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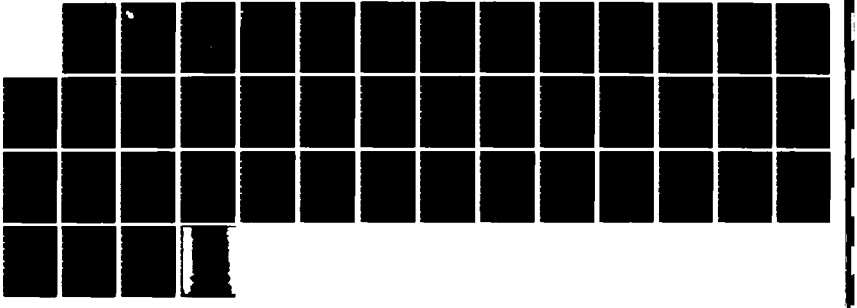

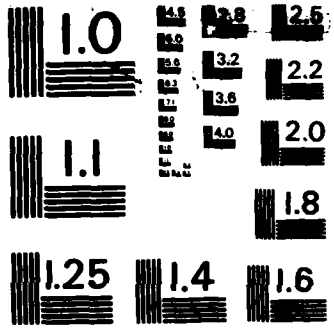

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INSTITUTE REPORT NO. 172

AD-A142 926

DERMAL SENSITIZATION POTENTIAL OF THE HOLSTON COMPOUNDS: VIRGIN DMSO, DMSO RECYCLE SOLVENT, AND DMSO EVAPORATOR SLUDGE

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TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

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JUNE 1984

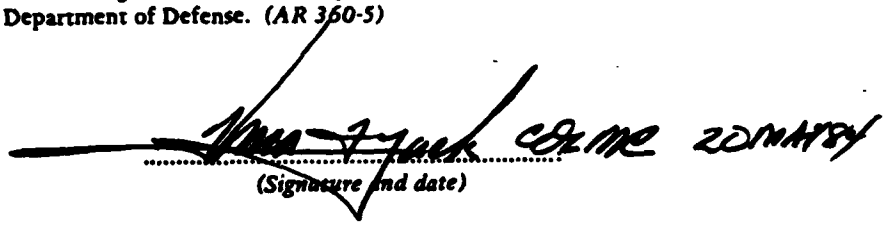
Toxicology Series 70

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Dermal Sensitization Potential of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge (Toxicology Series 70)--Lewis, Johnson, and Korte

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*



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

The Holston Compounds designated Virgin DMSO (TP014), DMSO Recycle Solvent (TP013) and DMSO Evaporator Sludge (TP015) were tested for dermal sensitization potential on guinea pigs. The study was conducted in compliance with the Good Laboratory Practice Regulations. The results from this study were not conclusive, but there was some evidence which suggests that the test compounds might be weak sensitizers. The test compounds caused mild irritation responses which were difficult to distinguish from weak sensitizing responses.

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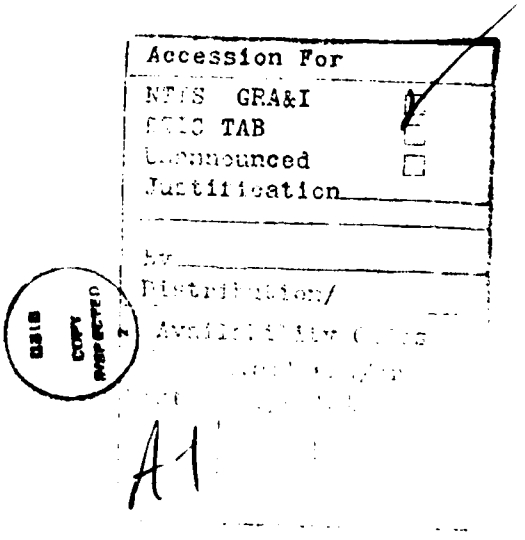
ABSTRACT

The Holston Compounds designated Virgin DMSO (TP014), DMSO Recycle Solvent (TP013), and DMSO Evaporator Sludge (TP015) were tested for dermal sensitization potential on guinea pigs. The study was conducted in compliance with the Good Laboratory Practice Regulations. The results from this study indicate that the test compounds are mild irritants (under conditions of the study) and provide little evidence of a sensitizing potential.

Key Words: Dermal Sensitization, DMSO, DMSO Recycle Solvent, DMSO Evaporator Sludge.



PREFACE

TYPE REPORT: Dermal Sensitization GLP Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Division of Research Support
Presidio of San Francisco, CA 94129

SPONSOR: U.S. Army Medical Research and Development Command
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, Frederick, MD 21701

PROJECT: DMSO Recrystallization Solution
TL01

GLP STUDY NO.: 83002

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: Carolyn M. Lewis, MS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compounds will be retained in the LAIR Archives.

TEST SUBSTANCE: The Holston Compounds (Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge).

INCLUSIVE STUDY DATES: 8 June - 22 July 1983

OBJECTIVE: The objective of the study was to evaluate the dermal sensitization potential of DMSO recrystallization solvents which are designated DMSO Recycle Solvent (TP013), Virgin DMSO (TP014), and DMSO Evaporator Sludge (TP015).

