose times and the overall score for each animal was compared to that for untreated groups using Dunnett's pairwise t-test and Tukey's Studentized Range multiple comparison test performed at the $p = 0.05$ level. The ANOVA and pairwise comparisons were performed using SAS. Lethality rates were compared using Fisher's exact test at the 5% significance level.

Results: Relative to the unprotected animals, ICD 2289 demonstrated significant protection against both TGD and VX in terms of absolute and relative AChE activity (Tables 3.12.1, 3.12.2 & 3.12.3).

In terms of lethality, ICD 2289 was effective in significantly reducing the lethal effects of VX by protecting over 90% of the animals from death in comparison to the 100% mortality observed within 4 hours in the unprotected animals (Table 3.12.4). At 1 hour post-challenge, all animals treated with TGD retained greater than 10% relative AChE activity, and only 1/24 animals (4%) treated with VX had a relative AChE level below 10%.

Table 3.12.1: Absolute and relative AChE levels in rabbits following topical exposures to 3.35 mg/kg TGD.

Time (h)	Unprotected		[redacted]		ICD 2289	
	AChE	%	AChE	%	AChE	%
0	2.2 ± 0.5	-	2.0 ± 0.4	-	2.1 ± 0.4	-
1.0	0.4 ± 0.4	20 ± 20	1.6 ± 0.2	80 ± 10	1.8 ± 0.4	90 ± 10
2.0	0.3 ± 0.3	10 ± 10	1.3 ± 0.4	70 ± 20	1.5 ± 0.4	70 ± 20
3.0	0.2 ± 0.1	10 ± 10	1.1 ± 0.5	60 ± 20	1.3 ± 0.5	60 ± 20
4.0	0.2 ± 0.2	10 ± 10	1.0 ± 0.3	50 ± 20	1.2 ± 0.4	60 ± 20

Arithmetic mean ± sem

Table 3.12.2: Absolute and relative AChE levels in rabbits following topical exposures to 0.5 mg/kg VX.

Time (h)	Unprotected		[redacted]		ICD 2289	
	AChE	%	AChE	%	AChE	%
0	2.2 ± 0.4	-	2.1 ± 0.4	-	2.0 ± 0.4	-
1.0	0.0 ± 0.0	0 ± 0	1.8 ± 0.4	90 ± 10	1.6 ± 0.5	80 ± 20
2.0	0.0 ± 0.0	0 ± 0	1.5 ± 0.4	70 ± 20	1.4 ± 0.8	70 ± 30
3.0	0.0 ± 0.0	0 ± 0	1.3 ± 0.6	70 ± 20	1.2 ± 0.7	60 ± 30
4.0	0.0 ± 0.0	0 ± 0	1.2 ± 0.7	50 ± 30	1.1 ± 0.8	50 ± 40

Arithmetic mean ± sem

Table 3.12.3: Number of animals with <50%, <10% and 0% AChE activity following 4-hour exposures to TGD and VX.

AChE	Unprotected		[redacted]		ICD 2289	
	TGD	VX	TGD	VX	TGD	VX
<50%	24/24 (100%)	24/24 (100%)	6/9 (60%)	5/9 (56%)	8/23 (35%)	11/24 (46%)
<10%	22/24 (92%)	24/24 (100%)	0/9 (0 %)	1/9 (11%)	1/23 (4 %)	7/24 (29%)
0%	2/24 (8 %)	24/24 (100%)	0/9 (0 %)	0/9 (0 %)	1/23 (4 %)	1/24 (4 %)

Table 3.12.4: Lethality rates at 4 hours and 24 hours after topical application of 3.35 mg/kg TGD or 0.5 mg/kg VX with or without TSP ICD 2289.

Time (h)	TGD		VX	
	With TSP	w/o TSP	With TSP	w/o TSP
4	1/24	2/24	1/24	24/24
24	2/24	3/24	2/24	24/24

Review Note: The dose of TGD was not high enough to observe any significant protective effect with ICD 2289. In general, there was no observable recovery of RBC AChE activity at 24 hours in those animals demonstrating significant reductions at earlier time points and a few animals which were above 50% activity at the earlier time points, were below 50% activity or dead at 24 hours.

