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HEALTH HAZARD EVALUATION OF LIQUID MONOPROPELLANTS. PHASE 2. EF--ETC(U)
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UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010

HEALTH HAZARD EVALUATION OF LIQUID MONOPROPELLANTS
SPECIAL STUDY NO. 75-51-0132-82
PHASE 2
EFFECTS OF DERMAL ADMINISTRATION OF
HYDROXYLAMMONIUM NITRATE
MAY 1980 - MARCH 1982

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Liquid Monopropellants	Hydroxylammonium nitrate	Dermal	Hydroxylamine
Liquid Gun Propellants	Nitrate	Rabbits	Destruction
LGP	HAN	dermatitis	Blood
LGP 1776	Acute	skin	Heinz bodies
LGP 1845	Subchronic	erythrocyte	Hemoglobin
	Inclusion bodies		Red blood cell

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Skin application of hydroxylammonium nitrate (HAN) to rabbits for 3 weeks induced a high incidence of chronic and ulcerative dermatitis in all treatment groups including the lowest dose tested, i.e., 0.7 mg/kg. Higher doses, 1.5 to 11.7 mg/kg, caused Heinz body formation and red blood cell destruction. It is recommended that extreme caution be taken to prevent HAN from coming into contact with the skin. Personal protection, to include rubber gloves and chemical splash goggles, should be worn when working with this material.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Asaki/ldr/AUTOVON
584-3627

REPLY TO ATTENTION OF
HSHB-LT/WP

16 AUG 1982

SUBJECT: Health Hazard Evaluation of Liquid Monopropellants Special Study No. 75-51-0132-82, Phase 2, Effects of Dermal Administration of Hydroxylammonium Nitrate, May 1980 - March 1982

Director
US Army Ballistics Research Laboratory
ATTN: DRDAR-BLP
Aberdeen Proving Ground, MD 21005

EXECUTIVE SUMMARY

The purpose, essential findings and recommendations of the inclosed report follow:

a. Purpose. This study was conducted to determine the toxicity of hydroxylammonium nitrate (HAN) following repeated dermal exposure to various dosages of the technical grade compound. This evaluation will assist in advising on the health hazards associated with handling this material.

b. Essential findings. Skin application of HAN to rabbits induced a high incidence of chronic and ulcerative dermatitis in all treatment groups including the lowest dose tested, i.e., 0.7 mg/kg. Higher doses, 1.5 to 11.7 mg/kg, caused red blood cell destruction and Heinz body formation.

c. Major recommendations. It is recommended that extreme caution be taken to prevent HAN from coming in contact with the skin. A standing operating procedure (SOP) should be written concerning safe use of this substance. This SOP should require that personal protection, to include rubber gloves and chemical splash goggles, be worn when working with this material. This information involving a potential hazard should be disseminated to the workers as part of their health education program.

FOR THE COMMANDER:

JOHN F. MAZUR
LTC, MSC
Director, Laboratory Services

1 Incl
as (5 cy)

CF:
HQDA (DASG-PSP) wo incl
Cdr, DARCOM (DRCSG)
Cdr, ARRADCOM
Cdr, HSC (HSPA-P)
Comdt, AHS (HSHA-IPM)
Cdr, WRAMC (PVNTMED Actv)
Cdr, MEDDAC, Ft Meade (PVNTMED Actv) (2 cy)

Sp Study No. 75-51-0132-82, May 80-Mar 82

CONTENTS

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

REPLY TO ATTENTION OF
HSHB-LT/WP

HEALTH HAZARD EVALUATION OF LIQUID MONOPROPELLANTS
SPECIAL STUDY NO. 75-51-0132-82
PHASE 2
EFFECTS OF DERMAL ADMINISTRATION OF
HYDROXYLAMMONIUM NITRATE
MAY 1980 - MARCH 1982

1. AUTHORITY. Letter, DRDAR-BLP, US Army Ballistics Research Laboratory, 21 August 1978, subject: Request for Toxicity Tests on a Liquid Monopropellant, with indorsement thereto.

2. REFERENCES. See Appendix A for a listing of references.

3. PURPOSE. The purpose of this study was to determine the toxicity of hydroxylammonium nitrate (HAN) following repeated dermal exposure to various dosages of the technical grade compound. This evaluation will assist in advising on the health hazards associated with handling this material.