ACKNOWLEDGMENTS

The authors wish to thank SP5 Evelyn Zimmerman, Susan Hernandez, Mr. Michael Sands, and Mr. Richard Spieler for their assistance in the weighing, dosing, observing and caring for the animals. We also wish to thank CPT Martha Hanes, DVM, and SP5 Leonard Sauers, MS, for their initial testing of intradermal injections and patches with the test compounds. Finally, we wish to thank CPT James Carroll and Dr. Jack Dacre, US Army Bioengineering Research and Development Laboratory, for their assistance as Project Consultants.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 83002 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies, outlined by the Food and Drug Administration.

DON W. KORTE JR. / DATE
MAJ, MS
Study Director

CAROLYN M. LEWIS, MS / DATE
DAC
Principal Investigator

YVONNE C. JOHNSON, BS / DATE
DAC
Co-Author

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA

3 April 1984

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 83002 the following inspections were made:

15 Apr 83
10 Jun 83
23 Jun 83
29 Jun 83
1 Jul 83

The report and raw data for this study were audited on 22 Mar 84.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Jul and Oct 83 reports to management and the Study Director.

NELSON R. POWERS, Ph.D.
DAC
Chief, Quality Assurance Unit

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APPENDICES

Dermal Sensitization Potential of the Holston Compounds: Virgin DMSO, DMSO Recycle Solvent, and DMSO Evaporator Sludge--Lewis et al

The Holston Defense Corporation has proposed that dimethyl sulfoxide (DMSO) be used as the replacement recrystallization process solvent for the synthesis of the explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro- 1,3,5,7-tetrazine (HMX). As a result of this proposal, a pilot recrystallization facility was put into small scale operation. Samples of the DMSO process stream were taken from two locations at the recrystallization facility. The solutions collected were designated DMSO Recycle Solvent and DMSO Evaporator Sludge. The industrial grade DMSO, also sampled, was designated Virgin DMSO. The process stream samples were analyzed by the Holston Defense Corporation and were found to contain major and minor cyclic and non-cyclic nitramines. Since nitramines have been reported to be neurotoxic (1-4), their presence in the samples represented a potential health hazard to workers utilizing this production process. Thus, it became necessary to delineate the acute toxicity of the DMSO solutions so that a complete health hazard assessment can be obtained before determining the DMSO process solvent procedure should be put into full scale operation.

The Toxicology Group of Letterman Army Institute of Research (LAIR) was designated by the U.S. Army Medical Research and Development Command to perform a major part of the initial toxicity testing on the DMSO samples. The initial data will provide a base for further toxicity testing leading to definitive health protection criteria. These criteria will be used to evaluate facility design and worker protection equipment.

Objective of the Study

The objective of the study was to evaluate the dermal sensitization potential of DMSO recrystallization solvents which are designated DMSO Recycle Solvent (TP013), Virgin DMSO (TP014), and DMSO Evaporator Sludge (TP015).

MATERIALS

Test Substances

1. Chemical name: DMSO Recycle Solvent (TP013)

2. Chemical name: Virgin DMSO (TP014)

3. Chemical name: DMSO Evaporator Sludge (T015)

Identification of nitramine impurities in the test samples by high pressure liquid chromatography (HPLC) was performed by the Holston Defense Corporation. Results from these analyses appear in Appendix A. Other information regarding chemical/physical characteristics of the test compound including stability are on file with the sponsor.

Animal Data

Seventy male, young adult Hartley guinea pigs (Charles River Breeding Laboratories, Inc., Wilmington, MA) were used for the dermal sensitization test. Each guinea pig was ear tagged (as per LAIR SOP OP-ARG-1). The animals weighed between 300 and 370 g upon receipt and between 400 and 500 g by the first dosing.

Husbandry

The guinea pigs were housed individually in stainless steel, screen-bottomed (no-bedding), battery type cages with automatic flushing. Water was provided ad libitum to the cage battery by automatic lick dispensers connected to a central line. During the 6-hour exposure periods the water was disconnected to prevent the wrappings from getting wet. The animals were fed ad libitum Purina Certified Guinea Pig Chow No. 5026 (Lot Numbers APR21832A and MAY168332A). The room temperature was maintained between 21-26°C. The relative humidity varied between 40% and 60%. The photoperiod in the animal room was between 0630 and 1900 hours each day.

A few minor deviations in these conditions are discussed in Appendix B. However, these should not have significantly affected the results of this study.

Acclimation and Group Assignment

The guinea pigs were quarantined for nine days, before being assigned to groups. During the quarantine period, they were checked daily for signs of illness and weighed twice a week. Based on their weights, ten animals were assigned to each of seven groups by a stratified randomization technique. The MINITAB statistical program (5) on the Data General Eclipse C/330 was used to rank animals

according to their weight. Extra animals were eliminated from the extremes (i.e., those whose weights deviated furthest from the mean). The RANDOM program (LAIR SOP OP-ISG-21) on the C/330 was used to generate ten random sequences of numbers one through seven.

Dose Levels

All three test substances were liquid and applied undiluted. The positive control substance, dinitrochlorobenzene (DNCB), was used at 0.1% concentration. During the induction phase, the experimental groups and the positive control group had a 0.5 ml dose applied topically under a one-inch square gauze patch once a week for three weeks (22 Jun 83, 29 Jun 83, and 6 Jul 83). The day before each dosing a three-inch square area on the left side of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI 53217) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT 06904). The patch was taped (Durapore® hypoallergenic surgical tape, 3M Corp., St. Paul, MN 55144) to the same site each time. The animal was wrapped several times with Conform® elastic tape (The Kendall Company, Boston, MA 02101) to occlude the patch. The patch was left in place for six hours. When the patch was removed, the area under the patch was marked off for scoring.