*Study 3.13** - **Letter Report No. 10:** ***Protection by TSP Against Dermal Exposure to either VX or GD as Measured by Reduction of Inhibition of Whole Blood Cholinesterase (ChE) Activity or Lethality.***

Task No: Task 93-34

Volume and Page: Vol. 2.19, page 110 [Ref. 5.4.43]

Conducting Laboratory and Location: Medical Research Evaluation Facility, Battelle, 505 King Ave., Columbus, OH [Contract DAMD 17-89-C-9050, Study No. SC94095]

Date of Study Initiation: May 19, 1998

GLP Compliance: Yes

QA Report: Yes ( ) No (x)

Methods: Per *in vivo* Protocol MREF II-012-00: A challenge dose of VX (0.5 mg/kg) or GD (8.906 mg/kg) was applied for 4 hours on unprotected sites on clipped rabbit backs or sites pretreated with either ICD 2289 or ——— 1 hour prior to challenge. The dose level for GD was selected as the 24-hour LD_{80} from lethality studies in unprotected rabbits, while the dose of VX represents 10 times the 24-hr LD_{50} dose for unprotected rabbits. To improve the sensitivity of the study, the endpoint was whole blood AChE activity instead of RBC AChE activity.

Species/Strain: New Zealand White Rabbits

#/group or time point: 2 animals/replicate group, 12 replicate groups per agent (24 rabbits per batch of TSP, 36 process ——— control rabbits, and 36 positive control rabbits)

Age, sex, weight: 2-4 kg

Drug Lot #, Radiolabel, and % Purity: ICD 2289 manufactured by McKesson BioServices: 12 kg Lot Nos. (Batch) – TSP001 (001.0298), TSP002 (002.0298), & TSP003 (003.0298); 300 kg Lot Nos. (Batch) – TSP004 (S-03946), TSP005 (S-03958), & TSP006 (S-03959).

Observations and Times: Blood samples were collected from the medial ear artery of the anesthetized rabbits prior to TSP application (-65, -35 and -5 minutes) and at 1, 2, 3 and 4 hours after CWA application. Evaluation of whole blood ChE activity was estimated using 6,6'-dithiodinicotinic acid (DTNA) in place of the standard DTNB assay.

The ANOVA and pairwise comparisons were performed using SAS. Lethality rates were compared using Fisher's exact test at the 5% significance level.

Results: Relative to the unprotected animals, all batches of ICD 2289 significantly reduced the effects of both GD and VX (Tables 3.13.1 & 3.13.2) on whole blood AChE activity. At 1 hour post-challenge, 15/144 animals (10%) treated with GD had a relative AChE level below 10%, 2 of these animals had no detectable AChE activity; and 29/144 animals (20%) treated with VX had a relative AChE level below 10%, 17 of these animals had no detectable AChE activity. At 4 hours challenge, 75% of the animals challenged with GD, and 54% of the animals challenged with VX had lost greater than 50% of their whole blood AChE activity (Table 3.13.3).

Table 3.13.1: Absolute and relative AChE levels in rabbits following topical exposures to 8.96 mg/kg GD.

TSP	Pre-challenge		1 hr		2 hrs		3 hrs		4 hrs	
	AChE	%	AChE	%	AChE	%	AChE	%	AChE	%
None	1.4 ± 0.1	-	0.0 ± 0.1	0	0.0 ± 0.1	0	0.0 ± 0.1	0	0.0 ± 0.1	
——	1.3 ± 0.2	-	1.1 ± 0.3	90 ± 30	0.9 ± 0.3	80 ± 40	0.8 ± 0.3	70 ± 40	0.7 ± 0.3	70 ± 40
ICD 2289 (Batch Nos. 1-6)										
1	1.4 ± 0.1	-	0.8 ± 0.4	60 ± 30	0.5 ± 0.3	40 ± 30	0.4 ± 0.3	30 ± 20	0.4 ± 0.3	30 ± 20
2	1.4 ± 0.1	-	0.8 ± 0.5	60 ± 30	0.5 ± 0.4	40 ± 30	0.5 ± 0.4	30 ± 30	0.4 ± 0.4	30 ± 30
3	1.3 ± 0.2	-	0.9 ± 0.3	70 ± 20	0.6 ± 0.3	50 ± 30	0.5 ± 0.3	40 ± 30	0.4 ± 0.3	30 ± 20
4	1.3 ± 0.1	-	0.8 ± 0.4	60 ± 30	0.5 ± 0.4	40 ± 30	0.4 ± 0.3	30 ± 20	0.4 ± 0.3	30 ± 20
5	1.3 ± 0.1	-	0.8 ± 0.4	60 ± 30	0.6 ± 0.4	50 ± 30	0.5 ± 0.4	40 ± 30	0.4 ± 0.4	30 ± 30
6	1.4 ± 0.1	-	1.0 ± 0.3	70 ± 20	0.7 ± 0.3	50 ± 20	0.5 ± 0.3	40 ± 20	0.5 ± 0.3	30 ± 20

