Institute Report No. 315

Mutagenic Potential of 1,3-Bis[3-(1-Octoxymethyl)Imidazolium]Propane Dichloride Dihydrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

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GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

October 1988

Toxicology Series: 198

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

Mutagenic Potential of 1,3-Bis[3-(1-Octoxymethyl)Imidazolium]Propane Dichloride Dihydrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (Toxicology Series 198)--Sebastian and Korte

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Edwin S. Beatrice 2 Nov 88

Edwin S. Beatrice **(date)**
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UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

ADA203591

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Institute Report No. 315	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Genetic Toxicology Branch, Division of Toxicology; 6b. OFFICE SYMBOL (If applicable): SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION: Walter Reed Army Institute of Research
6c. ADDRESS (City, State, and ZIP Code): Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code): Washington, D.C. 20307-5100
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 67234; PROJECT NO. A875; TASK NO. BC; WORK UNIT ACCESSION NO. DA OH0366

11. TITLE (Include Security Classification): Mutagenic potential of 1,3-bis[3-(1-octoxymethyl)imidazolium] propane dichloride dihydrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test

12. PERSONAL AUTHOR(S): Suzanne E. Sebastian, Don W. Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM 4/21/86 TO 8/23/86	October 1988	19

16. SUPPLEMENTARY NOTATION: Toxicology Series 198

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Mutagenicity, Genetic toxicology, Ames Test, ~~1,3-bis[3-(1-octoxymethyl)imidazolium]propane dichloride dihydrate~~ oximes, imidazoles, propanes, chlorides, onium

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

The mutagenic potential of 1,3-bis[3-(1-octoxymethyl)imidazolium]propane dichloride dihydrate was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1537, and TA1538 were exposed to doses ranging from 0.4 mg/plate to 0.0000128 mg/plate. The test compound was not mutagenic under conditions of this test.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Edwin S. Beatrice, COL MC	22b. TELEPHONE (Include Area Code): 415-561-3600; 22c. OFFICE SYMBOL: SGRD-ULZ

DD Form 1473, JUN 86 — Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

ABSTRACT

The mutagenic potential of 1,3-BIS[3-(1-OCTOXYMETHYL) IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1537, and TA1538 were exposed to doses ranging from 0.4 mg/plate to 0.0000128 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE, oxime.

PREFACE

TYPE REPORT: Ames Test 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
Walter Reed Army Institute of Research
Washington, D.C. 20307-5100

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLEO

GLP STUDY NUMBER: 86002

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

PRINCIPAL INVESTIGATOR: Suzanne E. Sebastian, BA, SPC, USA

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOP's, stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM] PROPANE DICHLORIDE DIHYDRATE

INCLUSIVE STUDY DATES: 21 April 1986 - 23 August 1986

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of 1,3-BIS[3-(1-OCTOXYMETHYL) IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE (LAIR Code TP67) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

MAJ John W. Harbell, PhD, MSC; SGT Lillie D. Witcher, BS; and Ms. Joanne Wong provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 86002 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr 27 OCT 88

DON W. KORTE, Jr, PHD / DATE
MAJ, MSC
Study Director

Suzanne E. Sebastian 24 October 88

SUZANNE E. SEBASTIAN, BA / DATE
SPC, USA
Principal Investigator

Conrad Wheeler 27 Oct 88

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Analytical Chemist

DEPARTMENT OF THE ARMY

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

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 1 November 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 86Ø02

1. This is to certify that in relation to LAIR GLP Study 86Ø02, the following inspections were made:

15 April 1986	-	Protocol Review
21 May 1986	-	Plate Incorporation (TP62)
17 March 1987	-	Plate Incorporation (TP64)
2Ø March 1987	-	Plate Counting (TP64)

2. The institute report entitled "Mutagenic Potential of 1,3-Bis[3-(1-Octoxymethyl) Imidazolium] Propane Dichloride Dihydrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 198, was audited on 23 April 1987.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

TABLE OF CONTENTS

Mutagenic Potential of 1,3-BIS[3-(1-OCTOXYMETHYL) IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test-- Sebastian and Korte

INTRODUCTION

1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE was synthesized for a United States Army Medical Research and Development Command program charged with developing more effective oximes for treatment of nerve agent poisoning. The Ames Test is one of a series of tests in which these compounds will be evaluated to determine their relative potential for further development.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames Test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (1).