4. GENERAL.

a. Hydroxylammonium nitrate is a constituent of substances being considered for use as liquid gun propellants (LGP) for the US Army. It is projected that the LGP's will serve the same function as the present powdered charge in propelling projectiles. Leakage of propellant materials from storage or reservoirs during loading, transportation, or transfer poses a potential occupational hazard from dermal exposure.

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* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health Education and Welfare Publication No. (NIH) 78-23, revised 1978.
† The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care. This report and data generated in this study are stored in Toxicology's file located in Room 3011, Building E2100, APG-EA 21010.

b. Previous studies performed by the Naval Medical Research Institute (reference 1, Appendix A) and the Navy Toxicology Unit (references 2 and 3, Appendix A) showed that similar compounds were moderately toxic to animals. Published reports (references 4 and 5, Appendix A) and studies currently being conducted by this Agency show that HAN is moderately toxic to rats and very toxic to rabbits. The major toxic signs were cyanosis, respiratory distress, and convulsions. It was caustic when applied and worked onto the shaved backs of rabbits. The LD_{50}'s‡ of HAN in rats and rabbits by various routes were as follows:

Oral Rat LD_{50} 882 mg/kg Slope = 9.1, SE of Slope = 2.56
Oral Rabbit ALD§ 100 mg/kg
Dermal Rabbit LD_{50} 70 mg/kg Slope = 4.46, SE of Slope = 1.45
(unoccluded)

5. MATERIALS AND METHODS.

a. Hydroxylammonium nitrate was received as a 13.24 molar aqueous solution containing approximately 80 percent HAN by weight. The chemical formula ($NH_3OH\ NO_3$) has a molecular weight of 96 (see Figure for structure) and density of 1560 mg/mL. It is a clear, odorless, and somewhat viscous liquid extremely soluble in water. It is unstable in the presence of heat, sulfuric acid, concentrated nitric acid, phosphorous pentoxide, and prolonged contact with iron, nickel, copper, other transition metals, and rust. Oxides of nitrogen are formed upon decomposition. The test material was received from Ballistics Research Laboratory (BRL), Aberdeen Proving Ground, MD with lot identification from the Naval Ordinance Station, Indian Head, MD, of NOSIH Batch R149/151.

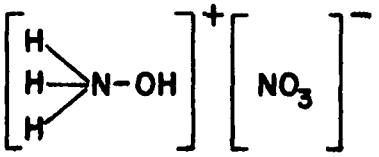

FIGURE. Hydroxylammonium Nitrate

‡ Acute lethal dose.
§ Approximate lethal dose.

b. Male New Zealand White rabbits were purchased from Dutchland Laboratories, Inc., Denver, PA, and ranged in weight from 2.2-2.7 kg and were identified by ear numbering with magic marker. All were housed individually in stainless steel cages with food and water available ad libitum (reference 7, Appendix A).

c. Rabbits were divided into six test groups of five animals each. The doses applied were fractions of the acute dermal unoccluded LD_{50}. The test solution was applied with a microsyringe onto the clipped mid-lumbar region of the rabbit 5 days a week for 3 weeks. Controls were treated with water. Following each daily topical application, the area was covered by a nonocclusive patch to prevent the animal from licking the area of application. Rabbits were weighed at the beginning of each week and doses for that week were calculated from this weight. On the weekly shaving day the compound was applied 4 hours after shaving. Dosages used in this study are fractions of the dermal LD_{50} (70 mg/kg).

d. Serum clinical chemistries were performed on blood from rabbits using the Abbott Bichromatic Analyzer 100 (ABA-100)™, Abbott Diagnostics, South Pasadena, CA. The Instrumentation Laboratory Flame Photometer Model 143, Instrumentation Laboratory Inc., Lexington, MA, was used in analyzing for sodium and potassium. Hematological parameters, except Heinz bodies and differential white blood cell count (WBC), were measured on a Coulter Counter Model 010ZBI and Hemoglobinometer, Coulter Electronics, Inc., Hialeah, FL (see reference 12, Appendix A). Stains for Heinz bodies and differential WBC were done according to Toxicology Division SOP (references 13 and 14, Appendix A). Blood samples were taken each week during a 4-week pretreatment period and at 1, 8, 15, and 21 days after the beginning of dosing. On day +4, blood samples were taken for Heinz bodies only.

e. Clinical chemistry parameters measured were:

serum glutamic oxaloacetic transamimase (SGOT)
serum glutamic pyruvic transaminase (SGPT)
blood urea nitrogen (BUN)
total protein
bilirubin
alkaline phosphatase
total lactic dehydrogenase (LDH)
alpha hydroxybutyric dehydrogenase (HBDH)
glucose
creatine phosphokinase (CPK)
calcium
cholesterol
triglycerides
sodium
potassium