Arithmetic mean ± sem

Table 3.13.2: Absolute and relative AChE levels in rabbits following topical exposures to 0.5 mg/kg VX.

TSP	Pre-challenge		1 hr		2 hrs		3 hrs		4 hrs	
	AChE	%	AChE	%	AChE	%	AChE	%	AChE	%
None	1.4 ± 0.2	-	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0
——	1.4 ± 0.1	-	1.2 ± 0.4	90 ± 30	1.1 ± 0.6	80 ± 40	1.0 ± 0.6	70 ± 40	1.0 ± 0.6	70 ± 40
ICD 2289 (Batch Nos. 1-6)										
1	1.4 ± 0.2	-	1.0 ± 0.5	70 ± 40	0.9 ± 0.6	60 ± 40	0.8 ± 0.6	60 ± 50	0.8 ± 0.6	50 ± 40
2	1.5 ± 0.1	-	0.9 ± 0.7	60 ± 40	0.7 ± 0.7	40 ± 40	0.6 ± 0.7	40 ± 40	0.5 ± 0.7	30 ± 40
3	1.4 ± 0.1	-	0.9 ± 0.6	70 ± 40	0.7 ± 0.6	60 ± 40	0.7 ± 0.7	50 ± 50	0.6 ± 0.6	50 ± 40
4	1.4 ± 0.2	-	0.8 ± 0.5	60 ± 50	0.7 ± 0.7	50 ± 50	0.7 ± 0.7	50 ± 50	0.6 ± 0.6	40 ± 40
5	1.4 ± 0.1	-	1.0 ± 0.5	70 ± 40	0.9 ± 0.6	60 ± 50	0.8 ± 0.6	60 ± 50	0.7 ± 0.7	60 ± 40
6	1.3 ± 0.2	-	0.9 ± 0.4	70 ± 40	0.8 ± 0.6	60 ± 50	0.8 ± 0.6	60 ± 50	0.7 ± 0.7	50 ± 40

Arithmetic mean ± sem

Table 3.13.3: Percent of all animals with <50%, <10% or 0% whole blood AChE activity at any time point (between 1 and 4 hrs) following topical application of 8.96 mg/kg GD or 0.5 mg/kg VX with or without TSP ICD 2289.

AChE Activity	GD			VX		
	w/o TSP	——	ICD 2289	w/o TSP	——	ICD 2289
<50%	100%	65%	75%	100%	32%	54%
<10%	100%	3%	26%	100%	26%	42%
0%	100%	0%	7%	100%	16%	34%

In terms of lethality, ICD 2289 was effective in significantly reducing the lethal effects of GD and VX by protecting over 90% of the animals exposed to GD and 60% of the animals exposed to VX at 24 hours in comparison to the 100% mortality seen in the unprotected animals at 4 hours (Table 3.13.4).

Table 3.13.4: Lethality rates at 4 hours and 24 hours after topical application of 8.96 mg/kg GD or 0.5 mg/kg VX with or without TSP ICD 2289.

TSP Treatment	GD		VX	
	4 hrs	24 hrs	4 hrs	24 hrs
w/o TSP	31/36 (86%)	32/36 (89%)	36/36 (100%)	36/36 (100%)
—	0/34 (0 %)	1/34 (3%)	6/32 (19%)	10/28 (36%)
ICD 2289				
1	1/24 (4%)	1/24 (4%)	7/24 (29%)	8/24 (33%)
2	2/24 (8%)	2/24 (8%)	11/24 (46%)	12/24 (50%)
3	0/24 (0%)	2/24 (8%)	8/24 (33%)	10/24 (42%)
4	2/24 (8%)	3/24 (12%)	10/24 (42%)	10/24 (42%)
5	4/24 (16%)	4/24 (16%)	7/24 (29%)	7/24 (29%)
6	1/24 (4%)	1/24 (4%)	7/24 (29%)	9/24 (38%)
Total	**10/144 (7%)**	**13/144 (9%)**	**50/144 (35%)**	**56/144 (39%)**