Animals were rested for two weeks following the third induction dose and then were given the challenge dose (20 Jul 83). The experimental groups and the positive control group had a 0.5 ml dose applied to the old site on the left side and to a new site on the right side. In addition, the negative control groups for each test compound had a 0.5 ml dose applied to the left side only. The procedures for clipping, shaving, wrapping and exposure period remained the same.

Slight changes in the dosing procedures were made after the first induction dose and are discussed in Appendix B. These changes should not have affected the results from this study.

Compound Preparation

The test compound, DMSO Recycle Solvent, was heated to 40°C and vortexed vigorously before application to help solubilize the nitramine impurities in it. The Virgin DMSO and DMSO Evaporator Sludge were applied at room temperature. The dinitrochlorobenzene dosing solution was prepared by first adding 30 mg DNCB to 1 ml of propylene glycol and heating it until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution also was heated to 40°C and vortexed before application to keep the DNCB in solution. The same solutions were used for all four applications.

Test Procedures

The closed patch dermal sensitization test developed by Buehler and Griffith (6-9) was used for this study. The Buehler test was used instead of the standard Landsteiner-Draize (10-11) test for several reasons. A topical route of administration was necessary because intradermal injections of 100% DMSO caused severe necrosis and even a 1% DMSO solution caused significant irritation. Furthermore, not all the nitramine impurities in the DMSO Recycle Solvent would go into solution at 40°C.

Following Buehler's technique, the test compounds were applied under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site. To distinguish between reactions from primary irritation and sensitization, negative control groups were added which received only the challenge dose.

In Buehler's procedures, skin reactions were scored 24 and 48 hours after the challenge dose only. We scored the skin reactions 24 and 48 hours after each induction dose as well. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema) and 3 (marked erythema). The results were expressed both in terms of incidence (the number of animals showing responses of 1 or greater at either 24 or 48 hours, divided by the number of animals tested) and severity (the sum of the test grades divided by the number of animals tested). Results from the left side were compared with right side and with the negative control group for each test compound.

Some modifications of Buehler's procedures were made. Instead of placing animals in restrainers during the 6-hour exposure period for each application, the animals were wrapped several times with elasticized adhesive tape to hold the patch in place and occlude it. Consequently, the animals were able to move about freely in their cage during the exposure period. Buehler and Griffith (8) also recommended depilating the hair the day before the challenge dose was applied, but we chose not to do this because dipilation might cause some skin irritation by itself and any residue left from the depilatory cream could possibly react with the test compound.

A historical listing of study events appears in Appendix C.

RESULTS

The incidence of reactions 24 hours after each dose is summarized in Table 1. The incidence on the left side of the guinea pigs 24 hours after the challenge dose was fairly high (>8/10) for all three test groups. The incidence at this time was noticeably higher than 24

hours after the third induction dose. However, the incidence of erythema on the right side was not as high (< 3/10) for the test groups.

Forty-eight hours after the challenge dose, the incidence of erythema in the test groups decreased (Table 2). The most significant change in incidence from 24 to 48 hours was in the Virgin DMSO group. After 24 hours, 9 out of 10 animals had erythema on the left side, but after 48 hours only 2 out of 10 animals had erythema on that side. Both the DMSO Recycle Solvent and the DMSO Evaporator Sludge still had moderately high incidences of erythema on the left side after 48 hours (6/10). The incidence of erythema on the left side was generally higher 48 hours after the challenge dose than after any of the induction doses for all three test groups. The DMSO Evaporator Sludge group was the only test group that had any animals with erythema on the right side after 48 hours.

The animals in the positive control group all had erythema on the left side 24 hours after the challenge dose and only one did not have any on the right side. The incidence of erythema, in the positive control group at 24 hours, increased the most between the first and second induction dose. All animals in the positive control group had some erythema 48 hours after the challenge dose on both the left and right sides. The incidence was only slightly lower 48 hours after the second and third induction dose.

The incidence of erythema in the negative control groups was low at both 24 and 48 hours. Only the Virgin DMSO group had any animals with erythema (3/10) after 24 hours. After 48 hours only the DMSO Evaporator Sludge group had any animals with erythema (2/10).

The severity of skin reactions 24 hours after each dose is summarized in Table 3. The test compound groups all showed an increase in the average 24-hour score on the left side from the third dose to the challenge dose. The average 24-hour score for the Virgin DMSO group increased 0.9 points while it only increased 0.4 points for both the DMSO Recycle Solvent group and the DMSO Evaporator Sludge group. However, during the induction phase these latter two groups generally had higher 24-hour scores. After the challenge dose, there was no obvious difference in the 24-hour scores for the left side between the three test groups. The 24-hour scores on the right side after the challenge dose were low for all three test groups. There were no obvious differences in these scores between the three test groups.