This evaluation of 1,3-BIS[3-(1-OCTOXYMETHYL) IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE utilizes a revision of the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (2). Two new tester strains, a frame-shift strain (TA97) and a strain carrying an ochre mutation on a multicopy plasmid (TA102), are added to the standard tester set.

Objective of the Study

The objective of this study was to determine the mutagenic potential of 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM] PROPANE DICHLORIDE DIHYDRATE (LAIR Code TP67) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical Name: 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM] PROPANE DICHLORIDE DIHYDRATE

LAIR Code Number: TP67

Physical State: White crystalline solid

Source: SRI International, Menlo Park, CA

Storage: 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE was received from SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 and assigned the LAIR Code number TP67. The test compound was stored at room temperature (21°C) until used.

Chemical Properties/Analysis: Data provided by SRI International characterizing the chemical composition and purity of the test material are presented in Appendix A along with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO). The test chemical was dissolved in glass distilled water. Reagent grade water used in this assay was first passed through a Technic Model 301 Reverse Osmosis Unit (Seattle, WA), then through a Corning MP-1 Mega Pure System glass distillation unit (Corning Glass Works, Corning, NY) (3).

Chemical Preparation

On the day of dosing, 100 mg of the test compound was measured into a sterile vial and dissolved in glass distilled water to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA97, TA98, TA100, TA102, TA1537, and TA1538 obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory in liquid nitrogen. Quality

control tests 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 (4).

Test Format

1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE was evaluated for mutagenic potential according to the revised Ames method (2). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (4).

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 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM] PROPANE DICHLORIDE DIHYDRATE 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 was 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 the three highest doses showed a decreased number of macrocolonies (below the spontaneous rate) and an observable reduction in the density of the background lawn, the highest dose selected for the mutagenicity test was 0.04 mg/plate.

Mutagenicity Test:

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 (batch R-315) was purchased from Microbiological Associates Inc. (Bethesda, MD). The optimal titer of this S-9, as determined by Microbiological Associates Inc., 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 (5). 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. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound 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 Maron and Ames (2). Sterility and strain verification controls were run concurrently. 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 integrity of the different *Salmonella* strains used in the assay was verified by the following standard tests:

- -Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer (LP) of the cell wall is present.

- -Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor in all strains except TA1537 and TA1538.

- -Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism (for all strains except TA102).

Six known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds, benzo[a]pyrene (lot 79C-05252), 2-aminofluorene (lot 021547), 2-aminoanthracene (lot 020797), mitomycin-C (lot 015F-0655), 4-nitroquinoline-n-oxide (lot 89C-0710) and N-methyl-N'-nitro-N-nitrosoguanidine (lot 127C-0342), 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 (6), a compound is considered mutagenic if 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 colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1537 and TA1538 (2,4). A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Maron and Ames (2) consider a compound mutagenic in tester strains TA97 and TA102 if a correlated dose response over three concentrations is achieved with the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count.

Deviations from the Protocol/SOP

Results with TA1535 were not obtained due to technical error. Since the test compound was negative in the other strains, conducting a special assay for TA1535 was deemed unnecessary.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

On 16 May 1986, the toxicity of 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE was determined (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 1). Exposure of the tester strain (TA100) to the three highest doses showed a decrease in the number of macrocolonies, and an observable reduction in the density of the background lawn, indicating chemical toxicity. Therefore, the highest dose selected for the mutagenicity test was 0.04 mg/plate. Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 20-22 August 1986 (Table 2). Some of the TA100 plates showed excessive clumping of the bacteria, presumably due to inadequate vortexing before plating. An accurate colony count on these plates could not be obtained. The data provided by the remaining strains are sufficient to reach a conclusion on the mutagenic potential of the test compound. 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3).