It is interesting to note here, that exposures to GD resulted in some reduction in whole blood AChE activity in over 99% of the animals (145/146), whereas exposures to VX resulted in reductions in whole blood AChE activity in only 67% of the animals (96/144), with 1/3 of the animals maintaining 100% activity over the 4 hour challenge period (Table 3.13.5). There were no significant differences in the mortality rate at 4 hours and the percent of animals with no detectable AChE activity (0%), whereas most of the animals with AChE activity levels between 1 and 10 % were still alive at 24 hours.

Table 3.13.5: Recap of final outcome following 4 hour exposures of GD and VX with and without ICD 2289.

Parameter	Time (h)	GD		VX	
		w/o TSP	ICD 2289	w/o TSP	ICD 2289
Mean AChE Levels (U/ml)	4	0.0 ± 0.1	0.4 ± 0.3	0 ± 0	0.6 ± 0.6
Mean AChE Activity (%)	4	0	30 ± 20 %	0	50 ± 40 %
No. of Animals w/100% AChE Activity	4	0	<1.0 %	0	33 %
No. of Animals w/<50% AChE Activity	4	100 %	75 %	100 %	54 %
No. of Animals w/<10% AChE Activity	4	100 %	26 %	100 %	42 %
No. of Animals w/ 0% AChE Activity	4	100 %	7 %	100 %	34 %
Mortality Rate	4	100 %	7 %	100 %	35 %
	24	100 %	9 %	100 %	39 %

It appears that there was bimodal distribution pattern in the protective effect of ICD 2289 following challenge with VX. One third of the exposed animals showed no detectable change in AChE activity while another 1/3 of the animals experienced complete loss and lethality within 4 hours of VX challenge.

AChE Activity	100%	99-50%	49-10%	9-1%	0%
Animals Treated with ICD 2289 and VX	33%	13%	12%	8%	34%

Review of the data suggested that the differences observed in VX breakthrough may have resulted from faulty application of TSP, since on some test days there was breakthrough in almost 100% of the animals while on other days only minimal breakthrough was observed in some of the animals. Due to the high concentration of VX applied, even a minute unprotected area would result in significant AChE inhibition.

Review Note: Although there was significant mortality observed in rabbits pretreated with TSP and challenged with VX, it should be noted that this dose is 10 fold greater than the LD_{50} dose in rabbits and expected to be significantly greater than exposures in the field. In humans, the percutaneous LD_{50} for GD is estimated to be 100 mg (or approximately 1.67 mg/kg). Furthermore, extrapolation of these findings to humans exposed to VX in the field is difficult since the rabbits were restrained and anesthetized, while in humans, TSP will be used in conjunction with protective clothing and under combat situations. It does however emphasize the need for complete coverage of exposed areas for optimal protection by TSP.

*Study 3.14** - **Letter Report Number 11:** ***Protection by TSP Against HD Using the Lesion Area Ratio (LAR) Test in Rabbits.***

Task No: 93-34

Volume and Page No.: Vol. 2.19, page 376 [Ref. 5.4.46]

Conducting Laboratory and Location: Medical Research Evaluation Facility, Battelle, 505 King Ave., Columbus, OH [Contract DAMD 17-89-C-9050, Study No. SC940095]

Date of Study Initiation: May 12, 1998

GLP Compliance: Yes

QA Report: Yes ( ) No (x)

Methods and Dosing: Eight 2.5 x 5 cm sites on the dorsum of each sedated rabbit were marked for treatment.

A	C	E	G
B	D	F	H

Sites (A-F) were randomly pretreated with one of the ICD 2289 test lots while Site G received no TSP and site H was pretreated with — on all animals. Sites G and H were compared with an historical database for process quality control. TSPs were applied at a depth of 0.1 mm (0.01 ml/cm^2). One hour after application of TSP, 1 μl HD was applied to each site. After 4 hours, all test sites except site G were decontaminated and rabbits were placed in stainless steel stanchions for the remainder of the 24 hour experimental period. At twenty-four hours after treatment, exposure sites were measured and LAR calculated.

