

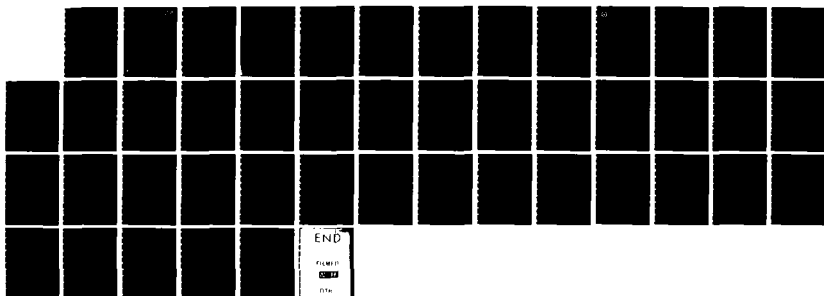
AD-A137 228

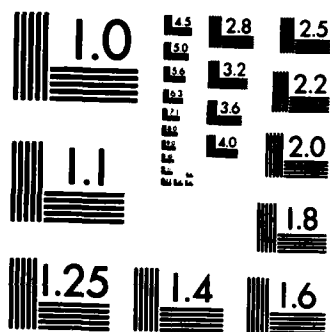
ACUTE ORAL TOXICITY OF DMSO (DIMETHYL SULFOXIDE)
PROCESS STREAM SAMPLES I. (U) LETTERMAN ARMY INST OF
RESEARCH PRESIDIO OF SAN FRANCISCO CA C W WHITE ET AL.
DEC 83 LAIR-167 F/G 6/28

1/1

UNCLASSIFIED

NL





MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

12

AD A 137228

INSTITUTE REPORT NO. 167

ACUTE ORAL TOXICITY OF DMSO PROCESS STREAM SAMPLES
IN MALE AND FEMALE MICE

CRAIG W. WHITE, DVM, CPT VC
JUSTO RODRIGUEZ, BS, SP5
and
GLEN E. MARRS, JR, DVM, MS, MAJ VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

DTIC
ELECTE
S JAN 26 1984 D

DECEMBER 1983

Toxicology Series 66

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

DISTRIBUTION STATEMENT
Approved for public release
Distribution Unlimited

84 01 26 010

DTIC FILE COPY

Acute Oral Toxicity of DMSO Process Stream Samples in Male and Female Mice--White et al

Reproduction of this document in whole or in part is prohibited except with the permission of the Commander, Letterman Army Institute of Research, Presidio of San Francisco, California 94129. However, the Defense Technical Information Center is authorized to reproduce the document for United States Government purposes.

Destroy this report when it is no longer needed. Do not return it to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

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)

 14 Dec 83
(Signature and date)

This document has been approved for public release and sale; its distribution is unlimited.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER LAIR Institute Report No. 167,	2. GOVT ACCESSION NO. AD-A137 228	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Acute Oral Toxicity of DMSO Process Stream Samples in Male and Female Mice		5. TYPE OF REPORT & PERIOD COVERED Final, 22 Dec 82 - 18 Jan 83 and 23 Mar - 12 Apr 83
7. AUTHOR(s) Craig W. White, DVM, CPT VC Justo Rodriguez, SP5, BS Glen E. Marrs, DVM, MS, MAJ VC		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS APC TL01
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE December 1983
		13. NUMBER OF PAGES 45
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) THIS DOCUMENT HAS BEEN CLEARED FOR PUBLIC RELEASE AND SALE: ITS DISTRIBUTION IS UNLIMITED.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Toxicity, Dimethyl Sulfoxide, Nitramine, HMX, RDX, TAX, DMSO, Holston Army Ammunition Plant, CAS Reg. No. 121-82-4 (RDX), 2691-41-0 (HMX), 14168-42-4 (TAX)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The acute toxicity of a single oral dose of the DMSO process stream samples DMSO Evaporator Sludge, DMSO Recycle Solvent, and Virgin DMSO was determined in male and female albino ICR mice. The DMSO Evaporator Sludge solution produced no deaths in male or female mice at a limit dose level of 5.0 ml/kg. The Virgin DMSO solution produced mortalities in one of seven males (14%) and two of seven females (29%) at a limit dose of 5.0 ml/kg. The DMSO Recycle Solvent LD50 with 95 percent confidence limit was calculated by probit analysis. The		

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

Item 20 (continued)

DMSO Recycle Solvent LD₅₀ was 4.0 ml/kg in male mice, 95 percent confidence limit (3.2 ml/kg, 5.1 ml/kg) and 2.5 ml/kg in female mice, 95 percent confidence limit (2.2 ml/kg, 2.8 ml/kg).

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

ABSTRACT

dimethyl sulfoxide

The acute toxicity of a single oral dose of the ¹DMSO process stream samples DMSO Evaporator Sludge, DMSO Recycle Solvent, and Virgin DMSO was determined in male and female albino ICR mice. The DMSO Evaporator Sludge solution produced no deaths in male or female mice at a limit dose level of 5.0 ml/kg. The Virgin DMSO solution produced mortalities in one of seven males (14%) and two of seven females (29%) at a limit dose of 5.0 ml/kg. The DMSO Recycle Solvent LD₅₀ with 95 percent confidence limit was calculated by probit analysis. The DMSO Recycle Solvent LD₅₀ was 4.0 ml/kg in male mice, 95 percent confidence limit (3.2 ml/kg, 5.1 ml/kg) and 2.5 ml/kg in female mice, 95 percent confidence limit (2.2 ml/kg, 2.8 ml/kg).