Hematological parameters measured were:

red blood cell count (RBC)
hematocrit
mean corpuscular volume (MCV)
WBC
hemoglobin
Heinz bodies
differential WBC

f. Each rabbit was necropsied at the end of the study and selected organs and tissues were prepared for routine histopathologic examination and evaluated by Findley Research, Inc., P.O. Box 375, Assonet, MA, 4 December 1980, under Contract No. DAAD05-80-D-0481. The organ-to-body weight ratios were calculated for the kidneys, liver, spleen, testes, and heart from each group.

6. FINDINGS. Results from each compound test group were compared with the control group on the same day. Any differences from controls were reported at a given dose level only if all higher dose groups were also different from controls. Significance is reported at the 0.05 level. The Mann-Whitney U-test was used for all comparisons. The dosages and the major effects produced after 21 days at each level are shown in the Table.

TABLE. COMPARISON OF TOXIC RESPONSES IN SUBCHRONIC DERMAL STUDIES

Dosage mg/kg	Necrosis of skin	RBC Destruction	Heinz bodies	Enlarged spleen Splenic hemato-poiesis	Hepatic hemato-poiesis	Enlarged heart
11.7 (1/6 LD_{50})	X	X	X	X	X	X
5.9 (1/12 LD_{50})	X	X	X	X	X	
2.9 (1/24 LD_{50}	X	X	X	X		
1.5 (1/48 LD_{50})	X	X	X			
0.7 (1/96 LD_{50})	X					

X - positive reaction

a. The time of appearance of skin erythema and edema responses was dose related, with the response at the highest dose appearing after the first application. Three applications produced well developed erythema and edema skin reactions in all test groups. Eschar and necrosis appeared after five applications (see Table B-1, Appendix B, for irritation scores and definitions).

b. Body weights of rabbits in the high dose group (11.7 mg/kg) were significantly lower than controls after 1 week of dosing and remained reduced throughout the study. Some decrease in food consumption also occurred during the middle period of dosing (see Tables B-2 and B-3, Appendix B).

c. Blood samples taken 24 hours after application of the highest dose contained Heinz bodies in the red blood cells. One week after dosing, Heinz bodies were present in the next three higher groups but never appeared in the lowest dose group. All groups except the lowest (0.7 mg/kg) exhibited anemia at the 2- and 3-week bleeding periods, with the RBC's, hemoglobin concentration, and hematocrit lower than control and the MCV's higher (Tables B-4 to B-9, Appendix B). No clinical chemistry changes were detected in any of the test groups.

d. At necropsy, the spleen organ-to-body weight ratios were larger than controls in the three highest dose groups (2.9 - 11.7 mg/kg) and the heart organ-to-body weight ratio higher only in the highest dose group (see Table B-10, Appendix B).

e. The following findings were extracted from Findley Research, Inc., Histopathological Report (reference 16, Appendix A):

> *Three weeks of daily dermal application of HAN, 5 day/week, resulted in a high incidence of dermatitis in all treatment groups. No skin lesions were noted in controls. The diagnosis of dermatitis encompassed two types of inflammatory response, chronic dermatitis and ulcerative dermatitis. Ulcerative dermatitis occurred in greater incidence and in greater severity in the highest dose group [11.7 mg/kg/day] than in the other treatment groups. Chronic dermatitis did not show a definite dose response among the treated groups.*
>
> *Chronic dermatitis was characterized by acanthosis, hyperkeratosis, and dermal infiltrates of predominantly mononuclear cells with a few heterophils. Infiltrates varied from focal to diffuse and extended with varying distributions from the epidermis to the underlying musculature. Ulcerative dermatitis was a much more severe lesion. There was marked necrosis and loss of epidermis with intense acute and chronic inflammatory infiltrates extending from the base of the epithelial necrosis into the dermis. Often there was superficial crusting composed of necrotic cellular debris and keratin.*
>
> *Splenic hematopoiesis occurred in high incidence and comparable degree in the three higher dose groups but not in the two lowest groups or the controls. Hematopoiesis of the liver also occurred in the two highest dose groups but not in the remaining experimental groups or controls.*

f. All other lesions were felt to be spontaneous in nature and not due to administration of the test compound. The necropsy report is summarized in Table B-11, Appendix B.

