THE MUTAGENIC POTENTIAL OF
(E)-1234-4-TETRAHYDRO-6-METHYL-1-(2-METHYL-1-OXO..(U)
LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN
UNCLASSIFIED FRANCISCO CA L J SAUERS ET AL. NOV 82 F/G 6/20 NL
INSTITUTE REPORT NO. 136

THE MUTAGENIC POTENTIAL OF:
(E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5)

LEONARD J. SAUERS, BA, SP5
and
JOHN T. FRUIN, DVM, PhD, COL VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

NOVEMBER 1982

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

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The mutagenic potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5) was assessed by using the Ames salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from \(0.05\) mg/plate to 3.2 x \(10^{-3}\) mg/plate. It was determined that the test substance did not have mutagenic potential.
The mutagenic potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from $10^{-1}$ mg/plate to $3.2 \times 10^{-3}$ mg/plate. It was determined that the test substance did not have mutagenic potential.
PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Same as above

PROJECT: 3M162779A871, Development of Repellents Against Medically
Important Arthropods, WU 201, APC TLO1

GLP STUDY NUMBER: 82012

STUDY DIRECTOR: COL John T. Fruin, D.V.M., PhD, VC, Diplomate of
American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: SP5 Leonard J. Sauers, BA

RAW DATA AND DATA MANAGEMENT: A copy of the final report, retired
SOPs, raw data, and chemical, analytical, stability, and
purity data of the test compound will be retained in the
LAIR Archives.

TEST SUBSTANCE: (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-
oxo-2-butenyl) quinoline (CHR 5)

INCLUSIVE STUDY DATES: 21 April - 21 May 1982

OBJECTIVE: To determine the mutagenic potential of the above
compound using the Ames Assay. Tester strains TA 98,
TA 100, TA 1535, TA 1537 and TA 1538 were used. The
plate incorporation method was followed. The test
substance was dissolved in ethanol and this diluent
was checked for sterility.
ACKNOWLEDGMENTS

The authors wish to thank PFC Paul Mauk, BS; Carolyn Lewis, MS; and John Dacey for their assistance in performing the research.
Signatures of Principal Scientists involved in the Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

LEONARD J. SAVERS, BA/DATE
SP5
Principal Investigator

JOHN T. FRUIN, DVM, PHD/DATE
COL, VC
Study Director
MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study #82012 the following inspections were made:

21 Apr 82
23 Apr 82

The report and raw data for this study were audited on 5 Aug 82.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 7 July 82 report to management and the Study Director.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer
TABLE OF CONTENTS

Abstract..................................................................................1
Preface..................................................................................iii
Acknowledgments..................................................................iv
Signatures of Principal Scientists.........................................v
Report of Quality Assurance Unit.......................................vi
Table of Contents..................................................................vii

BODY OF REPORT

INTRODUCTION

Rationale for using the Ames Assay......................................1
Description of Test, Rationale for strain selection..............1
Description of Strains, History, Methods, and Data.............2

METHODS

Rationale for Dosage Levels and Response Tabulations........3
Test Format............................................................................3
Statistical Analysis...............................................................4
Chemical Analysis.................................................................4

RESULTS..............................................................................4

DISCUSSION.....................................................................4

CONCLUSIONS...................................................................4

RECOMMENDATION..........................................................5

REFERENCES.......................................................................6

APPENDICES

Appendix A (Chemical Analysis)........................................7
Appendix B (Tables 1 through 5).........................................13

DISTRIBUTION LIST..........................................................21
Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay, which we use for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsomal enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon’s mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.
Description of Strains (History of the strains used, method to monitor the integrity of the organisms, and data pertaining to current and historical control and spontaneous reversion rates)

The test consists of using five different strains of *Salmonella typhimurium* that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained by using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred. These records are kept in the archives of the Quality Assurance Unit.