Accession For	
NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A/1	



PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study 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/WORK UNIT/APC: USAMBRDL DMSO Process Stream Samples Project,
APC TL01

GLP STUDY NO.: 82040 and 83003

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC
Diplomate, American College of
Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Craig W. White, DVM, VC

CO-PRINCIPAL INVESTIGATOR: SP5 Justo Rodriguez, BS

PATHOLOGIST: MAJ Glen E. Marrs, Jr., DVM, MS, VC
Diplomate, American College of
Veterinary Pathologists.

STATISTICIAN: Virginia L. Gildengorin, PhD

REPORT AND DATA MANAGER: 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 compound will be
retained in the LAIR Archives as GLP
Studies 82040 and 83003

TEST SUBSTANCE: DMSO Process Stream Samples:
Recycle Solvent (TPO13)
Virgin DMSO (TPO14)
Evaporator Sludge (TPO15)

INCLUSIVE STUDY DATES: 22 December 1982 - 18 January 1983 (82040)
23 March 1983 - 12 April 1983 (83003)

OBJECTIVE: To determine the acute oral toxicity of DMSO Process
Stream Samples: Recycle Solvent (LAIR Code TPO13), Virgin
DMSO (LAIR Code TPO14), and Evaporator Sludge (LAIR Code
TPO15) - in male and female ICR mice.

ACKNOWLEDGEMENTS

The authors wish to thank SP5 Leonard Sauers, MS; SP5 Lawrence Mullen, BS; SP5 Thomas Kellner, BA; and SP5 Evelyn Zimmerman for assistance in performing this research. Additionally, the authors wish to thank Dr. Jack Dacre and CPT James Carroll of the U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD 21701 for consultation services rendered.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study numbers 82040 and 83003 described in this report to be scientifically sound and the results in this report and interpretations to be valid. The studies were conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

John T. Fruin 6 Aug 83
JOHN T. FRUIN / DATE
COL, VC
Study Director

Justo Rodriguez 7-6-83
JUSTO RODRIQUEZ, BS / DATE
SP5, USA
Co-Principal Investigator

Glen E. Marrs, Jr. 6 Jul 83
GLEN E. MARRS / DATE
MAJ, VC
Pathologist

Carolyn M. Lewis 6 Jul 83
CAROLYN M. LEWIS, MS / DATE
DAC
Data Manager

Craig W. White 6 Jul 83
CRAIG W. WHITE / DATE
CPT, VC
Principal Investigator

Virginia L. Gildengorin 7 July 83
VIRGINIA L. GILDENGORIN, PhD / DATE
DAC
Statistician



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

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

9 Aug 83

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82040 the following inspections were made:

4 Jan 83
10 Jan 83
18 Jan 83

I hereby certify that in relation to LAIR GLP study 83003 the following inspection was made:

31 Mar 83

The report and raw data for these studies were audited on 26 Jul 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the April 1983 report to management and the Study Director.

NELSON R. POWERS, Ph.D.
CPT, MSC
Quality Assurance Officer

TABLE OF CONTENTS

	Page
Abstract.....	i
Preface.....	iii
Acknowledgments.....	iv
Signatures of Principal Scientists.....	v
Report of Quality Assurance Unit.....	vi
Table of Contents.....	vii
BODY OF REPORT	
INTRODUCTION.....	1
Objective of the Study.....	1
METHODS.....	2
Test Substance.....	2
Animal Data.....	2
Environmental Conditions.....	2
Dosing.....	2
Observation.....	3
Statistical Methods.....	3
Duration of Study.....	3
Changes to Original Procedure.....	3
RESULTS.....	4
Mortality.....	4
Lethal Dose Calculations.....	6
Clinical Observations.....	7
Gross Pathological Observations.....	7
DISCUSSION.....	7
CONCLUSION.....	8
RECOMMENDATION.....	8
REFERENCES.....	9

Table of Contents (Cont'd)

	Page
APPENDICES	
Appendix A, Chemical Data.....	13
Appendix B, Animal Data.....	25
Appendix C, Environmental Conditions.....	27
Appendix D, Historical Listing of Study Events.....	29
Appendix E, Statistical Analysis.....	31
Appendix F, Pathology Report.....	33
OFFICIAL DISTRIBUTION LIST.....	39

Acute Oral Toxicity of DMSO Process Stream Samples in Male and Female Mice--White et al

Dimethyl sulfoxide (DMSO) is a widely used industrial solvent/reagent which is currently being considered for use by the Holston Army Ammunition Plant as a replacement process solvent in the recrystallization phase of HMX/RDX production. A pilot recrystallization facility was established and put into small scale operation to evaluate the projected advantages of DMSO as a process stream solvent. Samples of the DMSO process stream were taken from two locations at the recrystallization facility. The solution samples 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 Laboratory and were found to contain major and minor cyclic and non-cyclic nitramines. Since nitramines have been reported to be neurotoxic, their presence in the samples represents a potential health hazard to workers utilizing this production process. Thus, it becomes necessary to delineate the acute toxicity of the DMSO solutions so a complete health hazard assessment can be obtained before the DMSO process solvent procedure is put into full scale operation (1-4).