7. DISCUSSION. Subchronic dermal application of HAN to shaved skin of male rabbits induced blood cell changes, chronic and ulcerated dermatitis, and splenic and hepatic hematopoiesis.

a. Red blood cell destruction was evident in several blood parameters. The RBC and the hematocrit decrease were a direct measure of the decrease in the number of erythrocytes and the percent volume they occupy collectively. The concentration of hemoglobin in the blood dropped as the hemoglobin-carrying red blood cells were destroyed. The MCV increased probably because the larger, immature red blood cells increased in the circulation to replace the smaller, mature red blood cells that were destroyed by the test compound.

b. Heinz bodies are particles of denatured protein comprised primarily of hemoglobin. They appear as dark spots in the red blood cell. Their presence is an indication of the oxidative denaturing of hemoglobin in the red blood cell by a toxic agent. When hemoglobin is oxidized to form Heinz bodies, there is an increase in the internal viscosity of the erythrocyte. Such an increase can affect the red blood cell's shape and, therefore, its ability to deform and pass through small openings such as capillaries or epithelial slits in the splenic sinuses. Heinz bodies, once formed, become attached to the interior of the cellular membrane. The binding of the Heinz body to the membrane changes the membrane's permeability and leads to a loss of water and to other changes that again decrease the ability to deform (see reference 10, Appendix A).

c. Red blood cells with Heinz bodies or the rigid Heinz bodies themselves cannot traverse the epithelial slits of the splenic sinuses. They are left behind in the perisinusoidal red pulp for phagocytosis by macrophages. Damaged red blood cells awaiting phagocytosis cause a backup or pooling of blood in the spleen. Minor damage to red blood cells sufficient to be detected by the phagocytizing macrophages can be caused by compression and congestion (see reference 11, Appendix A). The extent or impact of this extra damage is uncertain. The color of the spleens ranged from dark red to black in a dose-related response due to the accumulation of dark blood and the recycling of hemoglobin. Histopathologic examination of spleens indicated they were undergoing hematopoiesis, probably an attempt to replace damaged erythrocytes.

d. Both the liver and the spleen screen out imperfect erythrocytes. The liver screens out only grossly abnormal red blood cells while the spleen can detect minor imperfections. In humans, the liver receives 35 percent or more of cardiac output compared to less than 5 percent for the spleen (see reference 10, Appendix A). Visual examination of erythrocytes revealed gross damage. However, the livers appeared normal at gross necropsy and were not enlarged in any of the studies as might be expected. Histology revealed hematopoiesis in the liver to replace damaged erythrocytes.

e. The decrease in the number of erythrocytes can be expected to cause a decrease in the oxygen carrying capacity of the blood. The lack of oxygen in the blood causes an increase in the cardiac rate and stroke volume resulting in an increased workload and subsequent heart enlargement. This was borne out at necropsy by the fact that the hearts were enlarged.

f. Difficulties arise when extrapolating rabbit data to man (see reference 9, Appendix A). There are significant biochemical and physiological differences in the skin of the two species and the occupational human exposure may differ from the test conditions. For most compounds, rabbit skin is more permeable than human skin (see reference 8, Appendix A). In the present study, HAN was worked into the skin and not washed off as would presumably be the case in a human exposure, although in the field the availability of water and the opportunity to wash immediately is less certain.

8. RECOMMENDATIONS. The following paragraphs are based on good industrial hygiene practice. It is recommended that:

a. Extreme caution should be taken to prevent HAN from coming into contact with the skin. In the event of skin contamination, flush immediately with large volumes of water. Abrasive soap may increase absorption through the skin.

b. Protective clothing should be worn by workers when contact is possible and splash guards should prevent splashing onto people or onto equipment that people handle. Work clothing should be changed if it becomes contaminated with HAN.

c. There should be no smoking, drinking, or eating in the work area and workers should wash their hands before eating in the lunchroom. Workers should shower after work and change to street clothes.

d. An SOP should be written concerning safe use of this substance. This SOP should require that personal protection, to include rubber gloves and chemical splash goggles, be worn when working with this material. This information involving a potential hazard should be disseminated to the workers as part of their health education program.

