

AD-A165 756

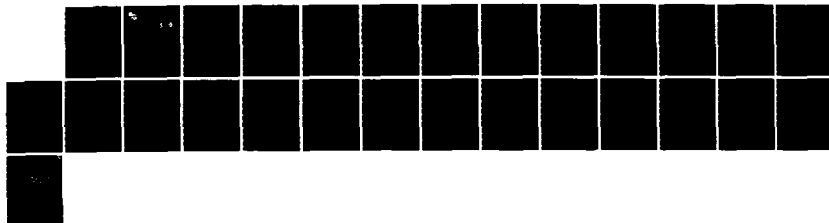
MUTAGENIC POTENTIAL OF P-DITHIANE(U) LETTERMAN ARMY
INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA
S K SANO ET AL. AUG 85 LAIR-207

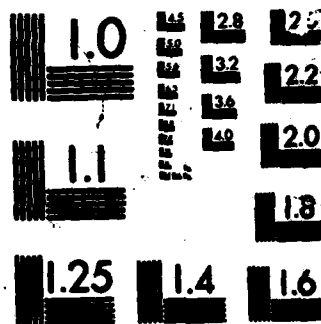
1/1

UNCLASSIFIED

F/G 6/20

NL





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

2



AD-A165 756

INSTITUTE REPORT NO. 207

DTIC
ELECTE
MAR 20 1986
S D

MUTAGENIC POTENTIAL OF p-DITHIANE

STEVEN K. SANO, BA, SP5

and

DON W. KORTE JR, PhD, MAJ MSC

TOXICOLOGY GROUP

DIVISION OF RESEARCH SUPPORT

DTIC FILE COPY

AUGUST 1985

Toxicology Series 95

GLP Study 84031

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

86 3 19 022

Mutagenic potential of p-dithiane (Toxicology Series 95)--Sano and Korte

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.

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)

 23 Aug '85
.....
(Signature and date)

EDWIN S. BEATRICE, M.D.
Colonel, MC
Commanding, LAIR

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

DISCLAIMER NOTICE

**THIS DOCUMENT IS BEST QUALITY
PRACTICABLE. THE COPY FURNISHED
TO DTIC CONTAINED A SIGNIFICANT
NUMBER OF PAGES WHICH DO NOT
REPRODUCE LEGIBLY.**

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Institute Report No. 207	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Mutagenic Potential of p-Dithiane		5. TYPE OF REPORT & PERIOD COVERED Final 24 Sep - 12 Oct 1984
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Steven K. Sano, BA SP4 Don W. Korte, Jr, PhD, MAJ, MS		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3516277A875 WU 308, APC TL05
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, MD 21701-5012		12. REPORT DATE August 1985
		13. NUMBER OF PAGES 30
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		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) Mutagenicity, Genetic Toxicology, Ames Assay, p-Dithiane		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Cont → The mutagenic potential of p-dithiane was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this assay. Keywords:		

DD FORM 1473

1 JAN 73

EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ABSTRACT

The mutagenic potential of p-dithiane was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this assay.

Key Words: Mutagenicity, Genetic Toxicology, Ames Assay,
p-Dithiane

PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Medical Bioengineering Research and
Development Laboratory
Fort Detrick, MD 21701-5010

WORK UNIT: 3516277A875 Medical Defense Against Chemical
Agents Projects; WU 308; APC TL05

GLP STUDY NUMBER: 84031

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD

PRINCIPAL INVESTIGATOR: SP4 Steven K. Sano, BA

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.

TEST SUBSTANCE: p-Dithiane (TA039)

INCLUSIVE STUDY DATES: 24 September - 12 October 1984

OBJECTIVE: The objective of this study was to determine the mutagenic
potential of p-dithiane (Batch Number 3030TH, LAIR Code TA039)
by using the Ames Salmonella/Mammalian Microsome
Mutagenicity Assay.