The Toxicology Group, Letterman Army Institute of Research, was assigned by the US Army Medical Research and Development Command the task of performing a major part of the Phase I toxicity testing. Phase I testing involves providing those data necessary to determine potential toxicity associated with accidental worker exposure to DMSO process streams. These initial toxicity assessments would be used to develop the appropriate exposure criteria and/or worker protection requirements.

Objective of the Study

The objective of this study was to determine the acute oral toxicity in male and female ICR mice of the DMSO Process Stream Samples: Recycle Solvent (LAIR Code TPO13), Virgin DMSO (LAIR Code TPO14), and Evaporator Sludge (LAIR Code TPO15).

METHODS

Test Substance

1. Chemical name: DMSO Recycle solvent (TP013)
2. Chemical name: Virgin DMSO (TP014)
3. Chemical name: DMSO Evaporator Sludge (TP015)

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. The samples were three years old at the time the study was conducted, thus analyses for chemical stability were not performed.

Animal Data

A combined total of 172 male and female ICR mice were obtained from Hilltop Lab Animals, Inc., P.O. Box 25, Chetsworth, CA, 91311. Additional animal data appear in Appendix B.

Environmental Conditions

A commercially available certified rodent ration and tap water were provided ad libitum for the animals during this study. Appendix C gives a complete listing of the environmental conditions of this study.

Dosing

Limit Test (GLP Study number 82040)

Animals were selected at random for five dose groups which consisted of seven male and seven female mice per group. The Recycle Solvent, Evaporator Sludge, Virgin DMSO, and Vehicle Control (reagent grade DMSO) were dosed at a 5.0 ml/kg limit level (5). The cage control group animals were untreated. No dilution of any of the dosing materials was made. The dosing material was heated to 40 C to optimize solute/solvent interactions at dosing (as requested by the sponsor). The volumes administered ranged from 0.08 ml to 0.15 ml.

All animals received a single dose of the appropriate sample on 4 January 1983. Sterile, disposable, 1 ml syringes (Becton, Dickinson & Co., Rutherford, NJ) fitted with 22-gauge, 2-inch ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized for the oral administration of the samples. The dosing procedures were conducted without animal sedation or anesthesia.

Recycle solvent LD₅₀ Test (GLP Study number 83003)

Six test groups were selected at random, consisting of eight male and eight female animals per group. Four dose levels were selected (3.0, 4.0, 5.0 and 6.0 ml/kg) based upon the fact that a 50 percent upon the fact that a 50 percent mortality occurred at the limit dose of 5.0 ml/kg. The Recycle Solvent was not diluted before dosing. The vehicle control animals received 0.2 ml of reagent grade DMSO per animal. The dose volumes ranged from 0.05 to 0.14 ml. The Recycle Solvent and the Vehicle Control were heated to 40 C to optimize the solvent/solute interaction at dosing (as requested by the sponsor).

All animals received a single dose of the Recycle Solvent on on 30 March 1983. Sterile, disposable, syringes (Becton, Dickinson & Co., Rutherford, NJ) fitted with 22-gauge, 2-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized for the oral administration of the samples. The dosing procedure was conducted without animal sedation or anesthesia.

Observations

The animals were observed for mortality and signs of acute toxicity throughout the dosing procedure, one hour after dosing and during the afternoon of the day of dosing. Observations were also conducted daily for the remainder of the study. Animals were observed, undisturbed in cages, outside of cages, and after replacement in cages. Body weights were recorded before dosing and twice weekly until death or termination of the study. Appendix D contains a complete listing of observation periods and related study events.

Statistical Methods

Statistical analyses were performed on the Recycle Solvent oral LD₅₀ study data. The LD₁, LD₅₀, and LD₉₅ were derived by Bliss probit analysis as described by Finney (6).

Duration of the Study

The study period for the Limit Study (GLP Study number 82040) was 15 days with 7 days of quarantine/acclimation before the study. The study period for the LD₅₀ Study (#83003) was 14 days with 7 days of quarantine/acclimation prior to the study onset. Appendix D is a historical listing of study events.

Changes to Original Procedures

Any changes to the original procedures are stated and documented in protocol amendments.

White--4

RESULTS

Mortality

Table 1 lists the compound related deaths by group and the percent mortality for the Oral Limit Test, GLP Study 82040.

Table 1

Compound Related Deaths by Group
Oral Limit Test, 82040

Group	DMSO Process Stream Samples	Compound Related Deaths/No. in Group		Percent Mortality	
		Male	Female	Male	Female
1	Cage Control	0/7	0/7	0	0
2	Vehicle Control (Reagent Grade DMSO)	1/7	1/6*	14	16
3	DMSO Recycle Solvent TP013	5/7	2/7	71	29
4	Virgin DMSO (Industrial Grade DMSO) TP014	1/7	2/7	14	29
5	Evaporator Sludge TP015	0/7	0/7	0	0

*One animal was removed from the study due to obvious improper administration of the dosing material.

Table 2 lists the compound related deaths by group and the percent mortality for the acute oral LD₅₀ toxicity study (GLP Study Number 83003) of the DMSO Recycle Solvent (TP013).

Table 2

Recycle Solvent, TP013

Compound Related Deaths by Group
Acute Oral Toxicity (LD₅₀) Study, 83003

Group	Dose Level	Compound Related Deaths/No. in Group		Percent Mortality	
		Male	Female	Male	Female
1	Cage Control	0/8	0/8	0	0
2	Vehicle Control (Reagent Grade DMSO)	0/8	0/8	0	0
3	3.0 ml/kg	2/8	5/8	25	63
4	4.0 ml/kg	3/8	4/8	38	50
5	5.0 ml/kg	7/8	6/7*	88	86
6	6.0 ml/kg	6/8	6/8	75	75

*One animal was removed from study due to improper administration of the dosing material.