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APPROVED:

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APPENDIX A

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APPENDIX B

SUMMARY OF DATA

TABLE B-1. SKIN REACTIONS* FOLLOWING DAILY SUBCHRONIC DERMAL APPLICATION OF HAN

		Erythema								Edema							
	Rabbit	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	148	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	149	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	154	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	159	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.73	129	0	0	2	3	3	4	4	4	0	0	2	2	2	4	4	4
mg/kg	131	0	0	0	1	2	4	4	4	0	0	0	0	0	4	4	4
	138	0	2	2	3	2	4	3	3	0	1	1	3	3	4	3	3
	158	0	0	4	4	4	4	4	4	0	1	2	2	3	4	4	4
	160	0	0	0	2	2	3	4	4	0	0	0	2	1	4	4	4
1.47	135	0	1	4	4	4	4	4	4	0	1	3	3	3	4	4	4
mg/kg	137	0	1	2	2	4	4	4	4	0	1	1	2	2	4	4	4
	139	0	1	1	2	4	4	4	4	0	0	1	3	3	4	4	4
	142	0	1	4	4	4	4	4	4	0	1	2	3	3	4	4	4
	153	0	1	2	3	3	4	4	4	0	0	2	3	3	4	4	4
2.93	128	0	2	2	2	3	4	4	4	0	1	2	3	3	4	4	4
mg/kg	133	0	4	4	4	4	4	4	4	0	1	3	3	3	4	4	4
	145	0	2	4	4	4	4	4	4	0	0	3	3	4	4	4	4
	147	0	1	2	3	2	4	4	4	0	0	1	2	1	4	4	4
	156	0	0	2	2	3	4	4	4	0	0	1	3	2	4	4	4
5.85	127	0	2	4	4	4	4	4	4	0	0	1	2	2	4	4	4
mg/kg	136	0	2	2	3	3	4	4	4	0	1	3	2	2	4	4	4
	143	0	0	2	2	2	4	4	4	0	0	0	2	2	4	4	4
	152	2	2	2	3	3	4	4	4	0	1	2	2	3	4	4	4
	162	0	2	2	3	3	4	4	4	0	0	1	3	3	4	4	4
11.7	130	0	1	2	2	2	4	4	4	0	1	0	2	3	4	4	4
mg/kg	132	0	4	4	4	4	4	4	4	0	1	3	3	3	4	4	4
	134	0	1	2	3	3	4	4	4	0	0	3	3	3	4	4	4
	140	0	4	1	2	4	4	4	4	0	1	2	2	3	4	4	4
	144	0	2	2	3	2	4	4	4	0	2	2	3	3	4	4	4

* Reaction scored according to Draize (ref 6, Appendix A).

Erythema:
0 - No Erythema
1 - Very slight Erythema
2 - Well defined Erythema
3 - Moderate to severe Erythema
4 - Severe Erythema (Beet redness to slight Eschar formation injurious in depth)

Edema:
0 - No Edema
1 - Very slight Edema (barely perceptible)
2 - Slight Edema (edges of area well defined by raising)
3 - Moderate Edema (edges raised approximately 1 mm)
4 - Severe Edema (raised more than 1 mm and extending beyond area of exposure)

TABLE B-2. BODY WEIGHTS (kg) SUBCHRONIC DERMAL

	Week -4	Week -3	Week -2	Week -1	Day +0	Week +1	Week +2	Week +3
Control	2.51 ±0.17	2.69 ±0.12	2.85 ±0.97	3.05 ±0.89	3.19 ±0.26	3.26 ±0.81	3.37 ±0.95	3.34 ±0.51
0.73 mg/kg	2.52 ±0.12	2.68 ±0.13	2.84 ±0.11	3.07 ±0.16	3.18 ±0.12	3.16 ±0.13	3.28 ±0.13	3.26 ±0.68
1.47 mg/kg	2.54 ±0.11	2.68 ±0.17	2.77 ±0.26	2.99 ±0.21	3.10 ±0.18	3.12 ±0.23	3.24 ±0.22	3.22 ±0.22
2.93 mg/kg	2.47 ±0.14	2.86 ±0.31	2.83 ±0.20	3.01 ±0.24	3.07 ±0.26	3.09 ±0.32	3.18 ±0.32	3.15 ±0.32
5.85 mg/kg	2.54 ±0.69	2.69 ±0.12	2.81 ±0.11	3.04 ±0.16	3.12 ±0.17	3.11 ±0.20	3.17 ±0.26	3.19 ±0.26
11.7 mg/kg	2.53 ±0.10	2.67 ±0.11	2.79 ±0.15	2.98 ±0.12	3.07 ±0.11	2.87* ±0.17	3.00* ±0.20	2.99* ±0.23

