

DTIC FILE COPY

AD-A203 825

Institute Report No. 324

Mutagenic Potential of  
2-(E)-Hydroxyiminomethyl-3-methyl-1-(1,2,2-  
trimethylpropyloxymethyl)imidazolium Chloride  
in the Ames *Salmonella*/Mammalian Microsome  
Mutagenicity Test

Steven K. Sano, BA, SGT, USA  
and  
Don W. Korte, Jr., PhD, MAJ, MSC

GENETIC TOXICOLOGY BRANCH  
DIVISION OF TOXICOLOGY

DISTRIBUTION STATEMENT A

Approved for public release;  
Distribution Unlimited

November 1988

Toxicology Series: 124

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

89

1

23

07

DTIC  
ELECTE  
JAN 25 1989  
S a H D

Mutagenic Potential of 2-(E)-Hydroxyiminomethyl-3-methyl-1-(1,2,2-trimethyl-propyloxymethyl)imidazolium Chloride in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (Toxicology Series 124)--Sano and Korte

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

Destroy this report when it is no longer needed. Do not return 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)

*Edwin S. Beatrice* 5 Dec 88

Edwin S. Beatrice  
COL, MC  
Commanding

(date)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION / AVAILABILITY OF REPORT	
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE		APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)  Institute Report No.: 324		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, DC, 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, Maryland 21701-5012		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO. 62734	PROJECT NO. A875
		TASK NO. BG	WORK UNIT ACCESSION NO. DA0H0366
11. TITLE (Include Security Classification) (U) Mutagenic Potential of 2-(E)-Hydroxyimino-methyl-3-methyl-1-(1,2,2-trimethylpropyloxymethyl)imidazolium Chloride in the Ames Salmonella/Mammalian Microsome Mutagenicity Test			
12. PERSONAL AUTHOR(S) SK Sano and DW Korte, Jr.			
13a. TYPE OF REPORT Institute	13b. TIME COVERED FROM 25FEB85 to 22MAR85	14. DATE OF REPORT (Year, Month, Day) November 1988	15. PAGE COUNT 20
16. SUPPLEMENTARY NOTATION  Toxicology Series No. 124			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	Mutagenicity, Genetic Toxicology, Ames Test, 2-(E)-Hydroxyiminomethyl-3-methyl-1-(1,2,2-trimethylpropyloxymethyl)imidazolium chloride, Oximes,	
		imidazoles, methyl radicals, chlorides, (Ames)	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The mutagenic potential of 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5.0 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this test.			
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL DORWIN C. 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 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM CHLORIDE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5.0 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM CHLORIDE, Oxime



Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Avail and/or	
Dist	Special
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 Officer: H.A. Musallam

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

GLP STUDY NUMBER: 85007

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

PRINCIPAL INVESTIGATOR: Steven K. Sano, BA, SGT, USA

REPORT AND DATA MANAGEMENT:

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

TEST SUBSTANCE: 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM CHLORIDE

INCLUSIVE STUDY DATES: 25 February 1985 - 22 March 1985

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM CHLORIDE (LAIR Code TP52) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

## ACKNOWLEDGMENTS

MAJ John W. Harbell, PhD, MSC, and Mr. John Dacey provided scientific guidance and research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE  
STUDY

We, the undersigned, declare that GLP Study 85007 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. 30 Nov 88

DON W. KORTE JR, PhD / DATE  
MAJ, MSC  
Study Director

Steven K. Sang 5 MAR 86

STEVEN K. SANG, BA / DATE  
SGT, USA  
Principal Investigator

Conrad Wheeler 14 July 88

CONRAD WHEELER, PhD / DATE  
DAC  
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

3 December 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Statement of Compliance

1. This is to certify that the protocol for GLP Study 85007 was reviewed on 21 February 1985.
2. The institute report entitled "Mutagenic Potential of 2-(E)-Hydroxyiminomethyl-3-methyl-1-(1,2,2-trimethylpropyloxymethyl)imidazolium Chloride in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 124, was audited on 14 November 1988.

