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**Mutagenic Potential of 1,4-Bis[3-(1-  
Phenylmethoxymethyl)Imidazolium]Butane  
Dichloride Hemihydrate in the Ames  
*Salmonella*/Mammalian Microsome Mutagenicity Test**

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*and*  
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GENETIC TOXICOLOGY BRANCH  
DIVISION OF TOXICOLOGY

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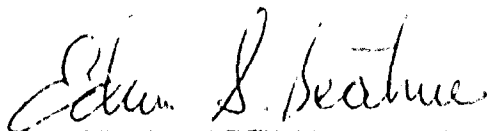
Mutagenic Potential of 1,4-Bis[3-(1-Phenylmethoxymethyl)Imidazolium]Butane Dichloride Hemihydrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (Toxicology Series 196)--Sebastian and Korte

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SECURITY CLASSIFICATION OF THIS PAGE

| REPORT DOCUMENTATION PAGE                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |       |                                                    |                                                                                                                                                                             | Form Approved<br>OMB No. 0704-0188                                            |                                |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|--------------------------------|
| 1a. REPORT SECURITY CLASSIFICATION                                                                                                                                                                                                                                                                                                                                                                                                                                                         |       |                                                    | 1b. RESTRICTIVE MARKINGS                                                                                                                                                    |                                                                               |                                |
| 2a. SECURITY CLASSIFICATION AUTHORITY                                                                                                                                                                                                                                                                                                                                                                                                                                                      |       |                                                    | 3. DISTRIBUTION / AVAILABILITY OF REPORT<br>Approved for public release; distribution is unlimited.                                                                         |                                                                               |                                |
| 2b. DECLASSIFICATION / DOWNGRADING SCHEDULE                                                                                                                                                                                                                                                                                                                                                                                                                                                |       |                                                    |                                                                                                                                                                             |                                                                               |                                |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S)<br>Institute Report No. 316                                                                                                                                                                                                                                                                                                                                                                                                                    |       |                                                    | 5. MONITORING ORGANIZATION REPORT NUMBER(S)                                                                                                                                 |                                                                               |                                |
| 6a. NAME OF PERFORMING ORGANIZATION<br>Genetic Toxicology Branch<br>Division of Toxicology                                                                                                                                                                                                                                                                                                                                                                                                 |       | 6b. OFFICE SYMBOL<br>(If applicable)<br>SGRD-ULE-T |                                                                                                                                                                             | 7a. NAME OF MONITORING ORGANIZATION<br>Walter Reed Army Institute of Research |                                |
| 6c. ADDRESS (City, State, and ZIP Code)<br>Letterman Army Institute of Research<br>Presidio of San Francisco, CA 94129-6800                                                                                                                                                                                                                                                                                                                                                                |       |                                                    | 7b. ADDRESS (City, State, and ZIP Code)<br>Washington, D.C. 20307-5100                                                                                                      |                                                                               |                                |
| 8a. NAME OF FUNDING / SPONSORING ORGANIZATION<br>US Army Medical Research & Development Command                                                                                                                                                                                                                                                                                                                                                                                            |       | 8b. OFFICE SYMBOL<br>(If applicable)               |                                                                                                                                                                             | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER                               |                                |
| 8c. ADDRESS (City, State, and ZIP Code)<br>Fort Detrick<br>Frederick, MD 21701-5012                                                                                                                                                                                                                                                                                                                                                                                                        |       |                                                    | 10. SOURCE OF FUNDING NUMBERS                                                                                                                                               |                                                                               |                                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |       |                                                    | PROGRAM<br>ELEMENT NO.<br>67234                                                                                                                                             | PROJECT<br>NO.<br>A875                                                        | TASK<br>NO.<br>BC              |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |       |                                                    | WORK UNIT<br>ACCESSION NO.<br>DA OH0366                                                                                                                                     |                                                                               |                                |