TABLE 1: TOXICITY LEVEL DETERMINATION FOR TP67

GLP STUDY NUMBER 86002

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)

CONCENTRATION	MEAN	±1SD	BACKGROUND LAWN*
START RUN NEGATIVE CONTROL	77	7.5	NL
5.0 mg/plate	0	0	NG
1.0 mg/plate	313	198.8	ST
0.2 mg/plate	206	62.5	ST
0.04 mg/plate	54	4.0	NL
0.008 mg/plate	83	4.6	NL
0.0016 mg/plate	89	10.8	NL
END RUN NEGATIVE CONTROL	92	10.1	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

	TA100*
HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG
STERILITY CONTROL	NG

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

*NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF TP67

GLP STUDY NUMBER 86002

STRAIN VERIFICATION

	OBSERVATIONS*				
STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA97	NG	G	NG	NG	NG
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA102	NG	G	G	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

*G = Growth, NG = No Growth

TABLE 3: Mutagenicity Assay for 1,3-BIS[3-(1-OCTOXYMETHYL) IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE (TP67)†

COMPOUND*	DOSE	TA97		TA98		TA100		TA102	
				WITHOUT S-9					
NEG CONTROL	0.0 mg	96	±5.8	17	±3.4	108	±15.0	76	±10.4
MITO C	0.5 µg							829	±117.5
MNNG	2.0 µg					1176	±50.0		
NQNO	0.5 µg	294	±14.5						
TP67	40.0 µg	106	±11.1	23	±2.5	119	±23.4	37	±2.0
TP67	8.0 µg	100	±8.6	17	±4.2	91	±13.5	63	±12.3
TP67	1.6 µg	98	±11.5	15	±0.7			71	±5.9
TP67	0.32 µg	91	±3.6	18	±3.6	114		51	±7.8
TP67	0.064 µg	114	±13.1	23	±4.0	103	±6.0	71	±14.0
TP67	0.0128 µg	89	±6.7	19	±3.6	109	±13.6	54	±11.2
				WITH S-9					
NEG CONTROL	0.0 mg	104	±8.1	26	±7.5	81	±9.9	92	±24.3
AA	2.0 µg			1797	±51.2	1652	±28.2		
AF	2.0 µg	264	±39.7	432	±12.5				
BP	2.0 µg	333	±34.6	202	±27.2				
TP67	40.0 µg	113	±15.0	24	±8.3	--		58	±5.5
TP67	8.0 µg	113	±16.7	28	±8.0	90	±11.3	86	±3.5
TP67	1.6 µg	110	±4.6	21	±0.6	--		93	±9.9
TP67	0.32 µg	103	±11.7	21	±1.0	92	±9.9	57	±8.2
TP67	0.064 µg	112	±11.1	27	±7.9	105	±5.7	99	±10.1
TP67	0.0128 µg	92	±7.1	28	±3.8	93	±15.3	74	±4.4

† Values represent the mean number of revertants/plate (± standard deviation)
* MITO-C=mitomycin C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline- n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

TABLE 3 (cont.): Mutagenicity Assay for 1,3-BIS[3-(1-OCTOXYMETHYL) IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE (TP67)†

COMPOUND*	DOSE/PLATE	TA1537		TA1538	
		WITHOUT S-9			
NEG CONTROL	0.0 mg	8	±1.8	14	±4.6
TP67	40 µg	8	±2.1	14	±1.2
TP67	8 µg	6	±3.1	15	±5.8
TP67	1.6 µg	7	±2.6	13	±1.5
TP67	0.32 µg	6	±2.1	13	±4.7
TP67	0.064 µg	7	±1.2	15	±1.5
TP67	0.0128 µg	8	±2.5	12	±0.6
		WITH S-9			
NEG CONTROL	0.0 mg	10	±3.2	21	±6.1
AA	2.0 µg	284	±14.5	1107	±15.6
AF	2.0 µg			535	±13.1
BP	2.0 µg	91	±6.6	105	±10.7
TP67	40 µg	10	±0.6	13	±1.5
TP67	8 µg	11	±3.8	21	±3.2
TP67	1.6 µg	8	±0.6	20	±6.7
TP67	0.32 µg	14	±2.1	24	±3.5
TP67	0.064 µg	19	±3.8	31	±6.7
TP67	0.0128 µg	14	±1.4	26	±4.0

†Values represent the mean number of revertants/plate (± standard deviation)
*MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

A tabular presentation of the raw data is included in Appendix B.