Lethal Dose Calculations

Lethal Dose (LD) values calculated by probit analysis for the DMSO Recycle Solvent (TPO13) are presented in Table 3.

Table 3*

Lethal Dose (LD) Levels
For the DMSO Recycle Solvent in Male and Female Mice

Percent Population	Lethal Dose ml/kg		95 Percent Confidence Limits (ml/kg)	
	Male	Female	Male	Female
LD 1	1.5	0.2	(0.3, 7.6)	(0.0, 5.9)
LD 50	4.0	2.5	(3.2, 5.1)	(2.2, 2.8)
LD 95	8.0	17	(3.0, 21)	(4.1, 74)

*Statistician's Report (Appendix E).

Clinical Observations

On the day of dosing, the animals were observed intermittently throughout the entire dosing procedure. Signs of inactivity, sluggishness, interrupted by periods of excitability and irritability, were common findings both in animals that eventually died and those which survived. The most striking signs ascribed to the dosing material were those of irritability and excitability. The affected animal would become highly agitated when disturbed in cages.

Gross Pathological Observations

The results of the limit test, which suggested that the mortality observed in the TP013 group was caused by the test substance, was confirmed by the gross pathological observations (Appendix F). The pattern of mortality implied that the medium lethal dose would be less than 5.0 ml/kg.

The mortalities which occurred appear to have been caused by the subject materials. The dose-response relationship demonstrated with the DMSO Recycle Solvent (TP013) was confirmed. No test compound-related gross lesions were observed when the surviving animals were sacrificed and necropsies were performed. The veterinary pathologist report appears in Appendix F.

DISCUSSION

In the limit study, the DMSO Evaporator Sludge (TP015) was not lethal at a dose of 5.0 ml/kg while the DMSO Recycle Solvent (TP013) produced 50 percent mortalities (5 of 7 males, 2 of 7 females) at an equivalent dose. There was also a 21 percent (1 of 7 males, 2 of 7 females) mortality rate in the Virgin DMSO (TP014) group and a 15 percent (1 of 7 males, 1 of 6 females) mortality rate in the vehicle control group. The pathology findings of the study indicate that mortalities which occurred were, in large part, due to improper administration of the dosing material. The mortality pattern of the DMSO Recycle Solvent (TP013) did, however, suggest a possible mean lethal dose below 5.0 ml/kg.

The LD₅₀ study was performed on the DMSO Recycle Solvent due to its toxicity at the 5.0 ml/kg dosage level. The calculated LD₅₀ for the DMSO Recycle Solvent (TP013) was 4.1 ml/kg in male mice and 2.5 ml/kg in female mice, with respective 95 percent confidence limits (3.2 ml/kg, 5.1 ml/kg) and (2.2 ml/kg, 2.8 ml/kg). The predominant clinical signs were depression, inactivity, excitation, and aggression, with mild to moderate loss of equilibrium. The toxicity of the DMSO Recycle Solvent sample can be attributed to the RDX and HMX in the sample, as the DMSO Evaporator Sludge contains only 0.4 percent nitramines versus 5.9 percent in the Recycle Solvent.

White--8

The Vehicle Control DMSO used in this study is reagent grade for laboratory use (0.08% water). Virgin DMSO is the bulk type or industrial grade used extensively as both a reaction medium and a reactant chemical (0.6% water). The mortalities observed with these compounds in the limit study were unexpected, as published toxicity data indicate the oral LD₅₀ of DMSO in the mouse approximately 21 gm/kg (7). This suggests that the mortalities were probably due to technical problems in the dosing of the mice, especially since no deaths were observed with the reagent grade DMSO (vehicle control) in the LD₅₀ study.

CONCLUSION

The higher toxicity of the DMSO Recycle Solvent compared to that of the Evaporator Sludge or Virgin DMSO is likely due to the greater amount of RDX and HMX present in the solution. The DMSO Recycle Solvent should be classified as moderately toxic (8).

RECOMMENDATION

No recrystallization pilot plant recommendation is warranted based solely upon this report. Further data are necessary for the health hazard assessment of these compound solutions.

REFERENCES

1. McNamara BP, Averill HP, Owens EJ, Callahan JF, Fairchild DG, Cinchta HP, Rengstroff RH, Biskup RK. The toxicology of cyclotrimethylenetrinitramine (RDX) and cyclotetremethylenetetranitramine (HMX) solutions in dimethyl sulfoxide [DMSO], cyclohexenone, and acetone. Edgewood Arsenal, MD.: April 1974; Technical Report EB-TR-73040.
2. Cholakis JM, Wong LC, Van Goethern DL, Minor J, Short R, Spring H, Ellis, HV. Mammalian toxicological evaluation of RDX. Fort Detrick, MD.: U.S. Army Medical Research and Development Command, September 1980.
3. Tyson CA, Dilley JV, Sasmore DP, Spangford RJ, Newell GW, Dacre JC. Single-dose and repeated-exposure toxicity of a complex waste water from munitions manufacturing plants. J Tox Environ Health 1982; 9:545-564.
4. Stidham, BR. Analysis of waste waters for organic compounds unique to RDX/HMX manufacturing processing. Washington DC: U.S. Army Medical Research and Development Command, December 1979. Technical Report No. HDC-51-79
5. Federal Register, Part IV, Environmental Protection Agency, 44;145 (para 772.112.21) July 1979.
6. Finney, DJ. Probit Analysis. 3d ed. Cambridge: Cambridge, University Press, 1971; 20-80.
7. Doull J, Klassen CD, Amdur MO. In: Casarett and Doulls Toxicology, 2nd ed. New York: MacMillan Publishing Co., Inc., 1980; 12, 18-22.

