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**MUTAGENIC POTENTIAL OF
2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-
PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE IN THE
AMES SALMONELLA/MAMMALIAN MICROsome
MUTAGENICITY TEST**

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DIVISION OF TOXICOLOGY**

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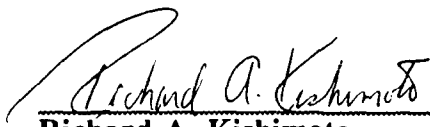
Mutagenic Potential of 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-
PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE in the Ames
Salmonella/Mammalian Microsome Mutagenicity Test (Toxicology Series 237)-Ormer *et al*

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Richard A. Kishimoto
COL, MSC
Acting Commander

28 July 1989
(date)

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ABSTRACT

The mutagenic potential of 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA100, TA104, 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 the conditions of this test.

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

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PREFACE

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GLP STUDY NUMBER: 88009

STUDY DIRECTOR: LTC Don W. Korte, Jr., PhD, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: Gayle A. Orner, BS, SGT, USA

CO-PRINCIPAL INVESTIGATORS: Joel B. Seewald, BS, SPC, USA
William J. Nieding, BS, SPC, 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-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-
PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE

INCLUSIVE STUDY DATES: 6 July - 27 October 1988

OBJECTIVE: The objective of this study was to determine the mutagenic potential of 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE (LAIR Code TP87) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

MAJ Gregory B. Knudson, PhD, MSC, provided research assistance.

**SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE
STUDY**

We, the undersigned, declare that GLP Study 88009 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

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REPLY TO
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SGRD-ULZ-QA

24 July 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 88009

1. This is to certify that in relation to LAIR GLP Study 88009 the following inspections were made:

22 June 1988	- Protocol Review
25 October 1988	- Dosing
27 October 1988	- Plate Counting

2. The institute report entitled "Mutagenic Potential of 2-Hydroxyiminomethyl-3-Methyl-1-(2'-Propargyloxyethyl)Imidazolium Chloride in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 237, was audited on 13 July 1989.

Carolyn M. Lewis

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

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Mutagenic Potential of 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE in the Ames Salmonella/Mammalian Microsome Mutagenicity Test-Orner et al.

INTRODUCTION

2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE was synthesized for a United States Army Medical Research and Development Command program charged with developing more effective oximes for the treatment of nerve agent poisoning. The Ames Salmonella/Mammalian Microsome Mutagenicity 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 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 therefore carcinogenic potential (1).

This evaluation of 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE utilizes a revision of the Ames Salmonella/Mammalian Microsome Mutagenicity Test (2).

Objective of the Study

The objective of this study was to determine the mutagenic potential of 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE (LAIR Code TP87) by using the revised Ames Salmonella/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical Name: 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE

LAIR Code Number: TP87

Physical State: Colorless crystalline solid

Source: Mr. Clifford D. Bedford
SRI International
333 Ravenswood Ave.
Menlo Park, CA 94025

Storage: 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE was received from SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 and assigned the LAIR Code number TP87. 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 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 100F-0269) obtained from Sigma Chemical Co. (St. Louis, MO). The test chemical was dissolved in glass distilled water. Reagent grade water used in this assay is first passed through a Technic Series 300 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, 300 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, TA100, TA104, 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 each strain's 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

2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL) IMIDAZOLIUM CHLORIDE was evaluated for mutagenic potential according to the revised Ames Test (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 (Table 1). This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of test compound ranging from 1.6×10^{-3} mg/plate to 5.0 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 decrease in the number of macrocolonies (below the number in the spontaneous reversion plates) or an observable reduction in the density of the background lawn, a maximum "limit" dose of 5.0 mg/plate was used in the mutagenicity test.

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 was purchased from Microbiological Associates Inc. (Bethesda, MD). 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). The water used in this medium and in all reagents came from a Technic Model 301 Reverse Osmosis Pre-Treatment Water System (Seattle, WA) (6). Plates were incubated upside down in the dark at 37°C for 48 hours. Plates were prepared in triplicate and the individual 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 minimal glucose agar 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 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 which was used in the toxicity test).