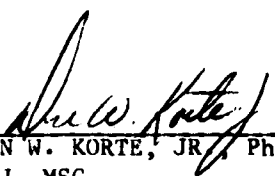
Accession For	
GRA&I	<input checked="" type="checkbox"/>
USARMC	<input type="checkbox"/>
USAMRIID	<input type="checkbox"/>
USAMRIID	<input type="checkbox"/>
Re: [illegible]	
Distribution/	
Availability Codes	
[illegible] or	
special	
A-1/24	

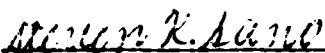
ACKNOWLEDGMENTS

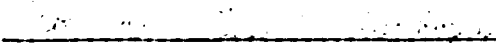
The authors wish to thank SP6 James Justus, BA; SP4 Paul Mauk, BA; PFC James Martin; and Mr. John Dacey, for their assistance in performing the research.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP study number 84031 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

 30 APR 85
DON W. KORTE, JR., Ph.D. / DATE
MAJ, MSC
Study Director

 25 APR 85
STEVEN K. SANO, B.A. / DATE
SP4, USA
Principal Investigator


CONRAD WHEELER, Ph.D. / DATE
DAC
Analytical Chemist



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

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

18 August 1985

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

1. I hereby certify that in relation to LAIR GLP Study 84031 the following inspections were made:

10 October 1984

12 October 1984

2. The report and raw data for this study were audited on 10 May 1984.

3. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 21 January 1985 report to Management and the Study Director.

GARY L. DUTCHER
SP6, USA
Quality Assurance Unit

TABLE OF CONTENTS

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	
Objective of the Study.....	1
METHODS	
Test Compound.....	1
Test Solvent.....	2
Chemical Preparation.....	2
Test Strains.....	2
Test Format.....	2
RESULTS.....	4
DISCUSSION.....	11
CONCLUSION.....	11
RECOMMENDATION.....	11
REFERENCES.....	12
APPENDIX.....	13

DISTRIBUTION LIST.....	18
------------------------	----

Mutagenic Potential of: p-Dithiane (TA039)--Sano and Korte

↙
The Ames Salmonella/Mammalian Microsome Mutagenicity Assay is a short-term screening assay that utilizes histidine auxotrophic mutant strains of Salmonella typhimurium to detect those compounds which are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the assay to increase sensitivity by simulating in vivo metabolic activation of the test compound. The Ames assay is an inexpensive yet highly predictive and reliable assay for detecting mutagenic activity and thus carcinogenic potential (4).

Cont'd 221473
Objective of the Study

The objective of this study was to determine the mutagenic potential of p-dithiane (Batch Number 3030TH, LAIR Code TA039) by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay.

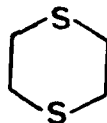
METHODS

Test Compound

Chemical name: p-Dithiane

Chemical Abstract Service Registry No.: 51330-42-8

Structural formula:



Empirical formula: $C_4H_5S_2$

Sano--2

Storage: Ten grams of p-dithiane (Batch Number 3030TH) were received from Aldrich Chemical Company, Inc (Milwaukee, WI) on 22 August 1984 and assigned the LAIR Code number TA039. The test compound was stored in a dessicator at room temperature (21°C) until use.

Chemical Properties/Analysis: Data characterizing the chemical composition and purity of the test material were obtained from Aldrich Chemical Co, Inc and confirmed by Infrared Spectrometer performed by the Toxicology Group, LAIR (Presidio of San Francisco, CA) (Appendix A).

Test Solvent

The test compound and the positive control chemicals were dissolved in grade I dimethyl sulfoxide (Lot Number 100F-0269) obtained from Sigma Chemical Co (St. Louis, MO).

Chemical Preparation

p-Dithiane was stored in a dessicator at room temperature (21°C) until used. On the day before dosing, 300 mg of the test compound was measured into a sterile vial and again stored at room temperature. On the day of dosing, the 300 mg sample was dissolved in a 6 ml volume of grade I dimethyl sulfoxide (Lot Number 100F-0269) to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates. The dosing procedure was completed within 20 minutes of dissolving the test compound.

Test Strains

Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538, obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality controls were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (2).

Test Format

p-Dithiane was evaluated for mutagenic potential according of Ames et al (3). A detailed description of the to the methods methodology is given in LAIR SOP, OP-STX-1 (2).