The 48-hour scores were often lower than the 24-hour scores for all the doses (Table 4). The difference between the 24-hour and 48-hour scores was the most dramatic after the challenge dose. The average score for Virgin DMSO group on the left side decreased 0.7 points from 24 hours to 48 hours. Consequently, the average 48-hour score for the DMSO Recycle Solvent and DMSO Evaporator Sludge groups were noticeably higher than for the Virgin DMSO group. The 48-hour scores on the left side after the challenge dose were still slightly higher than after the third dose in all test groups.

TABLE 1

Incidences of Skin Reactions

after Twenty-Four Hours

Test Substance	Induction First	Induction Second	Induction Third	Challenge Left	Challenge Right	Negative Control*
Virgin DMSO (TP014)	1†	1	0	9	3	3
DMSO Recycle Solvent (TP013)	3	1	4	8	3	0
DMSO Evaporator Sludge	2	1	5	8	2	0

* The Negative Control Group received a challenge dose of the test compound.

† Per 10 animals.

TABLE 2

Incidences of Skin Reactions

after Forty-Eight Hours

Test Substance	Induction First	Induction Second	Induction Third	Challenge Left	Challenge Right	Negative Control*
Virgin DMSO (TP014)	1†	0	0	2	0	0
DMSO Recycle Solvent (TP013)	4	0	4	6	0	0
DMSO Evaporator Sludge (TP015)	3	1	2	6	1	2
DNCB	5	8	9	10	10	---

* The Negative Control Group received a challenge dose of the test compound.

† Per 10 animals.

TABLE 3

Severity of Skin Reactions

after Twenty-Four Hours

Test Substance	Induction First	Induction Second	Induction Third	Challenge Left	Challenge Right	Negative Control*
Virgin DMSO (TP014)	0.1	0.1	0.0	0.9	0.3	0.3
DMSO Recycle Solvent (TP013)	0.3	0.1	0.4	0.8	0.3	0.0
DMSO Evaporator Sludge (TP015)	0.3	0.1	0.5	0.9	0.2	0.0
DNCB	0.4	0.9	1.3	1.3	0.9	---

* The Negative Control Group received a challenge dose of the test compound.

TABLE 4

Severity of Skin Reactions

after Forty-Eight Hours

Test Substance	Induction First	Induction Second	Induction Third	Challenge Left	Challenge Right	Negative Control*
Virgin DMSO (TP014)	0.1	0.0	0.0	0.2	0.0	0.0
DMSO Recycle Solvent (TP013)	0.4	0.0	0.4	0.6	0.0	0.0
DMSO Evaporator Sludge (TP015)	0.3	0.1	0.2	0.6	0.1	0.1
DNCB	0.5	0.8	1.0	1.2	1.0	---

* The Negative Control Group received a challenge dose of the test compound.

Only the DMSO Evaporator Sludge group had any reaction on the right side 48 hours after the challenge dose. In this group, only one animal had very slight erythema.

The positive control group did not show an increase in 24-hour scores on the left side from the third induction dose to the challenge dose. The largest increase in the 24-hour scores was between the first and second dose. The average 24-hour score on the right side after the challenge dose was slightly less than that on the left side. There was a steady increase in the 48-hour scores between each of the induction doses in the positive control group. The 48-hour scores on the left side after the challenge dose were only slightly higher than that after the third induction dose. The average 48-hour score on the right side following the challenge dose was only slightly less than that on left side.

The average 24-hour and 48-hour scores for the negative control groups were low. Only the Virgin DMSO Group had any positive reactions 24 hours after the challenge dose. The DMSO Evaporator Sludge was the only group with any positive reactions 48 hours after the challenge dose.

The individual 24-hour and 48-hour scores for all the doses appear by group, in Appendix D.

DISCUSSION

A major problem in interpreting the results from a dermal sensitization study is that the test compound may also be a primary irritant. To differentiate the two reactions Buehler and Griffith recommend comparing the results after the challenge dose for the experimental group with the results from the negative control group and if used, the vehicle control group (6-9). To aid in interpretation Griffith (7) provided the following guidelines for distinguishing between primary irritation and sensitization:

Primary Irritation

- Reactions fade within 24 to 48 hours unless skin damage is severe.

- Many moderate to strong reactions may be seen early in the test among a large proportion of animals.

- Disproportionate reactions may occur between the right and left flank at challenge. The stronger reactions can sometimes occur on the previously unpatched flank, due to accommodation of the skin on the repeatedly insulted flank brought about by recurring irritation and the stripping off of the stratum corneum around the patch site with the adhesive. This is by no means infallible, however; some animals become more reactive to irritants on the insulted flank.

Allergic Contact Sensitization

One or more of the following may occur:

- Stronger reactions to the challenge patches than to any of the induction patches.

- Delayed (96-hour) challenge reactions are stronger than those seen at 48 hours.

- A sudden increase in intensity of reactions is seen to a test material during the second or third week of the test; this can be indicative of a stronger sensitizer with a short induction period.

- One or only a very few animals show moderate or stronger response while the balance of the group does not develop reactions greater than a grade of 1 at any time during the test. This suggests a weak sensitizer.

- A very strong reaction by one animal following the first or second patch application suggests a preexisting sensitization.

Following Griffith's guidelines (7), all three test compounds in this study may be classified as weak primary irritants. One to three animals in each test group had slight erythema 24 to 48 hours after the first application. The incidence was greater in the DMSO Recycle Solvent and DMSO Evaporator Sludge groups than in the Virgin DMSO group. In fact, one animal in the DMSO Evaporator Sludge group had a moderate reaction 24 hours after the first dose. In most cases, the skin reactions disappeared by 48 hours after each dose. There was a much stronger reaction to the challenge dose on the left side than the right side.