* Significantly different than controls. Mann-Whitney U-Test. P = 0.05

TABLE B-3. FOOD CONSUMPTION SUBCHRONIC DERMAL

	Day +2	Day +4	Day +9	Day +11	Day +16	Day +18
Control	47 ±5.5	50 ± 3.3	49 ±4.9	54 ± 6.2	51 ±6.3	52 ±9.4
0.73 mg/kg	43 ±7.6	38 ±13	48 ±8.2	46 ± 6.5	48 ±6.4	48 ±4.9
1.47 mg/kg	50 ±5.8	43 ± 9.7	50 ±9.8	55 ±13.5	48 ±5.6	50 ±7.0
2.93 mg/kg	47 ±7.0	42 ± 9.6	48 ±6.5	47 ± 6.3	46 ±6.4	48 ±3.7
5.85 mg/kg	40 ±8.0	41 ± 7	42 ±4.7	49 ± 9.2	46 ±12	51 ±8.6
11.7 mg/kg	41 ±7.1	33 ± 8.6	36* ±7.5	40* ± 4.3	43 ±7.4	50 ±6.6

* Significantly different from controls. Mann-Whitney U-Test. P = 0.05

TABLE B-4. MEAN BLOOD VALUES - RBC ($10^6/mm^3$) SUBCHRONIC DERMAL

Treatment Group	6 May 80 Week - 4	28 May 80 Week - 1	8 Jun 80 Day +1	10 Jun 80 Day +8	17 Jun 80 Day +15	7 Apr 80 Day +21
Control	5.29 ±0.87	5.55 ±0.38	5.40 ±0.38	5.68 ±0.18	5.68 ±0.38	5.75 ±0.11
0.73 mg/kg	5.99 ±0.23	5.45 ±0.17	5.48 ±0.23	5.54 ±0.22	5.31 ±0.30	5.82 ±0.26
1.47 mg/kg	5.80 ±0.52	5.51 ±0.40	5.30 ±0.47	4.91* ±0.29	4.96 ±0.80	5.02 ±0.66
2.93 mg/kg	5.59 ±0.34	5.34 ±0.29	5.24 ±0.44	4.36* ±0.68	4.60* ±0.71	4.67* ±0.34
5.85 mg/kg	5.65 ±0.84	5.51 ±0.52	5.60 ±0.49	3.63* ±0.56	3.81* ±0.27	4.24* ±0.63
11.7 mg/kg	5.90 ±0.24	5.18 ±0.63	5.05 ±0.85	2.80* ±0.36	3.46* ±0.34	3.30* ±0.61

* Significantly different than controls. Mann-Whitney U-Test. P = 0.05

TABLE B-5. MEAN BLOOD VALUES - HEMOGLOBIN (g/dl) SUBCHRONIC DERMAL

Treatment Group	6 May 80 Week - 4	28 May 80 Week - 1	3 Jun 80 Day +1	10 Jun 80 Day +8	17 Jun 80 Day +15	7 Apr 80 Day +21
Control	11.94 ±1.58	12.62 ±0.66	12.56 ±0.85	13.03 ±0.55	12.95 ±0.31	12.96 ±0.36
0.73 mg/kg	13.32 ±0.45	12.76 ±0.46	12.98 ±0.37	12.97 ±0.30	12.55 ±0.72	13.40 ±0.83
1.47 mg/kg	12.92 ±0.92	13.18 ±0.64	12.64 ±0.55	12.04 ±0.97	12.24 ±1.25	12.02 ±0.93
2.93 mg/kg	12.44 ±0.65	12.72 ±0.66	12.52 ±0.82	10.80 ±1.58	11.10 ±1.03	11.24* ±0.74
5.85 mg/kg	12.60 ±1.94	13.76 ±1.18	12.80 ±1.21	9.28* ±0.53	10.10* ±1.31	10.06* ±0.86
11.7 mg/kg	13.50 ±0.95	12.32 ±1.66	12.06 ±1.66	8.03* ±0.80	9.80* ±0.81	9.78* ±1.05