| 11. TITLE (Include Security Classification) Mutagenic potential of 1,4-Bis[3-(1-phenylmethoxymethyl)imidazolium]butane dichloride hemihydrate in the Ames <u>Salmonella</u> /Mammalian Microsome Mutagenicity Test                                                                                                                                                                                                                                                                         |       |                                                    |                                                                                                                                                                             |                                                                               |                                |
| 12. PERSONAL AUTHOR(S)<br>Suzanne E. Sebastian and Don W. Korte, Jr.                                                                                                                                                                                                                                                                                                                                                                                                                       |       |                                                    |                                                                                                                                                                             |                                                                               |                                |
| 13a. TYPE OF REPORT<br>Institute                                                                                                                                                                                                                                                                                                                                                                                                                                                           |       | 13b. TIME COVERED<br>FROM 4/23/86 TO 9/12/86       |                                                                                                                                                                             | 14. DATE OF REPORT (Year, Month, Day)                                         |                                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |       |                                                    |                                                                                                                                                                             | 15. PAGE COUNT<br>19                                                          |                                |
| 16. SUPPLEMENTARY NOTATION<br>Toxicology Series 196                                                                                                                                                                                                                                                                                                                                                                                                                                        |       |                                                    |                                                                                                                                                                             |                                                                               |                                |
| 17. COSATI CODES                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |       |                                                    | 18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)                                                                                           |                                                                               |                                |
| FIELD                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | GROUP | SUB-GROUP                                          | Mutagenicity, Genetic toxicology, Ames Test, 1,4-bis[3-(1-phenylmethoxymethyl)imidazolium]butane dichloride hemihydrate oximes, imidazoles, butanes, phenyl radicals, (mgm) |                                                                               |                                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |       |                                                    |                                                                                                                                                                             |                                                                               |                                |
| 19. ABSTRACT (Continue on reverse if necessary and identify by block number)<br>The mutagenic potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was assessed by using the Ames <u>Salmonella</u> /Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1.0 mg/plate to 0.00032 mg/plate. The test compound was not mutagenic under conditions of this test. |       |                                                    |                                                                                                                                                                             |                                                                               |                                |
| 20. DISTRIBUTION / AVAILABILITY OF ABSTRACT<br><input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS                                                                                                                                                                                                                                                                                                        |       |                                                    | 21. ABSTRACT SECURITY CLASSIFICATION<br>unclassified                                                                                                                        |                                                                               |                                |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL<br>Edwin S. Beatrice, COL MC                                                                                                                                                                                                                                                                                                                                                                                                                           |       |                                                    | 22b. TELEPHONE (Include Area Code)<br>415-561-3600                                                                                                                          |                                                                               | 22c. OFFICE SYMBOL<br>SGRD-UL7 |