	Page
Appendix A, Chemical Data	
Sample Analysis.....	13
Holston Report.....	18
Chemical Data for Sample Constituents.....	20
Appendix B, Animal Data	
Study 82040.....	25
Study 83003.....	26
Appendix C, Environmental Conditions.....	27
Appendix D, Historical Listing of Study Events	
Study 82040.....	29
Study 83003.....	30
Appendix E, Statistical Analysis.....	31
Appendix F, Pathology Report.....	33

Toxicity Test Sample Composition^a

Concentration by HPLC, g/l

Sample	^b RDX	^c HMX	^d TAX	^e SEX	^g % H ₂ O	% DMSO
Virgin DMSO ^f	0	0	0	0	0.63 ¹	99.37 ^j
DMSO Recycle Solvent ^h	24.188	39.542	0.263	0	35.48 ¹	58.64 ^j
DMSO Evaporator Sludge ^f	0.548	0.942	3.521	0	5.35 ¹	94.19 ^j

Calculated Data In Weight Percent^a

Sample	RDX	HMX	TAX	SEX	H ₂ O	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

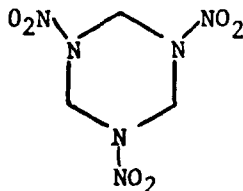
^a Data supplied by sponsor^b RDX: Hexahydro-1,3,5-Trinitro-1,3,5-Triazine^c HMX: Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine^d TAX: 1-Acetylhexahydro-3,5-Dinitro-1,3,5-Triazine^e SEX: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine^f At ambient temperature.^g By Karl Fisher^h Analysis of equilibrium 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: C₃H₆N₆O₆

Molecular weight: 222.13 g/mole

Physical State: White crystals varying in size

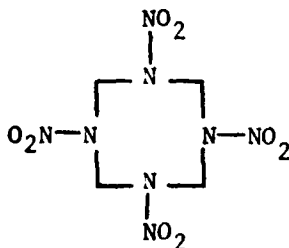
Melting point: 200-203 C

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:



APPENDIX A (cont.)

Empirical formula: $C_4H_8O_8N_8$

Molecular weight: 296.17 g/mole

Physical state: White crystals of varying size

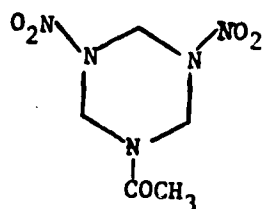
Melting point: 280 C

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

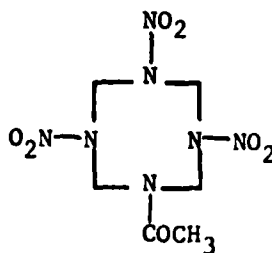
Melting point: 156 C

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: $\begin{array}{c} CH_3-S-CH_3 \\ | \quad \quad | \\ \quad \quad O \end{array}$

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: Dimethyl Sulfoxide (DMSO) reagent grade

Chemical Abstract Service Registry Number: 00006-76-85

Structural formula: $\begin{array}{c} CH_3-S-CH_3 \\ | \quad \quad | \\ \quad \quad O \end{array}$

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

HOLSTON DEFENSE CORPORATION

WEST STONE DRIVE
KINGSPORT, TENNESSEE 37660

June 22, 1983

TELEPHONE: AREA CODE 615 247-9111

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

Contracting Officer's Representative
June 22, 1983
Page 2

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
Plant Manager

Attachments (3)

APPENDIX A (cont.)

<u>HPLC Parameters</u>	<u>Components Detected</u>
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

<u>HPLC Parameters</u>	<u>Components Detected</u>
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

HPLC Analysis of Crude RDXHPLC ParametersComponents Detected

Column: Waters CN, 1/4" x 12" ss

Detector: UV, 215-290 nm in
10 nm incrementsSolvent System: 70% iso-octane
15% chloroform
10% acetonitrile
5% methanol

Flow Rate: 3.0 ml/min

Injection Volume: 10 microliters

RDX

HMX

SEX

HPLC Analysis of Crude HMXHPLC ParametersComponents Detected

Column: Waters CN, 1/4" x 12" ss

Detector: UV, 215-290 nm in
10 nm incrementsSolvent System: 70% iso-octane
15% chloroform
10% acetonitrile
5% methanol

Flow Rate: 3.0 ml/min

Injection Volume: 10 microliters

RDX

HMX

SEX

ATTACHMENT III

Quantitative Analysis of DMSO/Explosives SamplesSample 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:

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

ANIMAL DATA
GLP STUDY 82040

Study Type: Acute Oral Toxicity Limit Test of DMSO Process Stream
Samples in Male and Female Mice.

Species: Albino laboratory mouse (Mus musculus)

Strain: ICR

Source: Hilltop Laboratory Animals
P.O. Box 25
Chetsworth, CA 91311

Sex: Male and Female

Age: On Day of Dosing, 7 weeks

Dates of Birth: 16 November 1982

Method of Randomization: Weight bias stratified using the RANDOM
Computer Program (LAIR SOP OP-156-21).