* Significantly different than controls. Mann-Whitney U-Test. P = 0.05

TABLE B-6. MEAN BLOOD VALVES - HEMATOCRIT (%) SUBCHRONIC DERMAL

Treatment Group	6 May 80 Week - 4	28 May 80 Week - 1	3 Jun 80 Day +1	10 Jun 80 Day +8	17 Jun 80 Day +15	7 Apr 80 Day +21
Control	33.74 ±3.89	35.94 ±2.10	34.68 ±2.43	37.90 ±1.15	36.65 ±1.40	36.46 ±0.82
0.73 mg/kg	38.10 ±1.23	36.40 ±0.98	36.38 ±0.86	36.68 ±0.26	35.42 ±1.98	38.26 ±2.15
1.47 mg/kg	37.28 ±2.25	37.42 ±2.20	35.94 ±2.03	34.26 ±1.64	33.66 ±4.02	35.84 ±2.92
2.93 mg/kg	34.88 ±1.52	35.96 ±2.39	34.88 ±2.83	31.02 ±4.11	32.84 ±3.58	33.58* ±1.98
5.85 mg/kg	35.12 ±5.17	36.14 ±3.66	36.84 ±3.31	27.66* ±1.11	31.30 ±5.22	30.52* ±2.25
11.7 mg/kg	36.78 ±1.23	35.14 ±4.87	34.28 ±4.97	24.22* ±1.43	30.50* ±2.20	29.26* ±3.40

* Significantly different than controls. Mann-Whitney U-Test. P = 0.05

TABLE B-7. MEAN BLOOD VALUES - MCV (u^3) SUBCHRONIC DERMAL

Treatment Group	6 May 80 Week - 4	28 May 80 Week - 1	3 Jun 80 Day +1	10 Jun 80 Day +8	17 Jun 80 Day +15	7 Apr 80 Day +21
Control	63.00 ±4.64	64.40 ±2.07	64.00 ±2.00	66.33 ±4.04	64.25 ±1.89	63.20 ±1.79
0.73 mg/kg	62.20 ±2.28	66.40 ±2.88	66.00 ±2.34	65.75 ±2.50	66.50 ±2.08	65.40 ±1.52
1.47 mg/kg	63.40 ±4.28	69.40 ±6.73	67.80 ±3.70	69.40 ±2.88	70.20 ±4.32	71.60* ±5.36
2.93 mg/kg	61.20 ±1.79	66.80 ±1.48	66.20 ±0.84	81.00 ±2.74	71.60* ±3.51	71.60* ±3.78
5.85 mg/kg	61.00 ±3.16	65.20 ±2.86	66.00 ±3.46	77.20 ±12.01	81.25* ±8.22	75.60* ±2.25
11.7 mg/kg	61.00 ±2.45	67.40 ±1.82	67.80 ±3.90	108.00* ±17.83	88.00* ±7.62	89.20* ±6.30

* Significantly different than controls. Mann-Whitney U-Test. P = 0.05

TABLE B-8. MEAN BLOOD VALUES - WBC ($10^3/mm^3$) SUBCHRONIC DERMAL

Treatment Group	6 May 80 Week - 4	28 May 80 week -1	3 Jun 80 Day +1	10 Jun 80 Day +8	17 Jun 80 Day +15	7 Apr 80 Day +21
Control	6.60 ±2.02	6.06 ±1.64	6.68 ±2.28	8.00 ±1.15	8.20 ±2.25	6.82 ± 1.86
0.73 mg/kg	7.62 ±2.13	7.04 ±1.12	7.64 ±1.51	8.92 ±0.68	9.18 ±1.74	8.50 ± 1.28
1.47 mg/kg	9.14 ±2.09	8.74 ±2.49	8.04 ±1.32	11.14 ±2.13	11.24 ±1.25	10.24 ± 2.64
2.93 mg/kg	6.46 ±1.80	6.04 ±1.20	6.32 ±1.42	8.50 ±1.38	8.32 ±0.83	8.22 ± 0.92
5.85 mg/kg	7.88 ±1.40	7.26 ±1.34	7.12 ±1.23	24.50* ±15.28	11.43* ±3.17	12.74* ± 4.26
11.7 mg/kg	8.10 ±2.10	7.54 ±2.79	11.16 ±4.35	64.80* ±31.28	15.06* ±5.14	34.34* ±10.63

* Significantly different than controls. Mann-Whitney U-Test. P = 0.05

TABLE 8-9. MEAN BLOOD VALUES - HEINZ BODIES (% of RBC's containing Heinz Bodies) SUBCHRONIC DERMAL