## ABSTRACT

The mutagenic potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1.0 mg/plate to 0.00032 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE, oxime.

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| Dist          | Availability                        |
| A-1           |                                     |

## 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: 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)  
IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE

INCLUSIVE STUDY DATES: 23 April 1986 - 12 September 1986

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (LAIR Code TP65) 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

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1 November 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 86002

1. This is to certify that in relation to LAIR GLP Study 86002, the following inspections were made:

|               |                              |
|---------------|------------------------------|
| 15 April 1986 | - Protocol Review            |
| 21 May 1986   | - Plate Incorporation (TP62) |
| 17 March 1987 | - Plate Incorporation (TP64) |
| 20 March 1987 | - Plate Counting (TP64)      |

2. The institute report entitled "Mutagenic Potential of 1,4-Bis[3-(1-Phenylmethoxymethyl) Imidazolium] Butane Dichloride Hemihydrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 196, was audited on 23 April 1987.

*Carolyn M. Lewis*

CAROLYN M. LEWIS  
Chief, Quality Assurance



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**Mutagenic Potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE in the Ames Salmonella/Mammalian Microsome Mutagenicity Test--Sebastian and Korte**

**INTRODUCTION**

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE 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 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE 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 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (LAIR Code TP65) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

## **MATERIALS AND METHODS**

### Test Compound

Chemical Name: 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)  
IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE

LAIR Code Number: TP65

Physical State: White crystalline solid

Source: SRI International, Menlo Park, CA

Storage: 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]  
BUTANE DICHLORIDE HEMIHYDRATE was received from SRI  
International, 333 Ravenswood Ave., Menlo Park, CA 94025 and  
assigned the LAIR Code number TP65. 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, TA1535,  
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

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE 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 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE ranging from  $1.6 \times 10^{-3}$  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 highest dose showed neither a decreased number of macrocolonies (below the spontaneous rate) nor an observable reduction in the density of the background lawn, the highest dose selected for the mutagenicity test was 5.0 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 TA1535, 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 TA1535, 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

There were no deviations from the protocol or standard operating procedures.

### 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 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE 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 highest dose 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 1.0 mg/plate. Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 10-12 September 1986 (Table 2). 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3). A tabular presentation of the raw data is included in Appendix B.

**TABLE 1: TOXICITY LEVEL DETERMINATION FOR TP65**

GLP STUDY NUMBER 86002

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| <u>TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)</u> |             |             |                         |
|-------------------------------------------------------------|-------------|-------------|-------------------------|
| <u>CONCENTRATION</u>                                        | <u>MEAN</u> | <u>±1SD</u> | <u>BACKGROUND LAWN*</u> |
| START RUN NEGATIVE CONTROL                                  | 77          | 7.5         | NL                      |
| 5.0 mg/plate                                                | 37          | 8.0         | ST                      |
| 1.0 mg/plate                                                | 63          | 11.9        | NL                      |
| 0.2 mg/plate                                                | 62          | 4.5         | NL                      |
| 0.04 mg/plate                                               | 75          | 8.3         | NL                      |
| 0.008 mg/plate                                              | 84          | 10.2        | NL                      |
| 0.0016 mg/plate                                             | 69          | 10.2        | NL                      |
| END RUN NEGATIVE CONTROL                                    | 92          | 10.1        | NL                      |

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

|                            | <u>TA100*</u> |
|----------------------------|---------------|
| HISTIDINE REQUIREMENT      | NG            |
| AMPICILLIN RESISTANCE      | G             |
| UV                         | NG            |
| CRYSTAL VIOLET SENSITIVITY | NG            |
| STERILITY CONTROL          | NG            |

STERILITY CONTROL FOR TOXICITY DETERMINATION

| <u>MATERIAL TESTED</u>       | <u>OBSERVATION*</u> |
|------------------------------|---------------------|
| 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 TP65**

GLP STUDY NUMBER 86002

| STRAIN VERIFICATION |                          |                          |              |                   |                      |
|---------------------|--------------------------|--------------------------|--------------|-------------------|----------------------|
| STRAIN              | OBSERVATIONS*            |                          |              |                   |                      |
|                     | HISTIDINE<br>REQUIREMENT | AMPICILLIN<br>RESISTANCE | UV<br>REPAIR | CRYSTAL<br>VIOLET | STERILITY<br>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                   |
| TA1535              | NG                       | NG                       | NG           | NG                | NG                   |
| TA1537              | NG                       | NG                       | NG           | NG                | NG                   |
| TA1538              | NG                       | NG                       | NG           | NG                | NG                   |

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

| <u>MATERIAL TESTED</u>       | <u>OBSERVATION*</u> |
|------------------------------|---------------------|
| 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 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)†**