Animals in Each Group: 7 male and 7 female animals.

Condition of Animals at Start of Study: Normal

Body Weight Range at Dosing: Males 24 to 32 g
Females 17 to 26 g

Identification Procedures: Ear tagged (LAIR SOP OP-ARG-1), numbers
82C00243 to 82C00363, with exclusions.

Pretest Conditioning: Quarantine/acclimation 22 December 1982 to 3
January 1983

Justification: The laboratory mouse has been proven to be a sensitive
and reliable system for lethal dose determination.

ANIMAL DATA
GLP STUDY 83003

Study Type: Acute Oral Toxicity (LD₅₀) Test of DMSO Process Stream
Sample Recycle Solvent (TP013) in Male and Female Mice

Species: Albino laboratory mouse (Mus musculus)

Strain: ICR

Source: Hilltop Laboratory Animals
P.O. Box 25
Chetsworth, CA 91311

Sex: Male and Female

Age: On day of dosing (30 March 1983)
Male 6 weeks
Female 9 weeks

Dates of Birth: Male 16 February 1983
Female 29 January 1983

Method of Randomization: Weight bias stratified using the RANDOM
Computer Program (LAIR SOP OP-ISG-21).

Animals in Each Group: 8 males, 8 females

Condition of Animals at Start of Study: Normal.

Body Weight Range at Dosing: Males 18 to 24 g
Females 16 to 26 g

Identification Procedure: Ear tagged (LAIR SOP OP-ARG-1), numbers
83C00101 to 83C00202 without exclusions.

Pretest Conditioning: Quarantine/acclimation 22-29 March 1983.

Justification: The laboratory mouse has been proven to be a sensitive
and reliable system for lethal dose determination

ENVIRONMENTAL CONDITIONS

Caging: Number/cage - 1

Type of Cages Used: Stainless steel "mesh drawer rack" type. Bedding:
none.

Diet: Certified Purina Rodent Chow, Diet #5002
Ralston Purina Company
Checkerboard Square
St. Louis, MO 63188
ad lib.

Water: Automatic Lixit dispenser.

Temperature: 25.5 ± 1 C

Relative Humidity: $55 \pm 2\%$

Photoperiod: 0530 to 2000 hours per day (light 14-1/2 hours).

HISTORICAL LISTING OF STUDY EVENTS
GLP STUDY 82040

Date	Event
22 Dec 82	Animals arrived at LAIR. Sexes were identified. Mice were observed for illness, ear tagged, weighed, and caged in the GLP Suite. At least 56 animals were assigned to the study.
23 Dec 82 - 3 Jan 83	Animals checked once daily.
28 Dec 82	Animals weighed and randomized into dose groups.
23,27,30 Dec 82	Animals conditioned to dosing procedure using sterile gavage tubes (no material injected).
4 Jan 83	Animals weighed and dosed. Observations conducted one to two hours after dosing. Animals observed for clinical signs which were recorded.
5-17 Jan 83	Animals observed daily for clinical signs.
7,10,14 Jan 83	Animals weighed.
17 Jan 83	Food removed at 1600 hours.
18 Jan 83	Animals observed for clinical signs at 0700 hours and weighed. Animals delivered to PSG Necropsy Suite for sacrifice and gross necropsy by 0900 hours.

HISTORICAL LISTING OF STUDY EVENTS
GLP STUDY 83003

Date	Event
23 Mar 83	Animals arrived at LAIR. Sexes were identified. Mice were observed for illness, ear-tagged, weighed, and caged in the GLP Suite. At least 96 animals were assigned to the study.
24-29 Mar 83	Animals checked once daily.
25 Mar 83	Animals weighed and randomized into dose groups.
26 Mar 83	Animals weighed.
28,29 Mar 83	Animals conditioned to dosing procedure using sterile gavage tubes (no material injected).
30 Mar 83	Animals weighed and dosed. Observations conducted one to two hours after dosing. Animals observed for clinical signs which were recorded.
31 Mar - 11 Apr 83	Animals observed for clinical signs daily.
1,4,8 Apr 83	Animals weighed.
11 Apr 83	Food removed at 1600 hours.
12 Apr 83	Animals observed for clinical signs at 0700 hours and weighed. Animals delivered to PSG Necropsy Suite for sacrifice and gross necropsy by 0900 hours.

STATISTICAL ANALYSIS

Eight male and eight female animals were assigned to each of six study groups by simple random sampling techniques using a program, Random, on the Data General C 330 Computer.

Bliss method of probit analysis was used to determine the LD_1 , LD_{50} , and LD_{95} values along with the corresponding 95% confidence limits*. The program, PROBIT, was used to determine the probit curve and the lethal dose values. The probit regression line fit to the data was:

$$\text{Males } Y = 1.6 + 5.6 \text{ Log } X$$

$$\text{Females } Y = 4.2 + 1.9 \text{ Log } X$$

where X is the dose and Y the corresponding probit value.


VIRGINIA L. GILDENGORIN, PhD
DAC, Statistician
20 October 1983

*Results appear in Table 3, Body of Report, page 6.