Treatment Group	6 May 80 Week - 4	28 May 80 Week - 1	3 Jun 80 Day +1	6 Jun 80 Day +4	10 Jun 80 Day +8	17 Jun 80 Day +15	7 Apr 80 Day +21
Control	0 ±0	0 ±0	0.20 ±0.45	0.20 ±0.45	0 ±0	0 ±0	0 ±0
0.73 mg/kg	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0
1.47 mg/kg	0 ±0	0.20 ±0.45	0 ±0	0.40 ±0.89	1.00 ±2.24	1.20 ±2.17	4.00 ±8.94
2.93 mg/kg	0 ±0	0 ±0	0.20 ±0.45	6.00† ±8.94	1.20 ±2.17	0.20 ±0.45	1.20 ±2.17
5.85 mg/kg	0 ±0	0 ±0	1.00 ±2.24	17.00† 24.90	38.00* ±25.88	7.00* ±11.27	22.00* ±8.37
11.7 mg/kg	0 ±0	0 ±0	25.00† ±32.40	83.00* ±20.36	72.50* ±25.00	17.50* ±17.68	44.00* ±23.02

* Significantly different than controls. Mann-Whitney U-Test. P = 0.05
† Clinically significant but not statistically significant.

TABLE B-10. ORGAN-TO-BODY WEIGHT RATIOS (g/100g) SUBCHRONIC DERMAL

Dose Group	Spleen	Liver	Kidney	Heart
Control	0.054 ±0.051	2.92 ±0.57	0.58 ±0.06	0.19 ±0.01
0.73 mg/kg	0.055 ±0.30	2.76 ±0.33	0.57 ±0.04	0.20 ±0.03
1.47 mg/kg	0.062 ±0.013	3.15 ±0.27	0.61 ±0.07	0.20 ±0.02
2.93 mg/kg	0.084 ±0.015*	2.91 ±0.31	0.67 ±0.07	0.23 ±0.01*
5.85 mg/kg	0.126 ±0.024*	2.95 ±0.29	0.66 ±0.12	0.22 ±0.03
11.7 mg/kg	0.172 ±0.026*	2.91 ±0.25	0.66 ±0.12	0.29 ±0.05*

* Significantly different than controls. Mann-Whitney U-Test. P = 0.05

TABLE B-11. SUMMARY NECROPSY REPORT SUBCHRONIC DERMAL

Treatment	Rabbit Number	Gross Changes	Area Skin Necrosis	Spleen	Other
Control (H_2O)	148	none			
	149	none			
	154	none			
	155	none			
	159	none			
0.73 mg/kg	129		1x2 cm epidermis only		
	131		1x2 cm epidermis only		
	138		2x2 cm epidermis only		
	158		2x2 cm epidermis only	slightly enlarged	
	160		1x1 cm superficial		
1.47 mg/kg	135		1x2 cm to dermis base	moderately enlarged, dark	
	137		1x4 cm epidermis only	slightly enlarged	
	139		2x2 cm epidermis only	slightly enlarged, black	
	142		1x3 cm epidermis only		
	153		3x3x3 cm superficial	-	
2.93 mg/kg	128		2x2 cm to dermis base	enlarged, black,	
	133		1x3 cm epidermis only	dark	consolidation 1x1 cm rt cardiac lung lobe
	145		3 cm circular	enlarged slightly	
	147		2x2 cm epidermis only	moderately enlarged	2x2x3 caseous mass in rt inguinal area
	150		2x1 cm bus	slightly enlarged, dark	
5.85 mg/kg	127		1x5 cm bus	moderately enlarged, dark	
	136		2x5 cm bus	moderately enlarged, black	
	143		2x5 cm bus	moderately enlarged	
	152		2x5 cm bus	moderately enlarged, dark	
	152		2x5 cm bus	moderately enlarged, black	dark blood in pericardium
11.7 mg/kg	130		3x5 cm bus, hemorrhage	moderately enlarged, dark	
	132		3x3 cm bus, hemorrhage	mildly enlarged, black	
	134		3x5 cm bus	large, black	
	140		3x5 cm bus necrotic	moderately enlarged, black	
	144		2x3 cm bus	moderately enlarged, dark	

APPENDIX C

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Office certifies the following with regard to this study:

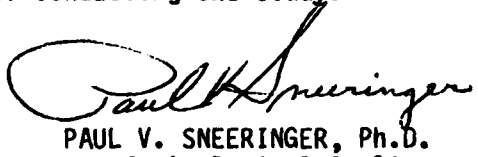
a. This study was conducted in accordance with:

(1) Standing Operating Procedures developed by the Toxicology Division, USAEHA.

(2) Title 21, Code of Federal Regulations, 1981 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratories Studies.

b. Facilities were inspected during its operational phase to insure compliance with paragraph a above.

c. The information presented in this report accurately reflects the raw data generated during the course of conducting the study.



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