Toxicity Tests

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by

using minimal glucose agar (MGA) plates, concentrations of p-dithiane ranging from 1.6×10^{-3} mg/plate to 5 mg/plate, and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin were placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since none of the plates showed decreased macrocolony formation (below the level of the spontaneous reversion plates) or an observable reduction in the density of the background lawn, a maximum "limit" dose of 5 mg per plate was used in the mutagenicity assay.

Mutagenicity Assay

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5 both with and without 0.5 ml of the S-9 microsome fraction. The S-9 was purchased from Litton Bionetics (Kensington, MD). The optimal titer of this S-9, as determined by Litton Bionetics, was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and in all reagents came from a Polymetric model 200-3 Water Purifier (Sunnyvale, CA). Plates were incubated upside down in the dark, at 37°C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound assay. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound assay plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Ames et al (3). Concurrent sterility and strain verification controls were run. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The Salmonella strains were verified by a standard battery of tests. The following tests were run to determine if:

- Lipopolysaccharide layer (LP) alteration causes growth inhibition in the presence of crystal violet.
- An ampicillin-resistant R factor has allowed growth in strains TA98 and TA100 in the presence of ampicillin impregnated disks.
- Absence of excision repair mechanism has inhibited growth in the presence of ultraviolet light.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. These compounds, benzo [a] pyrene, 2-aminofluorene, 2-aminoanthracene and N-methyl-n'-nitro-n-nitrosoguanidine, were obtained from Sigma Chemical Co (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (5), a compound is considered mutagenic if the following criteria are met:

1. For strain TA98 and TA100, a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous revertant colony count for the strain. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.
2. For strains TA1535, TA1537, and TA1538, a correlated dose response over three concentrations is achieved with at least one dose yielding a revertant colony count three times the spontaneous colony count for the strain.

RESULTS

On 3 October 1984, the toxicity level determination was performed on p-dithiane (Table 1). For this experiment all sterility, strain verification, positive and negative controls were normal (Table 2). No toxicity was observed after exposure of the tester strain (TA100) to the highest dose used (5 mg/plate).

Normal results were obtained for all sterility, strain verification, and negative controls during the Ames Assay performed during the 3-day period 10 to 12 October 1984 (Tables 3-4). p-Dithiane did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 5).

TABLE 1

TOXICITY LEVEL DETERMINATION

Substance assayed: p-DITHIANE (TA039) Substance dissolved in: DMSO
 Study Number: 84031 Date: 5 OCT 84 Performed by: SANO

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
5 mg/plate	86	95	104	95	NL
1 mg/plate	115	104	106	108	NL
0.2 mg/plate	105	97	108	103	NL
0.04 mg/plate	107	85	104	99	NL
0.008 mg/plate	78	82	97	86	NL
0.0016 mg/plate	108	95	113	105	NL

(1) NC = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 2
STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	G	G	NG	NG (16mm)	NG	+
Wild Type	NT	NT	G	NT	NT	+

STERILITY CONTROL FOR TOXICITY LEVEL DETERMINATION

His-Bio Mix Initial: NG End: NG MCA Plate: NG

Top Agar Initial: NG End: NG

Diluent: DMSO-NG Nutrient Broth: NG

TA037: TA038: NG (c) NG (d) NG (e) NG

Test Compound (a) NG (b) NG (c) NG (d) NG (e) NG

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable

Spontaneous Revertants: TA 100, No 5-9 (102,111, 90)101

(1) + = expected response - = unexpected response

Study Number: 84031 Date: 4 OCT 84 By: SANO

TABLE 3

STRAIN VERIFICATION CONTROL FOR ASSAY

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	NG (17mm)	NG	+
100	NG	G	NG	NG (20mm)	NG	+
1535	NG	NT	NG	NG (18mm)	NG	+
1537	NG	NG (15mm)	NG	NG (17mm)	NG	+
1538	NG	NT	NG	NG (16mm)	NG	+
Wild Type	NT	NT	G	NT	NT	+

STERILITY CONTROL FOR ASSAY

His-Bio Mix Initial: NG End: NG Diluent: DMSO: NG
 Top Agar Initial: NG End: NG NCA Plate: NG
 S-9 Mix Initial: NG End: NG Nutrient Broth: NG
 Test Compound (a) NG (b) NG (c) NG (d) (e) (f)