APPENDIX E

PATHOLOGY REPORT

GLP Study 82040

Acute Oral Toxicity Limit Test in Male and Female Mice of DMSO
Process Samples [a. DMSO Recycle Solvent (TPO13), b.
Virgin DMSO Solution (TPO14), and DMSO Evaporator Sludge (TPO15)]

History: The male and female albino ICR mice in this study were divided into 5 groups. All groups but the cage controls received a single dose of either 5 ml/kg of vehicle or one of the test solutions by oral gavage. The solution dosed with and the number of mice in each group were as follow:

- Group 1 (Cage controls) - 7 males, 7 females
- Group 2 (Vehicle controls) - 7 males, 7 females
- Group 3 (TPO13) - 7 males, 7 females
- Group 4 (TPO15) - 7 males, 7 females
- Group 5 (TPO14) - 7 males, 7 females

Seven male mice died: 1 mouse in Group 5 was found dead 21 minutes after being dosed, 1 mouse in Group 2 was found dead 25 hours and 26 minutes after dosing, and 5 mice in Group 3 were found dead between 17 hours and 27 minutes and 235 hours and 34 minutes after dosing.

Five female mice died: 2 mice in Group 5 were found dead, 1 at 5 minutes and 1 at 12 minutes after dosing, 2 mice in Group 2 were found dead, 1 at 1 hour and 27 minutes and 1 at 1 hour and 33 minutes after dosing, and 1 mouse in Group 3 was found dead 17 hours and 14 minutes after dosing.

All other mice survived until termination of the study, 14 days after dosing. The mice were killed by exsanguination from severed axillary vessels while under anesthesia produced by intraperitoneal injection of pentobarbital.

Gross necropsy findings: Most mice that died had lesions that may have been due to the toxic effects of tested compound and/or vehicle, but more than likely were due to aspiration of tested materials and/or vehicle or gouging with the stomach tube: 1/1* male and 1/2 females in Group 2, 3/5 males in Group 3, and 1/1 male and 2/2 females in Group 5 had lungs with red-mottled lobes, and 1/2 females in Group 2 and 1/1 male and 2/2 females in Group 5 had hydrothorax.

Three mice that died had lesions that were due to being gouged with the stomach tube: 1/2 females in Group 2 and 1/5 males in Group 3 had hemothorax and 1/1 female in Group 3 had a retroesophageal blood clot.

*Number of animals affected/Number of animals in the group or Number of animals affected/Number of animals that died.

The male in Group 3 that was found dead 235 hours after being dosed had black material in its stomach and small intestine that may have been blood. The reason for the presence of the black material was not determined.

The stomach of the male in Group 2 that died was distended with clear fluid which was probably water. Mild autolysis was present in several mice that died: 1/1 male in Group 2 and 3/5 males and 1/1 female in Group 3.

Necropsies revealed no gross lesions in male or female ICR mice that were killed at termination of the study. One of 7 male mice in Group 4 had pinworms.

Summary:

1. The number and pattern of deaths suggest that TPO13 kills male ICR mice.
2. Necropsies of mice that died indicate that almost all deaths were most likely due to aspiration of tested material and/or vehicle or trauma associated with administration of tested material and/or vehicle.
3. No gross lesions were observed in any mice killed at termination of the study.

Glen E. Marrs, Jr.

GLEN E. MARRS, JR., DVM, MS

Diplomate, A.C.V.P.

MAJ, VC

Assistant Chief, Pathology Services Group

Division of Research Support

7 March 1983

PATHOLOGY REPORT

GLP Study 83003

Acute Oral Toxicity Study (LD_{50}) of DMSO Process Stream Recycle Solvent Sample (LAIR Code No. TPO13) in Male Mice

History: The male albino ICR mice in this study were divided into 6 groups; a cage control group, a vehicle control group, and 4 dose groups. The mice in all groups but the cage controls received a dose of either reagent grade DMSO (vehicle controls) or DMSO Process Stream Recycle Solvent solution (TPO13) by oral gavage. The solution dosed with and number of mice in each group were as follow:

Group 1 (Cage controls) - 8 mice

Group 2 (Vehicle controls) - 8 mice

Group 3 (3.0 ml/kg) - 8 mice

Group 4 (4.0 ml/kg) - 8 mice

Group 5 (5.0 ml/kg) - 8 mice

Group 6 (6.0 ml/kg) - 8 mice

Eighteen mice died, 14 died between 5 minutes and 2 hours and 41 minutes after being dosed and 1 was found dead 46 hours and 12 minutes after being dosed with TPO13. The deaths of 2/8* mice in group 3, 3/8 mice in group 4, 7/8 mice in group 5, and 6/8 mice in group 6 were due to the TPO13 solutions. All other mice survived until the conclusion of the study. The surviving mice were killed by exsanguination from severed axillary vessels while under anesthesia produced by intraperitoneal injection of pentobarbital.

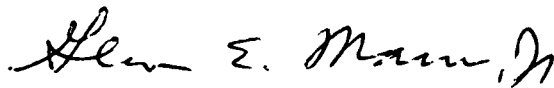
Necropsy findings: Necropsies revealed no gross lesions in 7/18 mice that died; 1/2 in group 3, 2/3 in group 4, and 4/7 in group 5. Eleven of 18 mice that died had gross lesions that were due to the tested solutions. The lungs of 2/6 mice in group 6 were mottled red and wet or mottled red and failed to collapse. The muzzle of 1/2 mice in group 3, 1/3 mice in group 4, 3/7 mice in group 5, and 5/6 mice in group 6 were covered by clear wet material.

*Number of mice affected/Number of mice in the group or Number of mice affected/Number of mice that died.