In this series of tests for the detection of mutagenic potential of different agents, we compare the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating
effectively, these strains detect substances that cause base pair
mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA
1538, and TA 98).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration
of the test substance had to be determined. This toxicity level was
found by using MGA plates, various concentrations of the substance,
and approximately 10^5 cells of TA 100 per plate, unless otherwise
specified. Top agar containing trace amounts of histidine and biotin
were placed on MGA plates. TA 100 is used because it is the most
sensitive strain. Strain verification was confirmed on the bacteria,
along with a determination of the spontaneous reversion rate. After
incubation, the growth was observed on the plates. (The auxotrophic
Salmonella will replicate a few times and potentially express a
mutation. When the histidine and biotin supplies are exhausted, only
those bacteria that reverted to the prototrophic phenotype will
continue to reproduce and form macrocolonies; the remainder of the
bacteria comprises the background lawn. The minimum toxic level is
defined as the lowest serial dilution at which decreased macrocolony
formation, below that of the spontaneous revertant rate, and an
observable reduction in the density of the background lawn occurs). A
maximum dose of 1 mg/plate is used when no toxicity is observed. The
densities were recorded as normal, slight, and no growth.

Test Format

After we validated our bacterial strains and determined the
optimal dosage of the test substance, we began the Ames Assay. In the
actual experiment, 0.1 ml of the particular strain of Salmonella (10^5
cells) and the specific dilutions of the test substance are added to 2
ml of molten top agar, which contained trace amounts of histidine and
biotin. Since survival is better from cultures which have just passed
the log phase, the Salmonella strains are used 16 hours (maximum)
after initial inoculation into nutrient broth. The dose of the test
substance spanned a 10^6-fold, decreasing from the minimum toxic level
by a dilution factor of 5. All the substances were tested with and
without S-9 microsome fraction. The optimal titer of the S-9 was
determined by the supplier, and 0.5 ml was added to the molten top
agar. After all the ingredients were added, the top agar was mixed,
then overlaid on minimum glucose agar plates. These plates contained
2% glucose and Vogel Bonner "E" Concentrate (4). The water used in
this medium and all reagents came from a polymetric system. 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 corresponding number of revertants obtained was
compared to the number of spontaneous revertants; the conclusions were
recorded statistically. A correlated dose response is considered
necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen in the Salmonella/Mammalian Microsome Mutagenicity Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by the method of Ames (2). He assumed that a compound which causes twice the spontaneous reversion rate and a correlated dose response is mutagenic.

Chemical Analysis

Our information on the chemical analysis of CHR5 was obtained from Starks Associates (Appendix A).

RESULTS AND DISCUSSION

Throughout this report, (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline will be referred to by its respective code name, CHR 5.

On 21 April 1982, the toxicity level determination was performed on the test compound. All sterility, strain verification and negative controls were normal (Table 1). A toxic response was observed at the highest dose used; therefore, 10^{-1} mg/plate was designated as the initial dose for the assay (Table 2).

On 23 April 1982, the Ames Assay was performed on CHR 5. All strain verification and sterility controls were normal for this experiment (Table 3). Expected results were obtained for all positive and negative controls (Table 4). The bacterial strains were exposed to doses ranging from 10^{-1} mg/plate to 3.2 \times 10^{-5} mg/plate of test substance. In no case was a dose response or a doubling of the spontaneous reversion rate observed (Table 5).

CHR 5 was tested previously (LAIR Institute Report 109). At that time, a solution of an unknown concentration was assayed. The Ames test was repeated due to the new lot and controlled concentration.

CONCLUSION

Based on the Ames Assay, CHR 5 is not mutagenic at the levels tested.
RECOMMENDATION

We recommend that candidate insect repellent CHR 5 be tested further with other toxicological assays if efficacy tests show this compound to be a promising repellent.
REFERENCES