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable

Study Number: 84031 By: SANO
 Date: 11 OCT 84

(1) + = expected response

- = unexpected response

TABLE 4
POSITIVE AND NEGATIVE CONTROL TEST
(Revertants/plate)
mean

COMPOUND	DOSE LEVEL	S-9 ADDED	TA98	TA100	STRAIN NUMBER		
					TA1535	TA1537	TA1538
AF	2 ug/plate	YES	(772,825,982) 860	(1053,878,1216) 1049			(913,966,820) 900
BP	2 ug/plate	YES	(230,175,387) 264	(335,332,302) 323		(32, 25, 21) 26	(78, 46, 86) 70
AA	2 ug/plate	YES	(1488,1613,1754) 1618	(1725,1495,1994) 1738		(224,205,211) 213	(927,1073,1089) 1030
MNG	2 ug/plate	NO		(1935,1737,2129) 1934			
	20 ug/plate	NO			(1852,1783,2053) 1896		

SPONTANEOUS REVERSION RATE (NEGATIVE CONTROL)

Before Assay	YES	(15, 13, 15) (27, 16, 16) 17	(89,102, 94) (113,113,106) 103	(15, 13, 12) (20, 15, 16) 15	(5, 6, 1) (4, 3, 5) 4	(12, 14, 14) (16, 8, 8) 12
After Assay	YES					
Before Assay	NO	(13, 24, 18) (13, 17, 20) 18	(86, 88, 87) (99, 79,108) 91	(13, 13, 16) (17, 15, 16) 15	(1, 4, 6) (6, 4, 9) 5	(13, 11, 18) (9, 15, 8) 12
After Assay	NO					

Study Number: 84031

Date: 12 Oct 84

Performed by: SANO & MARTIN

Compounds: AF = 2-aminofluorene, BP = Benzo (a) pyrene, AA = 2-aminanthracene,
MNG = N-methyl-N'-nitro-N-nitrosoguanidine

TABLE 5
p-DITHIANE ASSAY
(Revertants/Plate)
Mean

COMPOUND	DOSE LEVEL	S-9 ADDED	TA98	TA100	STRAIN NUMBER		
					TA1535	TA1537	TA1538
TA039	5 mg/plate	YES	(16, 14, 16) 15	(84, 73, 79) 79	(9, 11, 9) 10	(3, 1, 2) 2	(14, 9, 11) 11
		NO	(15, 19, 13) 16	(92, 90, 96) 93	(11, 10, 9) 10	(5, 3, 2) 3	(10, 10, 20) 13
TA019	1 mg/plate	YES	(18, 15, 18) 17	(90, 70, 112) 91	(9, 12, 17) 13	(7, 5, 9) 7	(18, 12, 16) 15
		NO	(12, 16, 18) 15	(98, 84, 93) 92	(11, 16, 15) 14	(3, 3, 8) 5	(7, 14, 10) 10
TA019	0.2 mg/plate	YES	(19, 22, 18) 20	(98, 84, 104) 95	(15, 16, 12) 14	(4, 5, 5) 5	(6, 15, 8) 10
		NO	(17, 14, 12) 14	(99, 87, 98) 95	(14, 14, 17) 15	(7, 2, 4) 4	(9, 9, 8) 9

Study Number: 8-031 Date: 12 Oct 84 Performed by: SANO & MARTIN

TABLE 5 (cont.)
p-DITHIANE (TA039)
(Revertants/Plate)
Mean

COMPOUND	DOSE LEVEL	S-9 ADDED	TA98	TA100	STRAIN NUMBER TA1535	TA1517	TA1538
TA039	0.04 mg/plate	YES	(14, 18, 20) 17	(109, 92, 105) 102	(19, 12, 10) 14	(2, 4, 4) 3	(8, 11, 15) 11
		NO	(8, 15, 17) 13	(106, 95, 104) 102	(21, 24, 15) 20	(5, 3, 5) 4	(13, 5, 6) 8
TA039	0.008 mg/plate	YES	(22, 26, 10) 19	(101, 81, 105) 96	(13, 14, 15) 14	(9, 5, 5) 6	(10, 9, 18) 12
		NO	(7, 10, 27) 15	(86, 87, 79) 84	(15, 15, 11) 14	(14, 4, 2) 7	(6, 13, 18) 12
TA039	0.0016 mg/plate	YES	(16, 20, 18) 18	(112, 105, 125) 114	(9, 11, 15) 12	(1, 4, 3) 3	(11, 13, 10) 11
		NO	(25, 13, 7) 15	(99, 109, 85) 98	(15, 17, 16) 16	(9, 7, 7) 8	(18, 13, 9) 13