*Carolyn M. Lewis*

CAROLYN M. LEWIS, MS  
Diplomate, American Board of Toxicology  
Chief, Quality Assurance

## TABLE OF CONTENTS

Abstract .....	i
Preface .....	iii
Acknowledgments .....	iv
Signatures of Principal Scientists .....	v
Report of the Quality Assurance Unit .....	vi
Table of Contents .....	vii
INTRODUCTION .....	1
Objective of the Study .....	1
MATERIALS AND METHODS .....	1
Test Compound .....	1
Test Solvent .....	2
Chemical Preparation .....	2
Test Strains .....	2
Test Format .....	2
Data Interpretation .....	4
Deviations from the Protocol/SOP .....	4
Storage of the Raw Data and Final Report .....	4
RESULTS .....	5
DISCUSSION .....	5
CONCLUSION .....	10
REFERENCES .....	11
APPENDICES .....	12
Appendix A: Chemical Data .....	13
Appendix B: Individual Plate Scores .....	15
OFFICIAL DISTRIBUTION LIST .....	20

**Mutagenic Potential of 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM CHLORIDE in the Ames Salmonella/Mammalian Microsome Mutagenicity Test--Sano and Korte**

**INTRODUCTION**

2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM CHLORIDE 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).

Objective of the Study

The objective of this study was to determine the mutagenic potential of 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM CHLORIDE (LAIR Code TP52) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

**MATERIALS AND METHODS**

Test Compound

Chemical Name: 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM CHLORIDE

LAIR Code Number: TP52

Physical State: White crystalline solid

Source: SRI International, Menlo Park, CA

Storage: 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE was received from SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 and assigned the LAIR Code number TP52. The test compound was stored in a desiccator at 5°C until used.

Chemical Properties/Analysis: SRI International data 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 compound was dissolved in sterile deionized water obtained from a Polymetric model 200-3 Water Purifier (Sunnyvale, CA).

#### Chemical Preparation

On the day of dosing, 300 mg of the test compound was measured into a sterile vial and dissolved in 6 ml of sterile deionized water to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

#### 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 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 (2).

#### Test Format

2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE was evaluated for mutagenic potential according to the methods of Ames et al (3). A detailed description of the 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 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE 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 none of the plates showed a decreased number of macrocolonies (below the spontaneous rate) or 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 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). 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 Ames et al (3). 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 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 the TA98 and TA100 strains.
- Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism.

Four 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, 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 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. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

#### Deviations from the Protocol/SOP

There were no deviations from the protocol or the 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 8 March 1985, the toxicity of 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE 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 neither a decrease in the number of macrocolonies nor an observable reduction in the density of the background lawn. Therefore, the highest dose selected for the mutagenicity test was 5.0 mg/plate. Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 11-14 March 1985 (Table 2). 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE did not induce an 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.

## 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, 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE 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 (TA98, TA100) or three times (TA1535, TA1537, TA1538) the spontaneous revertant colony count (5). 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE 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 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE is not mutagenic when evaluated in the Ames Test.

**TABLE 1: TOXICITY LEVEL DETERMINATION FOR TP52**

GLP STUDY NUMBER 85007

---

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)

<u>CONCENTRATION</u>	<u>MEAN</u>	<u>±1SD</u>	<u>BACKGROUND LAWN*</u>
5.0 mg/plate	117	14.5	NL
1.0 mg/plate	106	12.0	NL
0.2 mg/plate	110	25.0	NL
0.04 mg/plate	127	21.4	NL
0.008 mg/plate	127	18.5	NL
0.0016 mg/plate	135	45.6	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
TCP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

---

\*NL=Normal Lawn, G=Growth, NG=NO Growth

**TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING  
FOR THE MUTAGENICITY DETERMINATION OF TP52**

GLP STUDY NUMBER 85007

STRAIN VERIFICATION					
STRAIN	OBSERVATIONS*				
	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	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 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE (TP52) \***