Species/Strain: New Zealand White Rabbits

#/group or time point: 8 animals per each of 3 replicate groups

Age, sex, weight: 2-4 kg

Drug Lot #, Radiolabel, and % Purity: ICD 2289 manufactured by McKesson BioServices:

12 kg Lot Nos. (Batch) – TSP001 (001.0298), TSP002 (002.0298), & TSP003 (003.0298);
300 kg Lot Nos. (Batch) – TSP004 (S-03946), TSP005 (S-03958), & TSP006 (S-03959).

Observations and Times: The endpoint reported for these *in vivo* tests was the lesion area ratio (LAR) at approximately 24 hours following HD exposure.

Univariate statistics on absolute lesion areas and LARs were tabulated by TSP across test days. Multiple comparisons between the candidate TSPs were made using the Tukey-Kramer method and ANOVA and multiple comparison procedures were performed using the SAS (version 6.08) GLM procedure.

Results: As can be seen in Table 3.14.1, pretreatment with ICD 2289 prior to challenge with HD effectively reduced lesion size by approximately 75-84 % compared to lesions on unprotected sites challenged by HD applications. All TSP results were statistically significant when compared to the unprotected site.

Table 3.14.1: Effects of TSP pretreatment on skin lesions (arithmetic mean ±sem) induced with HD.

Parameter	Unprotected		ICD 2899 Batches (Sites A-F)					
	Site G	Site H	001	002	003	004	005	006
Area (sq. mm)	328 ± 158	45 ± 27	51 ± 29	59 ± 31	65 ± 40	77 ± 69	52 ± 23	55 ± 32
LAR (%)	100	15 ± 4	16 ± 3	19 ± 5	22 ± 6	25 ± 11	20 ± 8	17 ± 4

*Study 3.15** - **Evaluation of Topical Skin Protectant-2 (TSP_2) to Protect Against Percutaneous Exposure to Mycotoxin on the Skin of Rabbits.**

Study No: UIT-717-5

Volume and Page No.: Vol. 2.18, page 183 [Ref. 5.4.35]

Conducting Laboratory and Location: U.S. Army Medical Research Institute of Infectious Disease, Fort Detrick, MD

Date of Study Initiation: June, 1994

GLP Compliance: No

QA Report: Yes ( ) No (x)

Methods and Dosing: Ten 2.5 x 5 cm sites on the dorsum of each sedated rabbit were marked for treatment and rabbits were sedated using 0.1 mg of Buprenex (buprenorphine) and immobilized in stanchions.

Five randomized sites were pretreated with TSP_2 (ICD 2289) at a depth of 0.1 mm (0.01 ml/cm^2). One hour after application of TSP, the stanchions were opened and 2 μl, containing 25 μg T-2 toxin in methanol, was applied to 8 sites (A-H) and methanol alone applied to the two most posterior sites (I & J). While still sedated, animals were secured to stanchions and allowed to regain consciousness and access to food and water for 6 hours. At 1, 2, 4 and 6

hour intervals after T-2 application, the stanchions were opened and 1 pair of test sites were washed with Dyna-Hex and patted dry with a gauze pad. TSP was then removed by gentle scrapping with a dry absorbent pad and sites were again washed with Dyna-Hex and rinsed with distilled water. Eight hours from the beginning of the study, animals were removed from the stanchions, injected with 0.03 mg/kg Buprenex and returned to their cages with full access to food and water. Twenty-four, thirty-six and forty-eight hours after treatment, exposure sites were analyzed for severity of skin reactions by 1) LAR and 2) edema/erythema scores (0-4).

Note: A preliminary study was conducted using 0.01 to 100 µg T-2 / 2 µl to determine the effects of analgesic on the dose and time response; to evaluate the use of a reflectance color meter to quantitate the vesicant effects of T-2 toxin; and to determine the minimal number of animals required for obtaining statistical differences between treatments. This study found that simple washing of the treatment site 6 hours after percutaneous exposure to T-2 toxin resulted in a significant 20 to 30% reduction in the severity of the lesions. Analgesic treatment had no effect on either the severity of the response or the timing of the response. The [redacted] was shown capable of distinguishing a dose-response pattern for edema/erythema scoring.