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. Benzo[a]pyrene (lot 18C-0378), N-methyl-N'-nitro-nitrosoguanidine (lot 127C-0342), and 4-nitroquinoline-n-oxide (lot 89C-0710) were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium azide (lot P2352) was obtained from Eastman Organic Chemicals (Rochester, NY). 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 TA104 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

Strains TA98 and TA1535 were not used. Strain TA1538 provides the same information (frameshift mutation detection) as TA98, and TA100 provides the same information as TA1535 (base pair substitution detection). Toxicity determination tests were performed on strain TA102 instead of TA100.

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 July, 1988, the toxicity of 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE was determined and for this experiment all sterility, strain verification, and negative controls were normal (Table 1).

Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 25-27 October, 1988 (Table 2). 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE 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 TP87

GLP STUDY NUMBER 88009

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA102)

<u>CONCENTRATION</u>	<u>MEAN</u>	<u>± 1SD</u>	<u>BACKGROUND LAWN*</u>
START RUN NEGATIVE CONTROL	14	± 3.8	NL
5.0 mg/plate	23	± 4.4	NL
1.0 mg/plate	21	± 1.0	NL
0.2 mg/plate	26	± 4.0	NL
0.04 mg/plate	25	± 3.5	NL
0.008 mg/plate	30	± 2.5	NL
0.0016 mg/plate	24	± 4.5	NL
END RUN NEGATIVE CONTROL	17	± 0.6	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATIONTA102*

HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	G
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.

**TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING
FOR THE MUTAGENICITY DETERMINATION OF TP87**

GLP STUDY NUMBER 88009

STRAIN VERIFICATION

OBSERVATIONS*

<u>STRAIN</u>	<u>HISTIDINE REQUIREMENT</u>	<u>AMPICILLIN RESISTANCE</u>	<u>UV REPAIR</u>	<u>CRYSTAL VIOLET</u>	<u>STERILITY CONTROL</u>
TA97	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA104	NG	G	NG	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)	G†
S-9	NG

† contamination occurred after dosing plates

* G=Growth, NG=No Growth

TABLE 3: Mutagenicity Assay for 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE (TP87)†

COMPOUND*	DOSE/PLATE	TA97	TA100	TA104
WITHOUT S-2				
NEG CONTROL	0.0 µg	73 ± 20.1	76 ± 7.0	105 ± 14.6
SA	1.5 µg	-	437 ± 6.4	-
MNNG	2.0 µg	-	-	887 ± 32.5
NQNO	0.5 µg	372 ± 7.8	-	-
TP87	5000.0 µg	79 ± 12.7	86 ± 5.5	118 ± 13.9
TP87	1000.0 µg	66 ± 5.1	63 ± 18.3	97 ± 7.8
TP87	200.0 µg	72 ± 12.3	71 ± 16.5	103 ± 10.0
TP87	40.0 µg	79 ± 8.6	72 ± 9.5	123 ± 14.4
TP87	8.0 µg	64 ± 11.7	78 ± 8.2	105 ± 11.0
TP87	1.6 µg	70 ± 6.2	68 ± 10.3	107 ± 17.4
WITH S-2				
NEG CONTROL	0.0 µg	88 ± 12.9	74 ± 8.5	140 ± 24.4
BP	2.0 µg	287 ± 27.0	392 ± 15.4	509 ± 60.4
TP87	5000.0 µg	93 ± 5.2	71 ± 12.7	147 ± 10.0
TP87	1000.0 µg	94 ± 8.5	78 ± 19.0	155 ± 21.2
TP87	200.0 µg	104 ± 8.9	72 ± 19.2	150 ± 11.0
TP87	40.0 µg	89 ± 1.7	67 ± 12.1	136 ± 2.9
TP87	8.0 µg	84 ± 1.5	84 ± 7.8	156 ± 15.1
TP87	1.6 µg	92 ± 10.6	73 ± 7.2	141 ± 6.0

† Values represent the mean number of revertants/plate (±standard deviation).