Slight erythema in guinea pigs after one topical application of pure DMSO has been reported in the literature (12). In fact, with repeated exposure, pure DMSO has shown a definite irritation of the skin as evidenced by erythema, edema, and inhibition of hair growth in a number of species including man (12-14). When our group tested the primary dermal irritation potential of the Holston compounds on rabbits, several animals in each test group had slight erythema after 24 hours (15). However, the incidence and severity were low enough to classify these compounds as non-irritating after one application.

There were some indications that the test compounds might be weak sensitizers. We observed stronger reactions to the challenge dose on the left side than to any of the induction doses in all three test groups. A few animals in the DMSO Evaporator Sludge group showed moderate reactions, otherwise the animals in this group showed no or slight erythema. However, comparison of the skin reaction to the challenge dose on the right side with reactions from the initial dose and with reactions from the negative control group, indicates that the test compounds have little if any sensitizing potential. The incidence and severity of the skin reactions in the positive control group confirm that DNCB was a sensitizer. There was a sudden increase in the incidence and severity from the first to the second application. Reactions were stronger in several cases after 48 hours. There was a 90% response to the challenge dose on the right side after 24 hours and a 100% response after 48 hours. The reactions to the DNCB were only slight to moderate, but this was consistent with the concentration of the dosing solution (0.1%) used.

CONCLUSION

All three test compounds, Virgin DMSO (TP014), DMSO Recycle Solvent (TP013), and DMSO Evaporator Sludge (TP015), are mild irritants but possess no sensitizing potential under the conditions of this study.

RECOMMENDATION

Certain precautions should be made to protect workers from these DMSO solutions since they are mild irritants. Based on these studies no additional precautions to reduce potential sensitization of workers is required.

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APPENDICES

Toxicity Test Sample Composition[a]

Concentration by HPLC, g/l

Sample	RDX[b]	HMX[c]	TAX[d]	SEX[e]	%H_2O	%DMSO
Virgin DMSO[f]	0	0	0	0	0.63[g]	99.37
DMSO Recycle Solvent[h]	24.188	39.542	0.263	0	35.48[i]	58.64[j]
DMSO Evaporator Sludge[f]	0.548	0.942	3.521	0	5.35[i]	94.19[j]

Calculated Data In Weight Percent[a]

Sample	RDX	HMX	TAX	SEX	H_2O	DMSO
Virgin DMSO	0	0	0	0	0.63	99.37
DMSO Recycle Solvent	2.22	3.64	0.02	0	35.48	58.64
DMSO Evaporator Sludge	0.05	0.09	0.32	0	5.35	94.19

[a] Data supplied by sponsor

[b] RDX: Hexahydro-1,3,5-Trinitro-1,3,5-Triazine

[c] HMX: Octhydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine

[d] TAX: 1-Acetylhexahydro-3,5-Dinitro-1,3,5-Triazine

[e] SEX: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine

[f] At ambient temperature.

[g] By Karl Fisher

[h] Analysis of equilibrium liquid at 40 C.

[i] Water content calculated by difference.

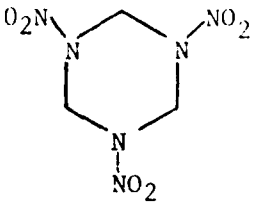
[j] DMSO content by gas chromatography using Virgin DMSO sample as the standard.

Chemical Data

1. Chemical name: Hexahydro-1,3,5-Trinitro-1,3,5-Triazine, Cyclotrimethylenetrinitramine, Cyclonite Hexogen, RDX

   Chemical Abstract Service Registry Number: 121-82-4

   Structural formula:



   Empirical formula: $C_3H_6N_6O_6$

   Molecular weight: 222.13 g/mole

   Physical State: White crystals varying in size

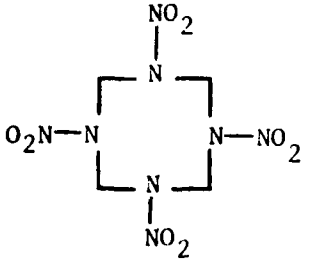
   Melting point: 200-203°C

   Manufacturer: Holston Army Ammunition Plant
   Kingsport, TN

2. Chemical name: Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazine HMX, Cyclotetramethylenetrinitramine

   Chemical Abstract Service Registry Number: 2691-41-0

   Structural formula:



Empirical formula: $C_4H_8O_8N_8$

Molecular weight: 296.17 g/mole

Physical state: White crystals of varying size

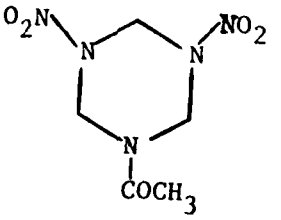
Melting point: 280°C

Manufacturer: Holston Army Ammunition Plant
Kingsport, TN

3. Chemical name: Hexahydro-1-(N)-Acetyl-3,5-Dinitro-1,3,5-Triazine, TAX

Chemical Abstract Service Registry Number: 14168-42-4

Structural formula:



Empirical formula: $C_5H_9O_5N_5$

Molecular weight: 219.17 g/mole

Physical state: White crystals of varying size

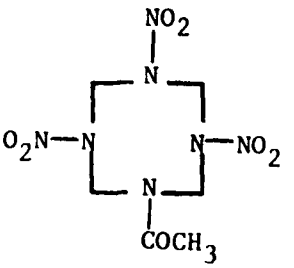
Melting point: 156°C

Manufacturer: By-product of the production/processing of HMX/RDX at the Holston Army Ammunition Plant, Kingsport, TN

4. Chemical name: Octahydro-1-(N)-Acetyl-3,5,7-Trinitro-1,3,5,7-Tetrazine, SEX

Chemical Abstract Service Registry Number: 13980-00-2

Structural formula:



Empirical formula: $C_6H_{11}O_7N_7$

Molecular weight: 293.21 g/mole

Physical State: White crystals of varying size

Melting point: 224.2-224.7°C

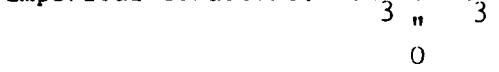
Manufacturer: By-product of the production/processing of HMX/RDX at the Army Ammunition Plant, Kingsport, TN

5. Chemical name: Dimethyl Sulfoxide (DMSO)

Chemical Abstract Service Registry Number : 00006-76-85

Structural formula: C_2H_6SO

Empirical structure:



Molecular weight: 78.02 g/mole

Physical state/color: Clear transparent liquid.

Freezing point: 18.55°C

Boiling point: 189°C

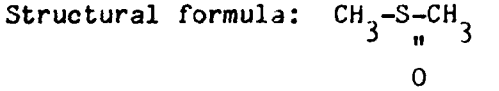
Contaminants: Water 0.63 percent

Manufacturer: Crown Zellerbach Corporation
Chemical Products Division
Camas, WA 98607

6. Chemical name: Dimethy Sulfoxide (DMSO) reagent grade

Chemical Abstract Service Registry Number: 00006-76-85

Structural formula:



Empirical formula: C_2H_6SO

Physical state: Clear transparent liquid

Freezing point: 18.3°C

Boiling point: 189°C

Density: 1.095 g/ml

Contaminants: Water 0.08%

Manufacturer: J.T. Baker Chemical Co.
Phillipsburg, NJ 08805

Lewis--20

HOLSTON DEFENSE CORPORATION

WEST STONE DRIVE

KINGSPORT, TENNESSEE 37660

TELEPHONE AREA CODE 615 247-9111

June 22, 1983

Contracting Officer's Representative
Holston Army Ammunition Plant
Kingsport, Tennessee 37660

Dear Sir:

Subject: DMSO Process Stream Toxicological Testing

Reference: USAMBRDL Letter to Commander, HSAAP, "DMSO Munition Process Solvent Toxicology Studies Laboratory Monitoring Visits and Technical Status Review Meetings," dated November 23, 1982

1. The meetings referred to in the above reference were attended as requested. At that time the toxicity studies at both LAIR and LEHR were just getting under way, and the meetings were used to review preliminary results then available as well as plans for completing the studies. Holston was also involved in a characterization screening study of the same test samples in an attempt to identify potentially toxic compounds which might be present and could contribute to the toxic or mutagenic results observed.

   The test samples had been previously analyzed for composition at Holston and shipped to LAIR. At the referenced meeting, Col. Fruin requested that in addition Holston furnish both the results of the characterization screening study and the details of the analytical methods used to perform the original quantitative analyses on the test samples at Holston. The screening study at Holston has now been completed, and the requested information is hereby transmitted.

2. The characterization screening study was performed on the composite recycle solvent sample from the DMSO pilot plant. Also, production crude/water-washed RDX and HMX samples were subjected to analyses to determine if any unusual compounds could be detected for comparison with any found in the DMSO sample. HPLC methods were used during the screening procedure varying the columns, solvent systems, wavelengths, and the other parameters such that any contaminant peaks found could be identified by component retention time.

   Initial HPLC analysis of the recycle solvent sample showed very large concentrations of RDX and HMX which interfered with analysis of other components. The sample was treated to remove the bulk of the RDX and HMX by heating to 40°C and then quenching one to one with water. The decanted liquid was then subjected to the remainder of the screening

study analyses. The sample was examined by several HPLC systems available at Holston which are normally used to analyze RDX, HMX, and related nitramines found in various plant process streams and products. These are presented in Attachments II and III. Other HPLC conditions presented in Attachment I, which do not represent proven HPLC methods, were also used to get as much system variability as possible. Note that Holston does not guarantee these results since these procedures in Attachment I were used only for screening and qualitative purposes. It should also be realized that most of Holston's routine procedures are used to detect nitramine or related compounds. Other impurities may not have been detected by these methods. The only compounds detected using any of the systems were RDX, HMX, SEX, and TAX. HPLC retention times for these compounds matched the known retention times for RDX, HMX, SEX, and TAX. Attachment I also presents the results obtained. Analysis of crude RDX and HMX by the methods described in Attachment II yielded no evidence of the presence of compounds other than RDX, HMX, and SEX.

3. Quantitative analyses of the test samples were performed by HPLC. Since no reliable method for direct analysis of DMSO by either HPLC or GC has been developed, DMSO values are by difference. Attachment III presents an outline of the quantitative methods used.