Species/Strain: New Zealand White Rabbits (VAF/Plus®)
#/group or time point: 8 Males, 10 sites/animal
Weight: 2.0-2.7 kg
Supplier: [redacted]
Drug Lot #, Radiolabel, and % Purity: WARST02/16-2

Observations and Times: Twenty-four, thirty-six and forty-eight hours (24, 36 & 48 hrs) after treatment, exposure sites were analyzed for severity of skin reactions by 1) LAR and 2) edema/erythema scores (0-4). After the last endpoint measurement, the rabbits were euthanized and postmortem skin samples were removed from all treated sites on 2 randomly selected rabbits.

Multiple comparison tests with a single control (i.e., one and two way ANOVA, Student-Newman-Keuls method of Pairwise Multiple Comparisons on GraphPAD or InStat or Sigma Stat programs) were utilized to determine difference in TSP treatment, exposure times, and endpoint measurements. All statistical tests were at the 95% confidence level.

Results: As can be seen in Table 3.15.1, pretreatment with TSP effectively blocked all macroscopic signs of dermal irritation up to 6 hours following T-2 toxin exposures. In addition, all the control edema/erythema scores and lesion areas were found to be significantly greater ($p<0.05$) at 32 and 48 hours when compared to the 24 hour control scores.

Similar protective effects were observed following microscopic examination of the skin, where histopathological findings were significantly ($p<0.05$) less from the TSP pretreated sites (Table 3.15.2).

Table 3.15.1: Effects of ICD 2289 pretreatment and post-exposure decontamination by washing with soap and water at 1, 2, 4 and 6 hours on the severity (arithmetic mean ± sem) of the percutaneous reactions following exposure to T-2 toxin.

Endpoint	Time (hr)	Saline Pretreated Sites				ICD 2289 Pretreated Sites			
		1 hr	2 hr	4 hr	6 hr	1 hr	2 hr	4 hr	6 hr
Edema + Erythema Scores	24	2.8 ± 0.2	2.8 ± 0.2	2.8 ± 0.2	2.8 ± 0.2	0	0	0	0
	32	3.5 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	0	0	0	0
	48	5.5 ± 0.3	5.5 ± 0.3	5.5 ± 0.3	5.5 ± 0.3	0	0	0	0
Lesion Area (units unspecified)	24	2.4 ± 0.4	2.3 ± 0.4	2.4 ± 0.4	2.4 ± 0.4	0	0	0	0
	32	3.7 ± 0.3	3.4 ± 0.3	3.5 ± 0.3	3.7 ± 0.3	0	0	0	0
	48	3.8 ± 0.4	3.7 ± 0.3	3.6 ± 0.4	3.6 ± 0.4	0	0	0	0

Table 3.15.2: Mean (arithmetic ± sem) number of histological responses in rabbit skin to T-2 toxin and efficacy of pretreatment with ICD 2289.

Histopathology *	Unprotected Sites	ICD 2289 Pretreated Sites	Methanol Control Sites
Edema	3.1 ± 0.2	0.9 ± 0.4	0
Superficial Dermatitis	3.4 ± 0.2	1.4 ± 0.2	1.1 ± 0.1
Deep Dermatitis	3.4 ± 0.5	0	0
Folliculitis	1.8 ± 0.3	0.1 ± 0.1	0.3 ± 0.3
Panniculitis	1.4 ± 0.4	0	0
Epidermal Necrosis	3.5 ± 0.2	0.7 ± 0.4	0.3 ± 0.3
Dermal Necrosis	3.5 ± 0.2	0	0
Intradermal Pustules	8/8	1/8	1/4

* Rating Scheme: 0 = Essentially normal tissue; 1 = minimal lesion, 1.5 = minimal to mild lesion; 2 = mild lesion; 2.5 = mild to moderate lesion; 3 = moderate lesion; 3.5 = moderate to marked lesion; 4 = marked lesion.

Study 3.16* - **Efficacy of Topical Skin Protectants (TSPs) Against CS and CR in the Rabbit.**
Study No: _?
Volume and Page No.: Vol. 2.18, page 250 [Ref. 5.4.36]
Conducting Laboratory and Location: Toxicology Team, Research Directorate, U.S. Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD
Date of Study Initiation: September, 1994 (Report dated 11/7/95)
GLP Compliance: Yes
QA Report: Yes (x) No ( )
Methods and Dosing: CS (o-chlorobenzylidene malononitrile) and CR (dibenz[b,f]-2,4-oxazepine) have both been standardized for military use and categorized as riot control agents. Since the main effects of these two irritant materials is to produce instant pain and potential dermal irritation, animals were anesthetized during treatment.