* SA=Sodium azide, BP=benzo[a]pyrene, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide

TABLE 3 (cont.): Mutagenicity Assay for 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE (TP87)†

COMPOUND*	DOSE/PLATE	TA1537	TA1538
WITHOUT S-2			
NEG CONTROL	0.0 µg	6 ± 1.6	9 ± 4.1
NQNO	0.5 µg	77 ± 12.7	83 ± 7.8
TP87	5000.0 µg	5 ± 2.9	12 ± 5.5
TP87	1000.0 µg	7 ± 2.1	10 ± 3.0
TP87	200.0 µg	4 ± 1.2	12 ± 1.7
TP87	40.0 µg	3 ± 3.1	10 ± 4.0
TP87	8.0 µg	4 ± 2.9	5 ± 1.7
TP87	1.6 µg	5 ± 0.6	9 ± 1.2
WITH S-2			
NEG CONTROL	0.0 µg	6 ± 2.5	21 ± 3.9
BP	2.0 µg	49 ± 5.5	86 ± 11.0
TP87	5000.0 µg	5 ± 1.7	16 ± 2.6
TP87	1000.0 µg	5 ± 0.6	20 ± 3.5
TP87	200.0 µg	8 ± 4.0	14 ± 4.2
TP87	40.0 µg	3 ± 2.3	18 ± 4.2
TP87	8.0 µg	5 ± 3.5	15 ± 3.2
TP87	1.6 µg	4 ± 2.1	15 ± 3.8

† Values represent the mean number of revertants/plate (± standard deviation).

* SA=Sodium azide, BP=benzo[a]pyrene, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide

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 lipopolysaccharide layer, and deficiency in DNA excision-repair. 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 TA102 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-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)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 (TA97, TA100, TA104) (1,6) or three times (TA1537, TA1538) (2,4) the spontaneous revertant colony count. 2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)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-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE is not mutagenic when evaluated in the Ames Test.

CONCLUSION

2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE was evaluated for mutagenic potential in the Ames Test, in both the presence and absence of metabolic activation, and did not induce a positive response under conditions of this study.

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APPENDICES

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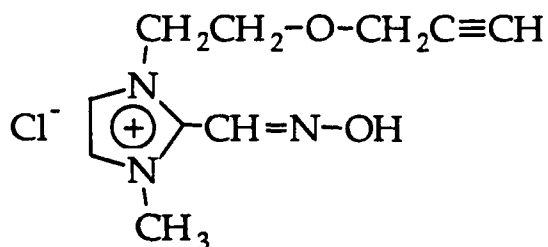
Appendix A: CHEMICAL DATA

Chemical name: 2-Hydroxyiminomethyl-3-methyl-1-(2'-propargyloxyethyl)imidazolium chloride

LAIR code number: TP87

WRAIR code number: WR256,038

Chemical structure:



Molecular formula: C₁₀H₁₄N₃O₂Cl

Molecular weight: 243.7

Physical state: Colorless crystalline solid

Melting point: 112-113°C

Analytical data:

IR(KBr): 3400, 3060, 1669, 1633, 1568, 1520, 1452, 1404, 1303, 992, 867, and 788 cm⁻¹.¹ IR spectrum was identical to that provided by the sponsor.

¹ Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.4, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

HPLC: The compound was analyzed by HPLC under the following conditions: column, 5 μ m silica (Brownlee, 100 x 4.6 mm): mobile phase, 82% A (0.01 M NaH₂PO₄, 0.0025 M tetramethylammonium hydrogen sulfate, pH adjusted to 3 with H₂SO₄), 18% B (acetonitrile); flow rate, 1.0 ml/min; wavelength monitored, 275 nm. The compound eluted at 2.71 min. No other peaks were observed to 15 min.²

Source: Mr. Clifford D. Bedford
SRI International
333 Ravenswood Ave.
Menlo Park, CA 94025

Lot number: BHH-0185

² Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.4, p 20. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: INDIVIDUAL PLATE SCORES

2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE

(Tp87)