3. LAIR SOP OP-STX-1, Ames Salmonella/mammalian microsome mutagenicity test, 15 February 1982


5. COMMONER, B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-022, 1976
DATA SHEET FOR COMPOUNDS

NAME OF COMPOUND: 3,4-Tetrahydro-6-methyl-1-(4-methyl-1-oxo-2-butenyl)quinoline

STRUCTURE

\[
\text{CH}_3
\]

\[
\text{CH}_3
\]

\[
\text{CH}_3
\]

\[
\text{CH}_3
\]

\[
\text{COCH}_3
\]

\[
\text{C}_13\text{H}_{16}\text{NO}
\]

\[
\text{C}_{16} \text{H}_{23} \text{NO}
\]

ANALYSES

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<th>ELEMENT</th>
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<th>FOUND</th>
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<td>C</td>
<td>78.56</td>
<td>78.90</td>
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<tr>
<td>H</td>
<td>8.35</td>
<td>8.52</td>
</tr>
<tr>
<td>N</td>
<td>6.31</td>
<td>6.00</td>
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STABILITY (Check When Applicable)

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<tr>
<th>ACID</th>
<th>BASE</th>
<th>HEAT</th>
<th>LIGHT</th>
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</tr>
<tr>
<td>LIGHT</td>
<td>64</td>
<td>64</td>
<td>71</td>
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</tbody>
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SYNTHESIS INDICATING SYNTHETIC ROUTE

1. \( \text{C}_6\text{H}_5\text{CH} = \text{CH} + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_5\text{CH} = \text{CH}_2 + \text{H}_2 \)
2. \( \text{C}_6\text{H}_5\text{CH} = \text{CH}_2 + \text{H}_2\text{N} \rightarrow \text{C}_6\text{H}_5\text{CH} = \text{CH} - \text{NH}_2 \)
3. \( \text{C}_6\text{H}_5\text{CH} = \text{CH} - \text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_5\text{CH} = \text{CH} - \text{NH}_2\text{H}_2 \)
4. \( \text{C}_6\text{H}_5\text{CH} = \text{CH} - \text{NH}_2\text{H}_2 + \text{H}_2 \rightarrow \text{C}_6\text{H}_5\text{CH} = \text{CH} - \text{NH}_2\text{H}_2 + \text{H}_2 \)

REFERENCE
1. Ether; RF=0.83
2. Ethyl acetate; RF=0.64
3. Methylene chloride; RF=0.30, elongated spot

PRIORITY 1
(E)-2,2,6,6-Tetramethyl-1,4,7,10-tetraoxa-3-endo-1,8-endododecane

CDCl₃

2/5/82
<table>
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<th>Description</th>
<th>Date</th>
<th>Page</th>
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<tr>
<td>Table 1</td>
<td>Strain Verification for Toxicity Level Determination</td>
<td>21 Apr 82</td>
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<td>Table 2</td>
<td>Toxicity Level Determination</td>
<td>21 Apr 82</td>
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</tr>
<tr>
<td>Table 3</td>
<td>Strain Verification Control</td>
<td>23 Apr 82</td>
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<td>Table 4</td>
<td>Quality and Positive Controls</td>
<td>23 Apr 82</td>
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<td>Table 5</td>
<td>Salmonella/microsome Assay Worksheet</td>
<td>23 Apr 82</td>
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### TABLE 1

**STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Histidine Requirement</th>
<th>Ampicillin Resistance</th>
<th>UV Sensitivity to Crystal Violet</th>
<th>Sterility Control</th>
<th>Response (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>NG</td>
<td>G</td>
<td>NG</td>
<td>14 mm</td>
<td>NG</td>
</tr>
<tr>
<td>1537</td>
<td>NG</td>
<td>16 mm</td>
<td>NG</td>
<td>15 mm</td>
<td>NG</td>
</tr>
<tr>
<td>WT</td>
<td>NG</td>
<td>14 mm</td>
<td>G</td>
<td>G</td>
<td>NG</td>
</tr>
</tbody>
</table>

**STERILITY CONTROL**

<table>
<thead>
<tr>
<th>His-Bio Mix</th>
<th>Initial: NG</th>
<th>End: NG</th>
<th>MGA Plate: NG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Agar</td>
<td>Initial: NG</td>
<td>End: NG</td>
<td></td>
</tr>
<tr>
<td>Diluent: NG</td>
<td>Nutrient Broths: NG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test Compound: (a)CHR-5, (b) NA, (c) NA, (d) NA, (e) NA