COMPOUND†	DOSE		TA98		TA100	
<b>WITHOUT S-2</b>						
NEG CONTROL	0.0 mg		17	± 5.9	119	± 16.7
MNNG	2.0 µg		-	-	1802	±305.5
MNNG	20.0 µg		-	-	-	-
TP52	5.0 mg		21	± 3.0	112	± 13.2
TP52	1.0 mg		18	± 3.5	93	± 14.7
TP52	0.2 mg		13	± 1.5	112	± 17.8
TP52	0.04 mg		12	± 1.5	78	± 27.8
TP52	0.008 mg		6	± 1.2	88	± 76.2
TP52	0.0016 mg		21	± 7.8	122	± 8.7
<b>WITH S-2</b>						
NEG CONTROL	0.0 mg		20	± 6.7	74	± 15.2
AA	2.0 µg		418	±132.1	575	± 28.2
AF	2.0 µg		353	± 64.4	137	± 9.8
BP	2.0 µg		240	± 43.5	164	± 19.1
TP52	5.0 mg		16	± 3.6	77	± 20.5
TP52	1.0 mg		22	± 3.2	78	± 9.6
TP52	0.2 mg		11	± 5.3	112	± 19.3
TP52	0.04 mg		20	± 3.5	25	± 3.2
TP52	0.008 mg		20	± 1.5	59	± 25.2
TP52	0.0016 mg		21	± 2.1	92	± 17.9

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

**TABLE 3 (cont.): Mutagenicity Assay for 2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE (TP52) \***

COMPOUND†	DOSE/PLATE	TA1535	TA1537	TA1538
<b><u>WITHOUT S-9</u></b>				
NEG CONTROL	0.0 mg	39 ± 6.0	6 ± 2.4	14 ± 2.5
MNNG	2.0 µg	-	-	-
MNNG	20.0 µg	1798 ± 255.1	-	-
TP52	5.0 mg	50 ± 7.4	7 ± 0.6	15 ± 3.6
TP52	1.0 mg	43 ± 1.0	9 ± 0.6	14 ± 0.6
TP52	0.2 mg	29 ± 0.6	2 ± 0.6	3 ± 0.6
TP52	0.04 mg	18 ± 6.9	3 ± 1.2	13 ± 4.0
TP52	0.008 mg	34 ± 5.6	4 ± 2.1	19 ± 2.5
TP52	0.0016 mg	44 ± 3.6	7 ± 0.6	18 ± 2.1
<b><u>WITH S-9</u></b>				
NEG CONTROL	0.0 mg	27 ± 17.1	6 ± 2.7	19 ± 6.6
AA	2.0 µg	-	164 ± 88.0	549 ± 5
AF	2.0 µg	-	-	320 ± 62.6
BP	2.0 µg	-	44 ± 13.1	102 ± 10.1
TP52	5.0 mg	22 ± 1.5	6 ± 2.0	32 ± 9.6
TP52	1.0 mg	24 ± 1.7	4 ± 1.0	37 ± 7.4
TP52	0.2 mg	12 ± 1.7	3 ± 1.5	13 ± 4.0
TP52	0.04 mg	14 ± 3.5	2 ± 1.0	20 ± 4.5
TP52	0.008 mg	22 ± 4.0	6 ± 2.1	22 ± 2.3
TP52	0.0016 mg	17 ± 0.6	3 ± 0.6	26 ± 3.6

\*Values represent the mean number of revertants/plate (± standard deviation)

†MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

**CONCLUSION**

2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM CHLORIDE 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.