Study Number: 84031 Date: 12 Oct 84 Performed by: SANO & MARTIN

DISCUSSION

Certain test criteria must be satisfied before an Ames assay can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, LP layer alterations, and DNA excision repair deficiencies. Second, the Salmonella strains must be responsive to the mutagenic process by exposing the strains to known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on macrocolony and microcolony formation. If these tests are performed and expected data are obtained, then the results of Ames assay can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, p-dithiane was evaluated in the Ames assay. Criteria for a positive response are a correlated dose-response relationship for the positive strains and a two-fold (strains TA98 or TA100) or three-fold (strains TA1535, TA1537, or TA1538) increase in revertant colony counts relative to the respective negative control counts (5). p-Dithiane did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this assay indicate that p-dithiane is not mutagenic when evaluated in the Ames assay.

CONCLUSION

p-Dithiane, both with and without metabolic activation, is not mutagenic in the Ames assay as conducted in this study.

RECOMMENDATION

p-Dithiane should be tested in other genetic toxicity assays in accordance with the Toxic Substance Control Act.

REFERENCES

1. McCann JE, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 1975;72:5135-5139.
2. Ames Salmonella/Mammalian Microsome Mutagenicity Assay. LAIR Standard Operating Procedure OP-STX-1, Letterman Army Institute of Research, Presidio of San Francisco, California, 15 November 1983.
3. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with Salmonella/Mammalian microsome mutagenicity test. Mutation Res 1975;31:347-364.
4. Vogel HJ, Bonner DM. Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem 1956;218:97-106.
5. Brusick D. Genetic Toxicology. In: Hayes AW, ed. Principles and Methods of Toxicology. New York: Raven Press, 1982: 223-272.

CHEMICAL DATA

Chemical name: 1,4-Dithiane

Chemical Abstracts Service Registry No.: 505-29-3

Chemical structure:



Molecular formula: $C_4H_8S_2$

Molecular weight: 120.24

Physical state: White crystals

Melting point: 110-112°C (data supplied by source)

Source: Aldrich Chemical Co.
Milwaukee, WI

Lot number: 3030TH

Analytical data: Compound was described as 97% pure by source. Analysis provided by sponsor demonstrated a purity of 99.92%.* NMR and IR analyses were performed after receipt of the compound: NMR (80 MHz, d_6 -DMSO): δ 2.82 (Singlet, 8 H, $-CH_2-$).† IR (KBr): 2945, 2905, 1410, 1280, 1270, 1150, 905, and 890 cm^{-1} .‡ NMR and IR data were identical to published standard IR[§] and NMR spectra.

Stability: No decomposition of 1,4-dithiane was detected by NMR after 66 h in DMSO.†

*Rosencrance AB. [Memorandum for Dr. Reddy]. SUBJECT: Results from the chemical analysis of three compounds slated for toxicity testing (24 July 1984). Frederick, Maryland: USAMBRDL.

†Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p74. Letterman Army Institute of Research, Presidio of San Francisco, CA.

‡Ibid. p75.

§Pouchert, CL. The Aldrich Library of NMR Spectra. Vol 1. 2nd ed. Milwaukee: Aldrich Chemical Co., 1981: 211, Spectrum B.

Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #7752.

APPENDIX A



Chemists Helping Chemists in Research and Industry

aldrich chemical company, inc.

ANALYTICAL DATA

Date June 18, 1984

Our: D21770-0 Para-dithiane, 97%

Batch No.: 3030TH

Analytical Results:

Appearance Off white crystals

m.p. 111-113 deg. C b.p.

n_D^{20} $[\alpha]_D^{20}$

Spectral Data:

I.R. Conforms to structure and standard as illustrated on page 160 B of Edition III, of "The Aldrich Library of Infrared Spectra".