Necropsies revealed no gross lesions in the male ICR mice that were killed at the conclusion of the study.

Summary:

1. The deaths of 18 male ICR mice were due to the tested solutions.
2. Gross lesions were observed in 11/18 male mice that died. The lung lesions in 2 group 6 mice and the clear wet material on the muzzle of some mice in each dose group that received TP013 were due to the tested solutions.
3. No gross lesions were observed in 7/18 male mice that died.
4. No gross lesions were observed in the male ICR mice that were killed at the conclusion of the study.



GLEN E. MARRS, JR., DVM, MS
Diplomate, A.C.V.P.
MAJ, VC
Assistant Chief, Pathology Services Group
Division of Research Support

10 June 1983

PATHOLOGY REPORT

GLP Study 83003

Acute Oral Toxicity Study (LD_{50}) of DMSO Process Stream Recycle Solvent Sample (LAIR Code No. TPO13) in Female Mice

History: The female albino ICR mice in this study were divided into 6 groups; a cage control group, a vehicle control group, and 4 dose groups. The mice in all groups but the cage controls received a dose of either reagent grade DMSO (vehicle controls) or DMSO Process Stream Recycle Solvent solution (TPO13) by oral gavage. The solution dosed with and number of mice in each group were as follow:

Group 1 (Cage controls) - 8 mice

Group 2 (Vehicle controls) - 7 mice

Group 3 (4.0 ml/kg) - 8 mice

Group 4 (5.0 ml/kg) - 7 mice

Group 5 (6.0 ml/kg) - 8 mice

Group 6 (3.0 ml/kg) - 8 mice

Twenty-one mice died between 5 minutes and 1 hour and 53 minutes after being dosed with TPO13. The deaths of 5/8* mice in group 6, 4/8 mice in group 3, 6/7 mice in group 4, and 6/8 mice in group 5 were due to the TPO13 solutions. All other mice survived until the conclusion of the study. The surviving mice were killed by exsanguination from severed axillary vessels while under anesthesia produced by intraperitoneal injection of pentobarbital.

Necropsy findings: Necropsies revealed no gross lesions in 14/21 mice that died; 5/5 in group 6, 2/4 in group 3, 3/6 in group 4, and 4/6 in group 5. Seven of 21 mice that died had gross lesions that were due to the tested solutions. The lungs of 1/4 mice in group 3 were mottled red. The muzzle of 2/4 mice in group 3, 2/6 mice in group 4, and 2/6 mice in group 6 were covered by clear wet material.

One of 6 mice in group 4 that died had a subcutaneous abscess in the area of the left axilla. This lesion most likely represented focal mastitis and was considered to be an incidental finding.

*Number of mice affected/Number of mice in the group or Number of mice affected/Number of mice that died.

Necropsies revealed no gross lesions in the female ICR mice that were killed at the conclusion of the study.

Summary:

1. The deaths of 21 female ICR mice were due to the tested solutions.
2. Gross lesions were observed in 7/21 female mice that died. The lung lesion in 1 group 3 mouse and the clear wet material on the muzzle of 2 mice in groups 3,4 and 5 were due to the tested solutions.
3. No gross lesions were observed in 14/21 female mice that died.
4. No gross lesions were observed in the female ICR mice that were killed at the conclusion of the study.

Glen E. Marrs Jr.

GLEN E. MARRS, JR., DVM, MS

Diplomate, A.C.V.P.

MAJ, VC

Assistant Chief, Pathology Services Group

Division of Research Support

10 June 1983

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
and Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, Frederick MD 21701

Defense Technical Information Center
ATTN: DTIC-DDA (12 copies)
Cameron Station
Alexandria VA 22314

Director of Defense Research and Engineering
ATTN: Assistant Director, Environmental
and Life Sciences
Washington DC 20301

The Surgeon General
ATTN: DASG-TLO
Washington DC 20314

HQ DA (DASG-ZXA)
WASH DC 20310

Commandant
Academy of Health Sciences
ATTN: HSHA-CDM
Fort Sam Houston TX 78234

Assistant Dean
Institute and Research Support
Uniformed Services University
of Health Sciences
6917 Arlington Road
Bethesda MD 20014

Commander
US Army Environmental Hygiene Agency
Aberdeen Proving Ground MD 21070

US Army Research Office
ATTN: Chemical and Biological Sciences
Division
P.O. Box 1221
Research Triangle Park NC 27709

Biological Sciences Division
Office of Naval Research
Arlington VA 22217

Director of Life Sciences
USAF Office of Scientific Research (AFSC)
Bolling AFB
Washington DC 20332

Director
Walter Reed Army Institute of Research
Washington DC 20307

Commander
US Army Medical Research Institute
of Infectious Diseases
Fort Detrick, Frederick MD 21701

Commander
US Army Research Institute
of Environmental Medicine
Natick MA 01760

Commander
US Army Institute of Surgical Research
Brooke Army Medical Center
Fort Sam Houston TX 78234

Commander
US Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick, Frederick MD 21701

Commander
US Army Aeromedical Research Laboratory
Fort Rucker AL 36362

Commander
US Army Research Institute
of Chemical Defense
Aberdeen Proving Ground
Edgewood Arsenal MD 21010

Commander
Naval Medical Research Institute
National Naval Medical Center
Bethesda MD 20014

Commander
USAF School of Aerospace Medicine
Aerospace Medical Division
Brooks Air Force Base TX 78235

FILMED

02 - 84