### REFERENCES

1. McCann J, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the *Salmonella*/Mammalian Microsome Mutagenicity Test: Test of 300 chemicals. Proc Nat Acad Sci, USA 1975;72:5135-5139.
2. Ames *Salmonella*/Mammalian Microsome Mutagenesis Test. LAIR Standard Operating Procedure OP-STX-1, Presidio of San Francisco, California: Letterman Army Institute of Research, 29 August 1986.
3. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with *Salmonella*/Mammalian Microsome Mutagenicity Test. Mutat 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.

APPENDICES

APPENDIX A: Chemical Data .....	13
APPENDIX B: Individual Plate Scores .....	15

**APPENDIX A: Chemical Data**

Chemical Name:

2-((hydroxyimino)methyl)-1-methyl-3-((1,2,2-trimethylpropoxy)methyl)-1H-imidazolium chloride

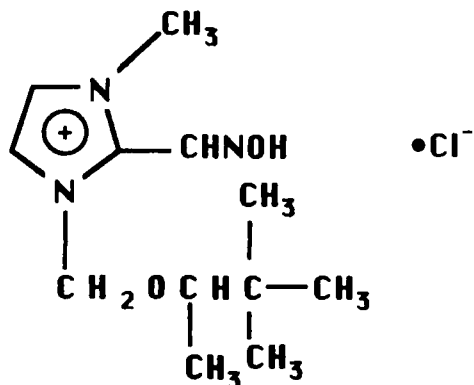
Alternate Chemical Names:

2-[(Hydroxyimino)methyl]-3-methyl-1-[(3,3-dimethyl-2-butoxy)methyl]imidazolium chloride,  
2-(E)-Hydroxyiminomethyl-3-methyl-1-(1,2,2-trimethylpropyloxymethyl)imidazolium chloride

Chemical Abstracts Service Registry Number: 91900-12-8

LAIR Code Number: TP52

Chemical Structure:

Molecular formula: C<sub>12</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>2</sub>

Molecular weight: 275.5

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

SRI Reference Number: BHH-0015

APPENDIX A (cont.): Chemical Data

Analytical Data: Data supplied by SRI International included melting point, elemental analysis, and NMR and IR spectra.<sup>1</sup> Melting point: 169-170°C (dec). Elemental analysis calculated for C<sub>12</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>2</sub>: C, 52.26; H, 8.04; N, 15.24; Cl, 12.86. Found: C, 52.44; H, 8.27; N, 15.47; Cl, 13.07. <sup>1</sup>H NMR (60 Mz, d<sub>6</sub>DMSO) δ 0.73 (s, 9H, CH<sub>3</sub>), 1.02 (d, 3H, J= 6.0 Hz, CH<sub>2</sub>), 3.33 (q, 1H, J= 6.0 Hz, CH), 4.00 (s, 3H, CH<sub>3</sub>), 5.83 (s, 2H, CH<sub>2</sub>), 8.05 (d, 1H, J= 2.0 Hz, aryl), 8.22 (d, 1H, J= 2.0 Hz, aryl), 8.60 (s, 1H, CH), 13.43 (s, 1H, NOH). IR (KBr) 2900, 1580, 1425, 1375, 1210, 1100, 1060, 1040, 925, 860, 740 cm<sup>-1</sup>. The IR spectrum obtained upon receipt of the compound confirmed the identity of the material.<sup>2</sup>

---

<sup>1</sup> Bedford CD. Notebook reference 5851-73. Stanford Research International, Physical Sciences Division, Menlo Park, CA.

<sup>2</sup> Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p20. Letterman Army Institute of Research, Presidio of San Francisco, CA.