4. This information should be transmitted to the following:

Col. John Fruin
Building 1110
Presidio of San Francisco
California 94129

Capt. James Carroll
USAMBRDL
Building 568
Fort Detrick
Frederick, Maryland 21701

Raymond Goldstein
ARRADCOM
Picatinny Arsenal
Dover, New Jersey

Yours very truly,

HOLSTON DEFENSE CORPORATION

M B Knowles

M B Knowles
Plant Manager

Attachments (3)

HPLC Parameters	Components Detected
1. Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 NM Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX SEX
2. Column: LiChrosorb-Amine, 1/4" x 12" ss Detector: UV, 230-260 nm in 10 nm increments Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX
3. Column: LiChrosorb-Diol, 1/4" x 12" ss Detector: UV, 230-260 nm in 10 nm increments Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX
4. Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 70% water 30% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX
5. Column: Waters CN, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX
6. Column: Waters CN, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 60% water 40% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX

	HPLC Parameters	Components Detected
7.	Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 50% water 50% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
8.	Column: LiChrosorb-Diol, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
9.	Column: LiChrosorb-Amine, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
10.	Column: LiChrosorb-RP18, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX SEX
11.	Column: LiChrosorb-RP18 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 60% water 40% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
12.	Column: LiChrosorb-RP 8 1/4" x 6" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.0 ml/min Injection Volume: 10 microliters	RDX HMX TAX SEX
13.	Column: LiChrosorb-RP 8 1/4" x 6" ss Detector: UV at 254 nm Solvent System: 60% water 40% methanol Flow Rate: 2.0 ml/min Injection Volume: 10 microliters	No component separation

ATTACHMENT II

HPLC Analysis of Crude RDX

HPLC Parameters	Components Detected
Column: Waters CN, 1/4" x 12" ss	
Detector: UV, 215-290 nm in 10 nm increments	RDX HMX SEX
Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol	
Flow Rate: 3.0 ml/min	
Injection Volume: 10 microliters	

HPLC Analysis of Crude HMX

HPLC Parameters	Components Detected
Column: Waters CN, 1/4" x 12" ss	
Detector: UV, 215-290 nm in 10 nm increments	RDX HMX SEX
Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol	
Flow Rate: 3.0 ml/min	
Injection Volume: 10 microliters	

ATTACHMENT III

Quantitative Analysis of DMSO/Explosives Samples

Sample Preparation

1. Weigh representative liquid sample.
2. Evaporate sample to dryness - weigh dried sample.
3. Add acetonitrile to sample sufficient to completely dissolve all solids.
4. Analyze for RDX, HMX, and SEX using Procedure A below.
5. Analyze for TAX using Procedure B below.

Procedure A - HPLC

Column: Waters CN, 1/4" x 12" ss (Waters No. 84082)
Detector: UV at 254 nm
Solvent System: 70% iso-octane
15% chloroform
10% acetonitrile
5% methanol
Flow Rate: 3.0 ml/min
Injection Volume: 10 microliters
Typical Retention Times (seconds): RDX - 195
SEX - 365
HMX - 423

Procedure B - HPLC

Column: Waters CN, 1/4" x 12" ss (Waters No. 84082)
Detector: UV at 254 nm
Solvent System: 80% water
20% methanol
Flow Rate: 2.5 ml/min
Injection Volume: 10 microliters

DMSO/Water Content

Karl Fischer titration was used to determine the water content of the liquid recycle solvent. DMSO was determined by difference as below:

$$\% \text{ DMSO} = 100\% - \% \text{ Solids} - \% \text{ Water}$$

DEVIATIONS IN HUSBANDRY AND DOSING PROCEDURES

1. The water to the cages was accidently left off one night (8 July 1983) during the rest period between the third dose and the challenge dose.

2. The relative humidity was 70% $\pm$ 10% for one week (11-18 July 1983) between the third induction dose. However, the hygrothermograph may have been calibrated wrong during this week since the next week the relative humidity was recalibrated and it was 10% lower.

3. With the first dose, only a 1-inch area was clipped for the patch. Due to problems with the patch slipping and with scoring the reactions, a larger area was clipped and then shaved with an electric razor for the remaining doses.

4. The patch tape was changed from Micropore® tape (3M Corp., St. Paul, MN 55144) to Durapore® tape after the first dose.

APPENDIX B

HISTORICAL LISTING OF EVENTS

8 Jun 83	A0	Animals arrived. They were examined, ear tagged, weighed, placed in cages and fed.
9 Jun 83	A1	QC animals were submitted for necropsy.
9 Jun - 22 Jul 83	A1-A13	Animals were checked daily.
13, 17, 20, 24, 27 Jun; 1, 4, 8, 11, 15, 18, 20 Jul 83	A5, A9, A12, 2, 5, 9, 12, 16, 19, 23, 26, 30	Animals were weighed.
17 Jun 83	A9	Animals were randomized into groups.
21, 28 Jun, 5 Jul 83	A13, 6, 13	All animals except those in the negative control groups were clipped.
22, 29 Jun, 6 Jul 83	0, 7, 14	All animals except those in the negative control groups were given induction dose.
23, 30 Jun, 7 Jul 83	1, 8, 15	24-hour scores were recorded for animals receiving induction dose.
24 Jun, 1, 8 Jul 83	2, 9, 16	48-hour scores were recorded for animals recieving induction dose.
19 Jul 83	27	All animals were clipped.
20 Jul 83	28	All animals were given challenge dose.
21 Jul 83	29	24-hour scores were recorded for challenge dose.
22 Jul 83	30	48-hour scores were recorded for challenge dose.