| COMPOUND*          | DOSE       | TA97      | TA98        | TA100       | TA102    |
|--------------------|------------|-----------|-------------|-------------|----------|
| <b>WITHOUT S-9</b> |            |           |             |             |          |
| NEG CONTROL        | 0.0 mg     | 115 ±11.7 | 17 ±5.1     | 113 ±10.8   | 56 ±8.5  |
| MITO C             | 0.5 µg     |           |             |             | 92 ±9.2  |
| MNNG               | 2.0 µg     |           |             | 1277 ±223.9 |          |
| NQNO               | 2.0 µg     | 535 ±15.7 |             |             |          |
| TP65               | 1.0 mg     | 103 ±12.1 | 28 ±7.8     | 97 ±11.0    | 71 ±9.0  |
| TP65               | 0.2 mg     | 129 ±7.6  | 28 ±7.8     | 129 ±11.0   | 61 ±5.7  |
| TP65               | 0.04 mg    | 98 ±2.5   | 23 ±6.0     | 96 ±7.0     | 50 ±6.7  |
| TP65               | 0.008 mg   | 105 ±3.2  | 20 ±8.6     | 112 ±3.6    | 48 ±5.5  |
| TP65               | 0.0016 mg  | 114 ±9.3  | 12 ±2.6     | 105 ±13.9   | 52 ±8.5  |
| TP65               | 0.00032 mg | 122 ±8.1  | 17 ±4.7     | 106 ±5.9    | 55 ±5.9  |
| <b>WITH S-9</b>    |            |           |             |             |          |
| NEG CONTROL        | 0.0 mg     | 112 ±11.0 | 37 ±5.0     | 102 ±18.2   | 74 ±17.0 |
| 2-AA               | 2.0 µg     |           | 1982 ±254.4 | 1945 ±231.7 |          |
| 2-AF               | 2.0 µg     | 477 ±27.6 | 780 ±67.3   | 177 ±17.6   |          |
| BP                 | 2.0 µg     | 427 ±32.5 | 234 ±3.5    |             |          |
| TP65               | 1.0 mg     | 125 ±15.0 | 54 ±4.7     | 116 ±7.2    | 112 ±6.7 |
| TP65               | 0.2 mg     | 113 ±21.4 | 30 ±11.9    | 104 ±8.7    | 84 ±6.6  |
| TP65               | 0.04 mg    | 111 ±7.8  | 36 ±1.5     | 84 ±7.2     | 72 ±8.1  |
| TP65               | 0.008 mg   | 106 ±10.0 | 21 ±4.2     | 82 ±1.2     | 70 ±8.5  |
| TP65               | 0.0016 mg  | 114 ±13.3 | 28 ±6.7     | 110 ±11.4   | 61 ±17.0 |
| TP65               | 0.00032 mg | 109 ±12.7 | 32 ±1.7     | 101 ±7.8    | 72 ±5.6  |

†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 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)†**

| COMPOUND*          | DOSE/PLATE | TA1535      | TA1537   | TA1538     |
|--------------------|------------|-------------|----------|------------|
| <b>WITHOUT S-9</b> |            |             |          |            |
| NEG CONTROL        | 0.0 mg     | 28 ±7.9     | 10 ±2.7  | 20 ±6.4    |
| MNNG               | 20.0 µg    | 2693 ±157.4 |          |            |
| TP65               | 1.0 mg     | 20 ±7.6     | 9 ±4.5   | 19 ±2.6    |
| TP65               | 0.2 mg     | 24 ±2.6     | 7 ±1.2   | 17 ±2.6    |
| TP65               | 0.04 mg    | 29 ±8.7     | 7 ±1.0   | 18 ±1.5    |
| TP65               | 0.008 mg   | 30 ±5.8     | 7 ±3.8   | 19 ±4.0    |
| TP65               | 0.0016 mg  | 25 ±3.1     | 9 ±2.5   | 10 ±0.6    |
| TP65               | 0.00032 mg | 25 ±5.7     | 11 ±2.1  | 10 ±3.5    |
| <b>WITH S-9</b>    |            |             |          |            |
| NEG CONTROL        | 0.0 mg     | 30 ±6.7     | 20 ±5.5  | 28 ±4.0    |
| 2-AA               | 2.0 µg     |             | 105 ±0.0 | 431 ±29.7  |
| 2-AF               | 2.0 µg     |             |          | 395 ±392.6 |
| BP                 | 2.0 µg     |             | 122 ±3.5 | 112 ±20.1  |
| TP65               | 1.0 mg     | 27 ±4.9     | 15 ±0.6  | 25 ±4.2    |
| TP65               | 0.2 mg     | 30 ±7.2     | 23 ±7.6  | 29 ±10.1   |
| TP65               | 0.04 mg    | 16 ±5.9     | 9 ±2.6   | 21 ±3.1    |
| TP65               | 0.008 mg   | 22 ±6.0     | 9 ±2.6   | 23 ±5.0    |
| TP65               | 0.0016 mg  | 22 ±8.5     | 7 ±1.0   | 23 ±7.6    |
| TP65               | 0.00032 mg | 29 ±3.5     | 13 ±6.2  | 23 ±4.9    |