## APPENDIX B: Individual Plate Scores

2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM  
CHLORIDE (TP52)

## TOXICITY DETERMINATION WITH TA100

DOSE/PLATE	5.0 mg	1.0 mg	0.2 mg	0.04 mg
PLATE 1	134	118	85	141
PLATE 2	108	105	109	102
PLATE 3	110	94	135	137
background lawn	NL*	NL	NL	NL
DOSE/PLATE	0.008 mg	0.0016 mg	NEG CONTROL	
PLATE 1	106	108	114	
PLATE 2	139	188	123	
PLATE 3	137	110	125	
background lawn	NL	NL	NL	

\* NL=Normal Lawn

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

2 - (E) -HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL) IMIDAZOLIUM  
CHLORIDE (TP52)

**NEGATIVE CONTROL DATA**

COMPOUND	DOSE/PLATE	TA98	TAL00	TA1535	TA1537	TA1538
<b><u>WITHOUT S-9</u></b>						
NEG CONTROL (START RUN)	0.0 mg	21	110	44	5	15
		17	129	39	3	13
		10	99	48	9	10
NEG CONTROL (END RUN)	0.0 mg	16	142	32	4	12
		12	130	36	7	15
		26	106	35	8	17
<b><u>WITH S-9</u></b>						
NEG CONTROL (START RUN)	0.0 mg	26	69	47	2	14
		25	64	30	6	12
		8	55	47	5	13
NEG CONTROL (END RUN)	0.0 mg	21	86	8	3	24
		19	74	16	10	25
		24	97	14	6	26

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

2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM  
CHLORIDE (TP52)

## POSITIVE CONTROL DATA

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
AA	2.0 µg	294	555		266	585
		403	562		114	486
		557	607		113	575
AF	2.0 µg	280	129			367
		402	148			344
		377	134			249
BP	2.0 µg	261	170		38	93
		190	180		59	113
		269	143		35	101
MNNG	2.0 µg		1521			
			1757			
			2127			
MNNG	20.0 µg			2063		
				1778		
				1554		

†AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine

APPENDIX B (cont.): Individual Plate Scores

2-(E)-HYDROXYIMINOMETHYL-5-METHYL-1-(1,2,2-TRIMETHYLPROPYLOXYMETHYL)IMIDAZOLIUM  
CHLORIDE (TP52)

MUTAGENICITY DATA WITHOUT S-9

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
TP52	5.0 mg	24	97	47	7	11
		21	122	58	6	16
		18	117	44	7	18
TP52	1.0 mg	21	88	42	9	13
		18	110	44	9	14
		14	82	43	8	14
TP52	0.2 mg	15	132	29	2	3
		13	98	29	2	3
		12	106	28	3	2
TP52	0.04 mg	13	110	14	4	18
		12	64	14	4	11
		10	60	26	2	11
TP52	0.008 mg	7	132	33	3	21
		7	132	40	6	16
		5	0	29	2	19
TP52	0.0016 mg	17	115	40	7	19
		16	132	45	7	16
		30	120	47	8	20

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

2-(E)-HYDROXYIMINOMETHYL-3-METHYL-1-(1,2,2-TRIMETHYLPROPXYLOXYMETHYL)IMIDAZOLIUM  
CHLORIDE (TP52)

MUTAGENICITY DATA WITH S-9

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
TP52	5.0 mg	13	53	23	4	21
		15	89	20	8	36
		20	88	22	6	39
TP52	1.0 mg	24	71	25	5	34
		23	74	25	4	31
		18	89	22	3	45
TP52	0.2 mg	13	129	13	5	17
		15	116	13	3	9
		5	91	10	2	14
TP52	0.04 mg	18	27	16	1	20
		18	24	16	2	16
		24	26	10	3	25
TP52	0.008 mg	20	43	24	5	23
		19	46	17	8	19
		22	88	24	4	23
TP52	0.0016 mg	22	96	17	3	22
		23	107	17	4	27
		19	72	16	3	29

OFFICIAL DISTRIBUTION LIST

Commander  
US Army Medical Research  
& 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

DASG-AAFJML  
Army/Air Force Joint Medical Library  
Offices of the Surgeons General  
5109 Leesburg Pike, Room 670  
Falls Church, VA 22041-3258

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  
and 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 Research 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

USAF School of Aerospace Medicine  
Document Section  
USAFSAM/TSKD  
Brooks Air Force Base, TX 78235-5301

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