APPENDIX C

LIST OF TABLES

Page

TABLE 1

GLP Study # 83002

BUEHLER SENSITIZATION TEST

Group Number _1_ Chemical Name Dinitrochlorobenzene

Dose Number	1		2		3		Challenge Dose			
							Left		Right	
Animal Number	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
83E00230	1	0	1	0	1	1	1	1	1	1
83E00244	0	0	1	1	2	2	2	2	1	1
83E00246	1	1	1	1	1	1	1	1	1	1
83E00253	1	1	1	1	2	1	1	1	1	1
83E00254	0	0	0	0	1	1	1	1	0	1
83E00278	1	1	1	1	2	1	2	1	1	1
83E00282	0	0	1	1	1	1	2	2	1	1
83E00293	0	1	1	1	1	1	1	1	1	1
83E00297	0	1	1	1	1	1	1	1	1	1
83E00298	0	0	1	1	1	0	1	1	1	1
Average	0.4	0.5	0.9	0.8	1.3	1.0	1.3	1.2	0.9	1.0

TABLE 2

GLP Study # 83002

BUEHLER SENSITIZATION TEST

Group Number 2 Chemical Name DMSO Recycle Solvent

Dose Number	1		2		3		Challenge Dose			
							Left		Right	
Animal Number	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
83E00225	1	0	0	0	0	0	1	1	0	0
83E00228	0	0	0	0	0	0	0	0	0	0
83E00233	0	0	0	0	0	0	1	1	0	0
83E00245	0	1	0	0	0	0	0	0	0	0
83E00268	1	0	0	0	0	0	1	0	0	0
83E00274	0	0	0	0	1	1	1	1	1	0
83E00277	0	1	0	0	1	1	1	1	0	0
83E00279	0	1	0	0	1	1	1	1	1	0
83E00290	1	1	1	0	1	1	1	1	1	0
83E00296	0	0	0	0	0	0	1	0	1	0
Average	0.3	0.4	0.1	0.0	0.4	0.4	0.8	0.6	0.3	0.0

TABLE 3

GLP Study # 83002

BUEHLER SENSITIZATION TEST

Group Number 3 Chemical Name Virgin DMSO

Dose Number	1		2		3		Challenge Dose			
							Left		Right	
Animal Number	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
83E00240	0	0	0	0	0	0	1	1	0	0
83E00241	0	0	0	0	0	0	1	0	0	0
83E00243	0	0	0	0	0	0	0	0	0	0
83E00255	0	0	0	0	0	0	1	0	0	0
83E00258	0	0	0	0	0	0	1	0	0	0
83E00262	0	1	0	0	0	0	1	0	1	0
83E00269	1	0	0	0	0	0	1	0	1	0
83E00270	0	0	0	0	0	0	1	1	0	0
83E00283	0	0	1	0	0	0	1	0	1	0
83E00292	0	0	0	0	0	0	1	0	0	0
Average	0.1	0.1	0.1	0.0	0.0	0.0	0.9	0.2	0.3	0.0

TABLE 4

GLP Study # 83002

BUEHLER SENSITIZATION TEST

Group Number 4 Chemical Name DMSO Evaporator Sludge

Dose Number	1		2		3		Challenge Dose			
							Left		Right	
Animal Number	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
83E00224	0	0	0	0	0	0	1	0	0	0
83E00227	0	0	0	0	0	0	0	0	0	0
83E00242	0	0	1	1	1	0	1	0	0	0
83E00257	0	1	0	0	0	0	1	1	0	0
83E00259	1	0	0	0	0	0	0	0	0	0
83E00275	2	1	0	0	1	0	1	1	0	0
83E00276	0	0	0	0	0	0	1	1	0	0
83E00281	0	1	0	0	1	0	1	1	0	0
83E0084	0	0	0	0	1	1	2	1	1	1
83E0087	0	0	0	0	1	1	1	1	1	0
Average	0.3	0.3	0.1	0.1	0.5	0.2	0.9	0.6	0.2	0.1

TABLE 5

SUMMARY OF SKIN REACTION AFTER THE CHALLENGE DOSE

FOR THE NEGATIVE CONTROL GROUPS

Group 5 - DMSO Recycle Solvent			Group 6 - Virgin DMSO			Group 7 - DMSO Evaporator Sludge		
Animal #	24hr	48hr	Animal #	24hr	48hr	Animal #	24hr	48hr
83E00229	0	0	83E00231	1	0	83E00226	0	0
83E00234	0	0	83E00235	1	0	83E00232	0	1
83E00237	0	0	83E00236	0	0	83E00239	0	1
83E00256	0	0	83E00238	0	0	83E00248	0	0
83E00263	0	0	83E00247	0	0	83E00249	0	0
83E00266	0	0	83E00252	0	0	83E00260	0	0
83E00271	0	0	83E00273	1	0	83E00261	0	0
83E00280	0	0	83E00288	0	0	83E00267	0	0
83E00286	0	0	83E00289	0	0	83E00272	0	0
83E00291	0	0	83E00299	0	0	83E00295	0	0
Average	0.0	0.0	Average	0.3	0.0	Average	0.0	0.2

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