†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.

## 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, 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE 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 (TA1535, TA1537, TA1538) (2,4) the spontaneous revertant colony count. 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE 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 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE is not mutagenic when evaluated in the Ames Test.

## CONCLUSION

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE 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

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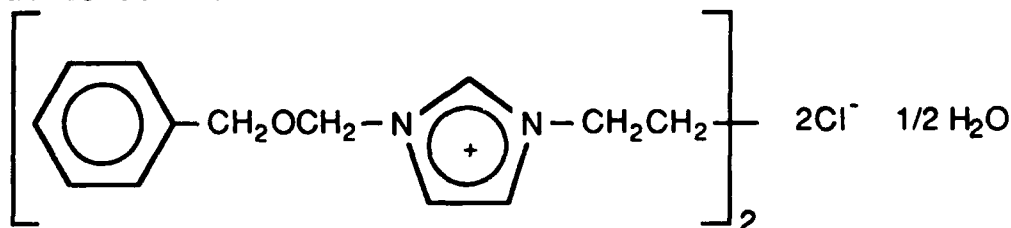
# APPENDIX A: Chemical Data

Chemical Name: 1,4-Bis[3-(1-phenylmethoxymethyl)imidazolium]  
butane dichloride hemihydrate

SRI Reference Number: 6868-32

LAIR Code: TP65

Chemical Structure:



Molecular Formula:  $C_{26}H_{32}N_4O_2Cl_2 \cdot 1/2 H_2O$

Molecular Weight: 512.5

Physical State: White crystalline solid

Analytical Data:

NMR (300 MHz,  $D_2O$ ):  $\delta$  1.81 (s, 4 H,  $CH_2CH_2CH_2CH_2$ ), 4.19 (s, 4 H,  $N-CH_2CH_2CH_2CH_2-N$ ), 4.73 (s, 4 H, phenyl- $CH_2-O$ ), 5.69 (s, 4 H,  $O-CH_2-N$ ), 7.36 (m, 10 H, phenyl), 7.46 (s, 2 H,  $O-CH_2-N-CH-CH-N$ ), 7.60 (s, 2 H,  $O-CH_2-N-CH-CH-N$ ).\* 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

\*Wheeler CR. Toxicity Testing and Antidotes for Chemical Warfare Agents. Laboratory Notebook #85-12-024, p 9. Letterman Army Institute of Research, Presidio of San Francisco, CA.

†Ibid. p 1.

## APPENDIX B: Individual Plate Scores

1, 4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

## TOXICITY DETERMINATION WITH TA100

| DOSE/PLATE      | 5.0 mg | 1.0 mg | 0.2 mg | 0.04 mg |
|-----------------|--------|--------|--------|---------|
| PLATE 1         | 29     | 58     | 57     | 68      |
| PLATE 2         | 45     | 77     | 62     | 72      |
| PLATE 3         | 37     | 55     | 66     | 84      |
| background lawn | ST*    | NL     | NL     | NL      |

| DOSE/PLATE      | 0.008 mg | 0.0016 mg | NEG START | NEG END |
|-----------------|----------|-----------|-----------|---------|
| PLATE 1         | 72       | 76        | 81        | 90      |
| PLATE 2         | 88       | 73        | 81        | 103     |
| PLATE 3         | 91       | 57        | 68        | 83      |
| background lawn | NL       | NL        | NL        | NL      |