DISCUSSION

Certain test criteria must be satisfied before an Ames Test 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, alterations in the LP layer, and deficiency in DNA excision-repair (except TA102). Second, the *Salmonella* strains must be susceptible to mutation by 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 formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of an Ames Test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, 1,3-BIS[3-(1-OCTOXYMETHYL) IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose response over three dose concentrations, and a revertant colony count at least two times (TA97, TA98, TA100, TA102) (1,6) or three times (TA1537, TA1538) (2,4) the spontaneous revertant colony count. 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE 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 test indicate that 1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE is not mutagenic when evaluated in the Ames test.

CONCLUSION

1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE was evaluated for mutagenic potential in the Ames Test, in both the presence and the absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

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APPENDICES

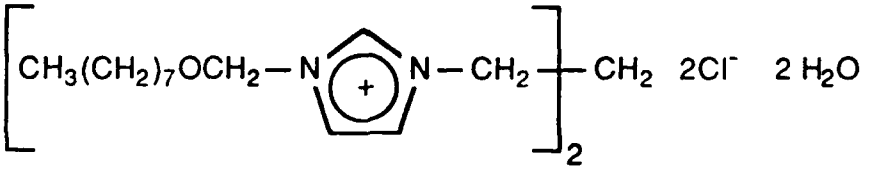
APPENDIX A: Chemical Data

Chemical Name: 1,3-Bis[3-(1-octoxymethyl)imidazolium]propane dichloride dihydrate

SRI Reference Number: 6868-40

LAIR Code Number: TP67

Chemical Structure:



Molecular Formula: $C_{27}H_{50}N_4O_2Cl_2 \cdot 2\ H_2O$

Molecular Weight: 569.6

Physical State: White crystalline solid

Analytical Data:

NMR (300 MHz, D_2O): δ 0.92 (t, J = 6.3 Hz, 6 H, CH_3-$(CH_2)_7$-O), 1.32 (s, 20 H, CH_3-$(CH_2)_5$-CH_2), 1.66 (t, J = 7.2 Hz, 4 H, CH_2-CH_2-O), 2.68 (m, J = 7.5 Hz, 2 H, N-CH_2-CH_2-CH_2-N), 3.72 (t, J = 6.2 Hz, 4 H, CH_2-CH_2-O-), 4.52 (t, J = 7.5 Hz, 4 H, N-CH_2-CH_2), 5.71 (s, 4 H, O-CH_2-N), 7.77 (d, J = 1.8 Hz, 2 H, CH-CH-N-CH_2-CH_2), 7.80 (d, J = 1.5 Hz, 2 H, OCH_2NCH-CH).* The NMR spectrum obtained upon receipt of the compound corresponded closely to the spectrum provided by the source (obtained in DMSO). Any discrepancies were due to the difference in solvents as well as the higher field strength and greater resolution of the NMR used to analyze the compound in our lab. No peaks other than those attributable to the compound were observed in the NMR spectrum.

Stability:

NMR data demonstrate that the compound is stable in water (D_2O) for at least 8 days.†

Source: Clifford D. Bedford
SRI International
Physical Sciences Division
Menlo Park, CA

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*Wheeler CR. Toxicity Testing and Antidotes for Chemical Warfare Agents. Laboratory Notebook #85-12-024, p 13. Letterman Army Institute of Research, Presidio of San Francisco, CA.

†*Ibid.* p 1.