U.V.

N.M.R.

Assay:

V.P.C.

Titration 99.9%, S-Content

Other

KB/kb

A. Napiorkowski
Anna Napiorkowski, Manager
Quality Control/Quality Assurance

APPENDIX A (cont.)

SGRD-UBG-L

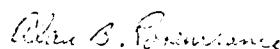
24 July 34

MEMORANDUM FOR DR. REDDY

SUBJECT: Results from the Chemical Analysis of Three Compounds Listed for Toxicity Testing

Benzothiazole, 1,4-thioxane and 1,4-dithiane were given by Dr. Reddy for analysis on 15 June 34. The following is a summary of the results from those analysis:

	% of Total	Formula	Compound	Other Possibilities
<u>Benzothiazole</u>				
	98.88	C ₇ H ₅ NS	Benzothiazole	
	0.61	C ₈ H ₇ NS	2-Methylbenzothiazole	(isomers)
	0.26	C ₆ H ₅ N ₃	Aniline	3 or 4-Cyanopyridine
	0.12	C ₁₂ H ₁₀ S ₂	Diphenyldisulfide	
	0.11	C ₇ H ₉ N	Toluidine (isomers)	Benzylamine, N-Methylaniline
	0.03	C ₈ H ₇ NS	Methylbenzothiazole	(isomers)
<u>1,4-Thioxane</u>				
	98.93	C ₄ H ₈ OS	1,4-Thioxane	
	1.06	C ₄ H ₈ S ₂	1,4-Dithiane	
<u>1,4-Dithiane</u>				
	99.92	C ₄ H ₈ S ₂	1,4-Dithiane	
	0.08	C ₄ H ₈ S ₃	Methyltrithiane	



ALAN B. ROSENBAUM
Research Chemist

CC:
Dr. Zulkarni
Dr. Rosenblatt

APPENDIX A (Not Included)

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
and Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, MD 21701-5012

Defense Technical Information Center
ATTN: DTIC/DDAB (2 copies)
Cameron Station
Alexandria, VA 22304-6145

Office of Under Secretary of Defense
Research and Engineering
ATTN: R&AT (E&LS), Room 3D129
The Pentagon
Washington, DC 20301-3080

The Surgeon General
ATTN: DASG-TLO
Washington, DC 20310

HQ DA (DASG-ZXA)
WASH DC 20310-2300

Commandant
Academy of Health Sciences
US Army
ATTN: HSHA-CDM
Fort Sam Houston, TX 78234-6100

Uniformed Services University
of Health Sciences
Office of Grants Management
4301 Jones Bridge Road
Bethesda, MD 20814-4799

US Army Research Office
ATTN: Chemical and Biological
Sciences Division
PO Box 12211
Research Triangle Park, NC 27709-2211

Director
ATTN: SGRD-UWZ-L
Walter Reed Army Institute
of Research
Washington, DC 20307-5100

Commander
US Army Medical Research Institute
of Infectious Diseases
ATTN: SGRD-ULZ-A
Fort Detrick, MD 21701-5011

Commander
US Army Medical Bioengineering
Research & Development Laboratory
ATTN: SGRD-UBG-M
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Medical Bioengineering
Research & Development Laboratory
ATTN: Library
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Research Institute
of Environmental Medicine
ATTN: SGRD-UE-RSA
Kansas Street
Natick, MA 01760-5007

Commander
US Army Institute of Surgical Research
Fort Sam Houston, TX 78234-6200

Commander
US Army Research Institute
of Chemical Defense
ATTN: SGRD-UV-AJ
Aberdeen Proving Ground, MD 21010-5425

Commander
US Army Aeromedical Research Laboratory
Fort Rucker, AL 36362-5000

AIR FORCE Office of Scientific
Research (NL)
Building 410, Room A217
Bolling Air Force Base, DC 20332-6448

Commander
USAFSAM/TSZ
Brooks Air Force Base, TX 78235-5000

Head, Biological Sciences Division
OFFICE OF NAVAL RESEARCH
800 North Quincey Street
Arlington, VA 22217-5000

END
FILMED

4-86

DTIC