\* NL=Normal Lawn, ST=Slight Toxicity

## APPENDIX B (cont.): Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

## NEGATIVE CONTROL DATA

| COMPOUND                   | DOSE/PLATE | TA97 | TA98 | TA100 | TA102 | TA1535 | TA1537 | TA1538 |
|----------------------------|------------|------|------|-------|-------|--------|--------|--------|
| <b><u>WITHOUT S-9</u></b>  |            |      |      |       |       |        |        |        |
| NEG CONTROL<br>(START RUN) | 0.0 mg     | 135  | 18   | 97    | 60    | 33     | 10     | 28     |
|                            |            | 111  | 25   | 119   | 66    | 38     | 6      | 21     |
|                            |            | 101  | 21   | 111   | 63    | 29     | 9      | 22     |
| NEG CONTROL<br>(END RUN)   | 0.0 mg     | 119  | 14   | 124   | 47    | 18     | 12     | 22     |
|                            |            | 108  | 13   | 104   | 52    | 19     | 14     | 10     |
|                            |            | 113  | 12   | 122   | 46    | 30     | 10     | 14     |
| <b><u>WITH S-9</u></b>     |            |      |      |       |       |        |        |        |
| NEG CONTROL<br>(START RUN) | 0.0 mg     | 110  | 31   | 82    | 88    | 20     | 26     | 22     |
|                            |            | 92   | 33   | 87    | 97    | 29     | 13     | 29     |
|                            |            | 112  | 37   | 89    | 75    | 25     | 13     | 29     |
| NEG CONTROL<br>(END RUN)   | 0.0 mg     | 121  | 39   | 127   | 67    | 31     | 23     | 30     |
|                            |            | 112  | 39   | 112   | 72    | 34     | 23     | 34     |
|                            |            | 123  | 45   | 114   | 47    | 39     | 19     | 26     |



## APPENDIX B (cont.): Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

## POSITIVE CONTROL DATA

| COMPOUND* | DOSE/PLATE | TA97 | TA98 | TA100 | TA102 | TA1535 | TA1537 | TA1538 |
|-----------|------------|------|------|-------|-------|--------|--------|--------|
| BP        | 2.0 µg     | 462  | 230  | -     | -     | 120    | 129    | 129    |
|           |            | 398  | 234  | -     | -     | 120    | 118    | 118    |
|           |            | 420  | 237  | -     | -     | 126    | 90     | 90     |
| MITO C    | 0.5 µg     | -    | -    | -     | 102   | -      | -      | -      |
|           |            | -    | -    | -     | 90    | -      | -      | -      |
|           |            | -    | -    | -     | 84    | -      | -      | -      |
| AA        | 2.0 µg     | -    | 2205 | 1680  | -     | 105    | 452    | 452    |
|           |            | -    | 2037 | 2111  | -     | 105    | 410    | 410    |
|           |            | -    | 1705 | 2043  | -     | -      | -      | -      |
| NQNO      | 2.0 µg     | 532  | -    | -     | -     | -      | -      | -      |
|           |            | 552  | -    | -     | -     | -      | -      | -      |
|           |            | 521  | -    | -     | -     | -      | -      | -      |
| MNNG      | 2.0 µg     | -    | -    | 1506  | -     | -      | -      | -      |
|           |            | -    | -    | 1260  | -     | -      | -      | -      |
|           |            | -    | -    | 1065  | -     | -      | -      | -      |
| MNNG      | 20.0 µg    | -    | -    | -     | -     | 2514   | -      | -      |
|           |            | -    | -    | -     | -     | 2810   | -      | -      |
|           |            | -    | -    | -     | -     | 2755   | -      | -      |
| AF        | 2.0 µg     | 488  | 812  | 157   | -     | -      | 171    | 171    |
|           |            | 498  | 826  | 190   | -     | -      | 848    | 848    |
|           |            | 446  | 703  | 184   | -     | -      | 165    | 165    |