APPENDIX B: Individual Plate Scores

1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE (TP67)

TOXICITY DETERMINATION WITH TA100

DOSE/PLATE	5.0 mg	1.0 mg	0.2 mg	0.04 mg
PLATE 1	0	325	143	54
PLATE 2	0	505	268	50
PLATE 3	0	108	206	58
background lawn	NG*	ST	NL	NL

DOSE/PLATE	0.008 mg	0.0016 mg	NEG START	NEG END
PLATE 1	84	86	81	90
PLATE 2	78	101	81	103
PLATE 3	87	80	68	83
background lawn	NL	NL	NL	NL

* NG=No Growth, ST=Slight Toxicity, NL=Normal Lawn.

APPENDIX B (cont.): Individual Plate Scores

1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE (TP67)

NEGATIVE CONTROL DATA

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1537	TA1538
			WITHOUT S-9				
NEG CONTROL (START RUN)	0.0 mg	93	22	121	61	10	15
		98	17	128	79	8	9
		92	17	89	86	10	18
NEG CONTROL (END RUN)	0.0 mg						
		98	12	108	66	6	19
		106	14	109	79	9	16
		90	18	94	86	6	8
			WITH S-9				
NEG CONTROL (START RUN)	0.0 mg	105	30	*	96	13	19
		111	29	*	108	9	27
		106	20	*	130	9	12
NEG CONTROL (END RUN)	0.0 mg	103	18	*	66	14	29
		88	20	74	72	10	21
		108	37	88	79	5	20

* Plates lost.

APPENDIX B (cont.): Individual Plate Scores

1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE (TP67)
POSITIVE CONTROL DATA

COMPOUND†	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1537	TA1538
AA	2.0 µg		1812	1631		299	1098
			1839	1684		270	1125
			1740	1641		284	1098
AF	2.0 µg	219	422	*	*		537
		295	446	*	*		547
		277	428	*	*		521
BP	2.0 µg	369	216	*		85	107
		300	171	*		98	114
		329	220	*		90	93
MITO-C	0.5 µg				773		
					750		
					964		
MNNG	2.0 µg			1127			
				1175			
				1227			
NQNO	0.5 µg	308					
		279					
		294					

† AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene, MITO-C=mitomycin C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide
* Plates lost.

APPENDIX B (cont.): Individual Plate Scores

1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE (TP67)

MUTAGENICITY DATA WITHOUT S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1537	TA1538
TP67	40 µg	96	23	146	39	10	13
		118	21	107	37	6	15
		104	26	104	35	7	15
TP67	8 µg	102	14	78	68	9	22
		91	22	105	72	3	12
		108	16	90	49	5	12
TP67	1.6 µg	109	15	*	73	9	13
		98	14	*	75	4	11
		86	*	*	64	8	14
TP67	0.32 µg	95	15	114	56	4	15
		88	17	*	55	7	8
		90	22	*	42	8	17
TP67	0.064 µg	99	25	97	82	8	14
		120	25	103	75	6	15
		123	18	109	55	8	17
TP67	0.0128 µg	82	16	95	66	11	11
		95	18	122	51	8	12
		91	23	111	44	6	12

* Plates lost.

APPENDIX B (cont.): Individual Plate Scores

1,3-BIS[3-(1-OCTOXYMETHYL)IMIDAZOLIUM]PROPANE DICHLORIDE DIHYDRATE (TP67)

MUTAGENICITY DATA WITH S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1537	TA1538
TP67	40 µg	117	17	*	64	9	12
		125	33	*	55	10	15
		96	21	*	54	10	13
TP67	8 µg	108	36	82	83	8	17
		132	27	98	88	9	23
		100	20	*	*	15	22
TP67	1.6 µg	111	21	*	88	9	18
		105	22	*	104	8	27
		114	21	*	86	8	14
TP67	0.32 µg	94	21	85	64	15	26
		98	20	99	59	12	21
		116	22	*	48	*	*
TP67	0.064 µg	100	30	109	108	22	28
		113	18	101	88	21	27
		122	33	*	101	15	39
TP67	0.0128 µg	98	32	101	79	13	22
		93	25	75	72	*	26
		84	26	102	71	15	30

* Plates lost.

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