Phase I was designed to determine 1) a dose level of CR/CS that would produce a reliable/repeatable medium-to-severe erythema/edema response; 2) the shortest exposure time that would produce a consistent irritant response; 3) test agent application and decontamination procedures; and 4) an effective analgesic/anesthetic regimen. In this phase, animals were exposed for varying amounts of time to both solid and liquid preparations of CS and CR. Exposure times consisted of 15, 30, 60 and 120 minutes. Removal of the solid agents was done using either masking tape or hypoallergenic surgical tape. Removal of liquid agents and TSP was accomplished using foam covered swabs.

Animals were exposed to both liquid and solid forms of CS and CR using ——— ——— Liquid solutions of CR and CS were prepared in an 80% propylene glycol/20% sterile water solution and trioctylphosphate (TOF), respectively. Solids or solutions were placed on a sorbent pad within occlusive ——— exposure chambers and chambers were secured to the animals with occlusive dressings. Animals were treated with a systemic analgesic/anesthetic preparation prior to dosing and secured to stainless steel tie down boards. In addition, buprenorphine hydrochloride was administered as needed to control the pain both during and after CS/CR treatments.

In Phase II, four pairs of test sites were located along the dorsal midline as follows:

Untreated Site	/	CS/CR Control Site
———	/	CS/CR + ———
ICD 2289	/	CS/CR + ICD 2289
———	/	CS/CR + ———

TSP was applied to clipped skin at a thickness of 0.15 mm. After allowing the TSP to dry for 15 minutes, ——— Chambers were loaded with CWA and secured over the treatment sites. After the 15-minute exposure period, the chamber was removed and the site decontaminated, removing both the CWA and TSP. Sites were scored at 15, 30, 60, 120 and 240 minutes and at 24 hours.

Species/Strain: Outbred New Zealand White Rabbits

#/group or time point: Phase I - 13 females; Phase II - 2 replicates of 10 females

Weight: 2.3-2.7 kg

Supplier: ———

Drug Lot #, Radiolabel, and % Purity: ICD 2289 Lot 305 —794 (51.4% PFPE:48.6% PTFE)

Observations and Times: Following the removal of test substances, initial dermal irritation evaluation was made at 15 minutes, followed with additional readings at 0.5, 1, 2, 4 and 24 hours. Sites were scored from 0-4 for erythema and eschar, and edema. For the purposes of this test, any irritation observed at any of the observation times was considered a positive breakthrough.

To assess differences between responses to 1.0% CS in Phase II, a repeated measure analysis was used. To assess differences in each of the three TSP formulations, a Friedman's block/treatment test was used at each time point observed.

Results: In Phase I, exposures to 0.2 ml of liquid 0.1 % CR or 1.0% CS demonstrated only minimal signs of dermal irritation up to 4 hours and no signs of persisting irritation at 24 hours. Likewise, concentrations of solid CR up to 40 mg/site for 2 hours produced no consistent, reproducible signs of dermal irritation. Persistent dermal irritation was achieved following exposures to 0.05, 0.1, 0.25 and

0.2 ml of 1.0% CS solution for 15 minutes and 40 mg solid CS for 2 hours. Based on these results, the only exposure proposed for TSP evaluation in Phase II was for 15 minute applications of 0.1 ml of 1.0% CS/TOF. Methods used for test agent application and decontamination procedures, and the analgesic/anesthetic regimen appeared adequate.

Results of the combined data from duplicate Phase II experiments consisting of 15 minute applications of 0.1 ml of 1.0% CS/TOF with and without TSP are presented in Table 3.16.1. Signs of erythema were very mild, and were accompanied by signs of edema only at the 24 hour observation point. In the second set of 10 animals, the first signs of erythema did not occur until 1 hour after administration of CS/TOF on the ICD 2289 pretreated site. Furthermore, at 24 hours, test sites protected by all three TSPs demonstrated significantly milder reactions (barely perceptible) than those at the unprotected sites.

Table 3.16.1: Number of animals (n=20) demonstrating erythema (with edema) following 15 minute challenges with 1.0% CS/TOF.