\*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

## APPENDIX B (cont.): Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

MUTAGENICITY DATA WITHOUT S-2

| <u>COMPOUND</u> | <u>DOSE/PLATE</u> | <u>TA97</u>       | <u>TA98</u>    | <u>TA100</u>      | <u>TA102</u>   | <u>TA1535</u>  | <u>TA1537</u> | <u>TA1538</u>  |
|-----------------|-------------------|-------------------|----------------|-------------------|----------------|----------------|---------------|----------------|
| TP65            | 1.0 mg            | 96<br>96<br>117   | 22<br>26<br>37 | 108<br>86<br>97   | 62<br>80<br>72 | 28<br>18<br>13 | 13<br>4<br>9  | 21<br>16<br>20 |
| TP65            | 0.2 mg            | 122<br>127<br>137 | 19<br>34<br>30 | 138<br>133<br>117 | 66<br>55<br>63 | 22<br>27<br>23 | 8<br>8<br>6   | 14<br>19<br>18 |
| TP65            | 0.04 mg           | 101<br>98<br>96   | 22<br>17<br>29 | 89<br>103<br>96   | 46<br>58<br>47 | 33<br>35<br>19 | 7<br>6<br>8   | 16<br>19<br>18 |
| TP65            | 0.008 mg          | 107<br>101<br>106 | 29<br>12<br>18 | 116<br>109<br>111 | 48<br>42<br>53 | 23<br>33<br>33 | 10<br>3<br>9  | 17<br>17<br>24 |
| TP65            | 0.0016 mg         | 117<br>104<br>122 | 13<br>14<br>9  | 117<br>109<br>90  | 44<br>61<br>51 | 28<br>24<br>22 | 7<br>9<br>12  | 9<br>10<br>10  |
| TP65            | 0.00032 mg        | 128<br>126<br>113 | 12<br>19<br>21 | 104<br>113<br>102 | 51<br>53<br>62 | 19<br>30<br>27 | 10<br>13<br>9 | 7<br>10<br>14  |

## APPENDIX B (cont.): Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

MUTAGENICITY DATA WITH S-9

| COMPOUND | DOSE/PLATE | TA97 | TA98 | TA100 | TA102 | TA1535 | TA1537 | TA1538 |
|----------|------------|------|------|-------|-------|--------|--------|--------|
| TP65     | 1.0 mg     | 125  | 49   | 110   | 108   | 23     | 14     | 28     |
|          |            | 140  | 56   | 124   | 120   | -      | 15     | 22     |
|          |            | 110  | 58   | 114   | 109   | 30     | 15     | -      |
| TP65     | 0.2 mg     | 108  | 22   | 94    | 91    | 34     | 15     | 31     |
|          |            | 136  | 44   | 106   | 83    | 22     | 25     | 18     |
|          |            | 94   | 25   | 111   | 78    | 35     | 30     | 38     |
| TP65     | 0.04 mg    | 120  | 37   | 89    | 68    | 20     | 11     | 20     |
|          |            | 109  | 34   | 88    | 66    | 9      | 6      | 24     |
|          |            | 105  | 36   | 76    | 81    | 18     | 10     | 18     |
| TP65     | 0.008 mg   | 106  | 16   | 81    | 80    | 21     | 10     | 23     |
|          |            | 116  | 22   | 83    | 64    | 16     | 11     | 18     |
|          |            | 96   | 24   | 83    | 67    | 28     | 6      | 28     |
| TP65     | 0.0016 mg  | 111  | 30   | 101   | 78    | 23     | 8      | 15     |
|          |            | 103  | 34   | 123   | 62    | 30     | 7      | 30     |
|          |            | 129  | 21   | 107   | 44    | 13     | 6      | 25     |
| TP65     | 0.00032 mg | 124  | 34   | 92    | 73    | 26     | 20     | 26     |
|          |            | 101  | 31   | 106   | 66    | 29     | 8      | 17     |
|          |            | 103  | 31   | 105   | 77    | 33     | 11     | 25     |

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