G = Growth, NG = No Growth, NT = Not Tested, NA = Not Applicable, WT = Wild Type

**Spontaneous Revertants:** TA 100, No S-9 91, 106, 97 average = 98

(1) + = expected response, - = unexpected response

**Study Number:** 82012 **Date:** 21 Apr 82 **By:** Sauers
TABLE 2

TOXICITY LEVEL DETERMINATION

Substance assayed: CHR 5
Substance dissolved in: ETOH
Study Number: 82012
Date: 21 Apr 82
Performed by: Sauers, Dacey

TA 100 REVERTANT PLATE COUNT

<table>
<thead>
<tr>
<th>Test Compound Concentration</th>
<th>Plate #1</th>
<th>Plate #2</th>
<th>Plate #3</th>
<th>Average</th>
<th>Background Lawn (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/plate</td>
<td>51</td>
<td>56</td>
<td>53</td>
<td>53</td>
<td>SL</td>
</tr>
<tr>
<td>10^-1 mg/plate</td>
<td>90</td>
<td>73</td>
<td>81</td>
<td>81</td>
<td>NL</td>
</tr>
<tr>
<td>10^-2 mg/plate</td>
<td>96</td>
<td>93</td>
<td>86</td>
<td>92</td>
<td>NL</td>
</tr>
<tr>
<td>10^-3 mg/plate</td>
<td>87</td>
<td>61</td>
<td>107</td>
<td>85</td>
<td>NL</td>
</tr>
<tr>
<td>10^-4 mg/plate</td>
<td>81</td>
<td>61</td>
<td>80</td>
<td>74</td>
<td>NL</td>
</tr>
<tr>
<td>10^-5 mg/plate</td>
<td>120</td>
<td>99</td>
<td>93</td>
<td>104</td>
<td>NL</td>
</tr>
<tr>
<td>10^-6 mg/plate</td>
<td>74</td>
<td>65</td>
<td>78</td>
<td>72</td>
<td>NL</td>
</tr>
<tr>
<td>10^-7 mg/plate</td>
<td>73</td>
<td>71</td>
<td>82</td>
<td>75</td>
<td>NL</td>
</tr>
</tbody>
</table>

(1) NG = No Growth   ST = Slight Growth   NL = Normal Lawn
### TABLE 3

**STRAIN VERIFICATION CONTROL**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Histidine Requirement</th>
<th>Ampicillin Resistance</th>
<th>UV</th>
<th>Sensitivity to Crystal Violet</th>
<th>Sterility Control</th>
<th>Response (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>NG</td>
<td>G</td>
<td>NG</td>
<td>NG (14 mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>NG</td>
<td>G</td>
<td>NG</td>
<td>NG (15 mm)</td>
<td>NG</td>
<td>+</td>
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<tr>
<td>1535</td>
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<td>NA</td>
<td>NG</td>
<td>NG (14 mm)</td>
<td>NG</td>
<td>+</td>
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<tr>
<td>1537</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG (13 mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>1538</td>
<td>NG</td>
<td>NA</td>
<td>NG</td>
<td>NG (14 mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>WT</td>
<td>G</td>
<td>NA</td>
<td>G</td>
<td>NA</td>
<td>NA</td>
<td>+</td>
</tr>
</tbody>
</table>

**STERILITY CONTROL**

- **His-Bio Mix**
  - Initial: NG
  - End: NG
  - Diluent: NG

- **Top Agar**
  - Initial: NG
  - End: NG
  - MGA Plate: NG

- **S-9 Mix**
  - Initial: NG
  - End: NG
  - Nutrient Broth: NG

**Test Compound**

- (a) CHR5 - NG (b) NA (c) NA (d) NA (e) NA (f) NA

- G = Growth
- NG = No Growth
- NT = Not Tested
- NA = Not Applicable
- WT = Wild Type