TOXICITY DETERMINATION WITH TA102

DOSES	5.0 mg/plate	1.0 mg/plate	0.2 mg/plate	0.04 mg/plate
PLATE 1	28	22	22	29
PLATE 2	21	21	25	25
PLATE 3	20	20	30	22
BACKGROUND	normal lawn	normal lawn	normal lawn	normal lawn

DOSES	0.008 mg/plate	0.0016 mg/plate	NEG START	NEG END
PLATE 1	27	24	11	17
PLATE 2	30	28	12	16
PLATE 3	32	19	18	17
BACKGROUND	normal lawn	normal lawn	normal lawn	normal lawn

Appendix B (cont.): INDIVIDUAL PLATE SCORES

2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL) IMIDAZOLIUM CHLORIDE
(TP87)

NEGATIVE CONTROL DATA

COMPOUND	DOSE/PLATE	TA97	TA100	TA104	TA1537	TA1538
WITHOUT S-9						
NEG CONTROL	0.0 mg	111	73	117	5	17
(START RUN)		72	79	112	8	7
		72	88	123	5	11
NEG CONTROL	0.0 mg	66	72	87	6	7
(END RUN)		52	77	93	6	7
		64	68	96	3	7
WITH S-9						
NEG CONTROL	0.0 mg	111	59	146	2	22
(START RUN)		95	81	175	9	19
		77	81	152	8	19
NEG CONTROL	0.0 mg	87	74	111	7	26
(END RUN)		79	69	113	7	16
		81	78	142	5	25

Appendix B (cont.): INDIVIDUAL PLATE SCORES

2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE
(TP87)

POSITIVE CONTROL DATA

COMPOUND†	DOSE/PLATE	TA97	TA100	TA104	TA1535	TA1537
SA	1.5 µg		432 441 *			
BP	2.0 µg	261 286 315	375 405 396	524 561 443	55 44 49	81 99 79
NQNO	2.0 µg	366 377 *			86 68 *	78 92 79
MNNG	2.0 µg			852 894 916		

† BP=benzo[a]pyrene, NQNO=4-nitroquinoline-n-oxide, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, SA=Sodium azide.
* Plate contaminated

Appendix B (cont.): INDIVIDUAL PLATE SCORES

2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE (TP87)						
MUTAGENICITY DATA WITHOUT S-9						
COMPOUND	DOSE/PLATE	TA97	TA100	TA104	TA1537	TA1538
TP87	5.0 mg	93	92	106	7	7
		74	82	114	2	18
		69	83	133	7	12
TP87	1.0 mg	60	42	102	9	13
		67	77	88	5	7
		70	69	101	6	10
TP87	0.2 mg	86	88	113	5	11
		69	71	104	3	14
		62	55	93	5	11
TP87	0.04 mg	71	72	115	0	9
		77	62	140	6	6
		88	81	115	2	14
TP87	0.008 mg	69	87	116	2	4
		51	71	104	2	7
		73	76	94	7	4
TP87	0.0016 mg	68	65	113	5	8
		77	59	87	5	10
		65	79	120	4	10

Appendix B (cont.): INDIVIDUAL PLATE SCORES

2-HYDROXYIMINOMETHYL-3-METHYL-1-(2'-PROPARGYLOXYETHYL)IMIDAZOLIUM CHLORIDE (TP87)

MUTAGENICITY WITH S-9

COMPOUND	DOSE/PLATE	TA97	TA100	TA104	TA1537	TA1538
TP87	5.0 mg	99	66	155	4	18
		90	85	151	4	13*
		90	61	136	7	17*
TP87	1.0 mg	100	59	165	5	24
		84	77	170	6	20
		97	97	131	5	17
TP87	0.2 mg	94	50	161	8	11
		107	81	139	4	13
		111	85	151	12	19
TP87	0.04 mg	90	54	139	2	19
		90	68	134	2	21
		87	78	134	6	13
TP87	0.008 mg	83	80	172	5	14
		86	79	142	2	19
		84	93	154	9	13
TP87	0.0016 mg	94	69	135	6	18
		81	68	140	2	17
		102	81	147	5	11

*Contaminated - colonies counted visually.

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