Treatment	15 min	30 min	1 hr	2 hr	4 hr	24 hr
Unprotected Sites	9	15	17	17	20	20 (16)
——— Pretreated Sites	0	1	1	1	10	12 (3)
ICD 2289 Pretreated Sites	1	1	3	3	6	9 (1)
——— Pretreated Sites	2	1	2	1	5	8

According to Friedman's block/treatment test, the median erythema score and area were significantly greater ($p<0.05$) for the control site than for ——— , IDC-2289 and ——— at all times measured; and the median edema score was significantly greater ($p<0.05$) for the control site than for ——— , IDC-2289 and ——— at 24 hours. In addition to ameliorating the effect of CS, all ——— TSPs were shown to delay the onset of dermal reactions.

3. Effect of Pesticides and Sun Screen on TSP

*Study 3.17** - **Letter Report Number 5:** ***Effect of Insect Repellent and Camouflage Paints on TSP Protection Against HD.***

Task No: Task 93-34

Volume and Page: Vol. 2.18, page 123 [Ref. 5.4.33]

Conducting Laboratory and Location: Medical Research Evaluation Facility, Battelle, 505 King Ave., Columbus, OH [Contract DAMD 17-89-C-9050]

Date of Study Initiation: August 24, 1995

GLP Compliance: No

QA Report: Yes ( ) No (x)

Methods: Per *in vivo* Protocol MREF 107: ICD 2289 was applied on rabbits with or without subsequent application of either insect repellent or one of four paints from two camouflage kits,

followed by a topical 4 hour HD challenge. Compatibility was evaluated in terms of TSP efficacy against HD when a test material was present relative to an unprotected test site. Although the presence of the test material may decrease the efficacy of the TSP, it was regarded as compatible with the TSP as long as the difference in lesions compared to unprotected sites remained significant. The endpoint reported was the lesion area ratio (LAR) at approximately 24 hours following HD exposure. Each animal served as its own control with 1 TSP unprotected site and 1 ——— control protected site. The six remaining test sites were pretreated with TSP and three of these sites were further treated with either 50 µl insect repellant or 1 ml camouflage paint. One hour after test agent applications, a 1 µl topical challenge dose of HD was applied at each of the test sites.

Species/Strain: New Zealand White Rabbits

#/group or time point: 8 animals/group, eight sites/animal

Age, sex, weight: 2-4 kg

Drug Lot #, Radiolabel, and % Purity: Test materials were supplied by the US Army Medical Material Command and identified as insect repellent lot no. IXDM; CP215-95 camouflage paint in a stick applicator (NSN 685-00161-6204, MIL P-2018F) and CP216-95 camouflage paint in a compact (NSN 685-001262-0635, MILP-2018H).

Observations and Times: Following a 4 hour exposure period, all test sites were decontaminated and lesion scores calculated at 24 hours.

Results (Table 3.17.1): There were notable decreases observed in the protective effects of ICD 2289 to 1 µl HD challenge when combined with applications of insect repellent (LAR increased by 400%), loam camouflage paint (LAR increased by 300 %) and sand camouflage paint (LAR increased by 39%). There were no significant effects observed on ICD 2289 efficacy with bright green or white camouflage paints.

Table 3.17.1: LARs following application ICD 2289 alone or in the presence of army issue repellent and camouflage paints and challenged with t 1 µl HD.

Pretreatment (n=8)	**LAR (%)***				
ICD 2289	16 ± 10	10 ± 4	24 ± 13	23 ± 4	20 ± 11
Test Agent	Repellent	Loam	Bright Green	Sand	White
+ ICD 2289	71 ± 26	30 ± 16	25 ± 8	32 ± 7	21 ± 8

* arithmetic mean ± sem

Although there were moderate reductions in the protective effects of ICD 2289 when used in the presence of loam and sand camouflage paints, there were still meaningful decreases in lesion sizes when compared to unprotected sites. HD-induced lesions were reduced by approximately 70 to 90% in the presence of ICD 2289 alone and when combined with bright green and white camouflage paints, and by greater than 50% when combined with loam and sand camouflage paints. Further testing should be done to evaluate the compatibility of ICD 2289 use with insect repellent which appeared to completely eliminate the protective effects of ICD 2289 in some animals. As a follow-up study, it was suggested that the application of insect repellent prior to application of TSP be investigated for similar effects. (See Study 3.18)