**Study Number:** 02012  
**By:** Sauers  
**Date:** 23 April 82

(1) + = expected response  
- = unexpected response
<table>
<thead>
<tr>
<th>Compd.</th>
<th>Amount of Compd. Added</th>
<th>S-9 Added</th>
<th>98</th>
<th>100</th>
<th>Strain Number 1532</th>
<th>Strain Number 1537</th>
<th>Strain Number 1538</th>
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<tbody>
<tr>
<td>AF</td>
<td>2 ug/plate yes</td>
<td>(703,865,697)</td>
<td>(619,545,533)</td>
<td></td>
<td></td>
<td>(652,933,959)</td>
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</tr>
<tr>
<td>DMBA</td>
<td>20 ug/plate yes</td>
<td>(999,999,999)</td>
<td>(999,999,999)</td>
<td></td>
<td></td>
<td>(28,157,294)</td>
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</tr>
<tr>
<td>BBNG</td>
<td>2 ug/plate no</td>
<td>(999,999,999)</td>
<td></td>
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<tr>
<td></td>
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<td>(999,999,999)</td>
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</tr>
</tbody>
</table>

**Strain Performance**

- **Spontaneous Revertants**
  - before: (21, 23, 17) (121,113,139) (18, 26, 20) (5, 6, 4) (28, 16, 18)
  - after: (16, 18, 20) (88,116,114) (13, 13, 22) (4, 5, 8) (16, 17, 21)
  - before: (27, 29, 38) (125,128,106) (16, 18, 14) (4, 5, 9) (24, 28, 16)
  - after: (22, 29, 26) (135,138,108) (17, 19, 13) (5, 3, 7) (11, 17, 27)

**Study Number:** 82012

**Date:** 23 April 82
**By:** Sauers, Maas, Lewis, Dacey

**Note:** 999 means > 999
<table>
<thead>
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Study No.: 82012  Date: 23 Apr 82  Performed by: Sayers, Mauk, Lewis, Dacey
**TABLE 5, concluded**

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Study No.: 82012  Date: 23 April 82 Performed by: Sauers, Leak, Lewis, Dorey
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Commander
US Army Medical Research
and Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, Frederick MD 21701

Defense Technical Information Center
ATTN: DTIC-DDA
Cameron Station
Alexandria VA 22314

Director of Defense Research and Engineering
ATTN: Assistant Director, Environmental
and Life Sciences
Washington DC 20301

The Surgeon General
ATTN: DASG-TLO
Washington DC 20314

HQ DA (DASG-ZXA)
WASH DC 20310

Commandant
Academy of Health Sciences
ATTN: HSHA-CDM
Fort Sam Houston TX 78234

Assistant Dean
Institute and Research Support
Uniformed Services University
of Health Sciences
6917 Arlington Road
Bethesda MD 20014

Commander
US Army Environmental Hygiene Agency
Aberdeen Proving Ground MD 21070

US Army Research Office
ATTN: Chemical and Biological Sciences
Division
P.O. Box 1221
Research Triangle Park NC 27709

Biological Sciences Division
Office of Naval Research
Arlington VA 22217

Director of Life Sciences
USAF Office of Scientific Research (AFSC)
Bolling AFB
Washington DC 20332

Director
Walter Reed Army Institute of Research
Washington DC 20012

Commander
US Army Medical Research Institute
of Infectious Diseases
Fort Detrick, Frederick MD 21701

Commander
US Army Research Institute
of Environmental Medicine
Natick MA 01760

Commander
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Fort Sam Houston TX 78234

Commander
US Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick, Frederick MD 21701

Commander
US Army Aeromedical Research Laboratory
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Commander
US Army Research Institute
of Chemical Defense
Aberdeen Proving Ground
Edgewood Arsenal MD 21010

Commander
Naval Medical Research Institute
National Naval Medical Center
Bethesda MD 20014

Commander
USAF School of Aerospace Medicine
Aerospace Medical Division
Brooks Air Force Base TX 78235