INSTITUTE REPORT NO. 205

MUTAGENIC POTENTIAL OF BENZOTHIAZOLE

STEVEN K. SANO, BA, SP5

and

DON W. KORTE JR, PhD, MAJ MSC

TOXICOLOGY GROUP
DIVISION OF RESEARCH SUPPORT

AUGUST 1985

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

Toxicology Series 93
Mutagenic potential of bensothiosole (Toxicology Series 93)--Sano and Korte

Reproduction of this document in whole or in part is prohibited except with the permission of the Commander, Letterman Army Institute of Research, Presidio of San Francisco, California 94129. However, the Defense Technical Information Center is authorized to reproduce the document for United States Government purposes.

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

(Signature and date)
EDWIN S. BEATRICE, M.D.
Colonel, MC
Commanding, LAIR

This document has been approved for public release and sale; its distribution is unlimited.
# Mutagenic Potential of Benzothiazole

**Authors:**
Steven K. Sano, BA SPA
Don W. Korte, Jr., PhD, MAJ MS

**Performing Organization Name and Address:**
Toxicology Group, Division of Research Support
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

**US Army Medical Research and Development Command:**
Fort Detrick, MD 21701-5012

**Abstract:**
The mutagenic potential of benzothiazole was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1 µl/plate to 1000 µl/plate. The test compound was not mutagenic under conditions of this assay.
ABSTRACT

The mutagenic potential of benzothiazole was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1 ul/plate to 3.2 x 10^-4 ul/plate. The test compound was not mutagenic under conditions of this assay.

Key Words: Mutagenicity, Genetic Toxicology, Ames Assay, Benzothiazole
PREFACE

TYPE REPORT: Ames Assay 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
US Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, MD 21701-5010

WORK UNIT: 3516277A875 Medical Defense Against Chemical Agents Projects; WU 308; APC TL05

CLP STUDY NUMBER: 84031

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD

PRINCIPAL INVESTIGATOR: SP4 Steven K. Sano, BA

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

TEST SUBSTANCE: Benzothiazole

INCLUSIVE STUDY DATES: 24 September - 12 October 1984

OBJECTIVE: The objective of this study was to determine the mutagenic potential of benzothiazole (Batch Number 1723LK, LAIR Code TA037) by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay.
ACKNOWLEDGMENTS

The authors wish to thank SP6 James Justus, BA; SP4 Paul Mauk, BA; PFC James Martin; and Mr. John Dacey, for their assistance in performing the research.
We, the undersigned, declare that GLP study number 84031 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., PhD / DATE
MAJ, MSC
Study Director

STEVEN K. SANO, BA / DATE
SP4, USA
Principal Investigator

CONRAD WHEELER, Ph.D. / DATE
DAC
Analytical Chemist
MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

1. I hereby certify that in relation to LAIR GLP Study 84031 the following inspections were made:

   10 October 1984
   12 October 1984

2. The report and raw data for this study were audited on 10 May 1984.

3. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 21 January 1985 report to Management and the Study Director.

GARY L. DUTCHER
SP6-46A
Quality Assurance Unit

13 August 1985
# TABLE OF CONTENTS

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

**BODY OF REPORT**

**INTRODUCTION**

Objective of the Study ........................................................... 1

**METHODS**

Test Compound ........................................................................ 1
Test Solvent .............................................................................. 2
Chemical Preparation ............................................................. 2
Test Strains ............................................................................. 2
Test Format ............................................................................... 2

**RESULTS** ............................................................................. 4

**DISCUSSION** ......................................................................... 11

**CONCLUSION** ......................................................................... 11

**RECOMMENDATION** ............................................................ 11

**REFERENCES** ......................................................................... 12

**APPENDIX** ............................................................................. 13

**DISTRIBUTION LIST** ............................................................... 18
The Ames Salmonella/Mammalian Microsome Mutagenicity Assay is a short-term screening assay that utilizes histidine auxotrophic mutant strains of Salmonella typhimurium to detect those compounds which are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the assay to increase sensitivity by simulating in vivo metabolic activation of the test compound. The Ames assay is an inexpensive yet highly predictive and reliable assay for detecting mutagenic activity and thus carcinogenic potential.

Objective of the Study

The objective of this study was to determine the mutagenic potential of benzothiazole (Batch Number 1723LK, LAIR Code TA037) by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay.

Methods

Test Compound

Chemical name: Benzothiazole

Chemical Abstract Service Registry No.: 95-16-9

Structural formula:

[Diagram of benzothiazole structure]

Empirical formula: C_7H_5NS
Storage: Ten milliliters of 99% benzothiazole (Batch Number 1723LK) was received from Aldrich Chemical Company, Inc, (Milwaukee, WI) on 22 August 1984 and assigned the LAIR Code number TA037. The test compound was stored in a dessicator at room temperature (21°C) until use.

Chemical Properties/Analysis: Data characterizing the chemical composition and purity of the test material was obtained from Aldrich Chemical Co, Inc and confirmed by Infrared Spectrometer performed by the Toxicology Services Group, LAIR (Presidio of San Francisco, CA), (Appendix A).

Test Solvent

The test compound and the positive control chemicals were dissolved in grade I dimethyl sulfoxide (Lot Number 100F-0269) obtained from Sigma Chemical Co (St. Louis, MO).

Chemical Preparation

Benzothiazole was stored in a dessicator at room temperature (21°C) until used. On the day before dosing, 0.5 ml of the test compound was measured into a sterile vial and again stored at room temperature. On the day of dosing, the 0.5 ml sample was dissolved in a 9.4 ml volume of grade I dimethyl sulfoxide (Lot Number 100F-0269) to achieve a 5% (v/v) solution. Aliquots of this solution were used to dose the test plates. The dosing procedure was completed within 20 minutes of dissolving the test compound.

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 at -80°C. Quality controls 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

Benzothiazole 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 benzanthrazone ranging from $1.6 \times 10^{-3}$ ul/plate to 5 ul/plate and approximately $10^8$ cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin were placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate.

**Mutagenicity Assay**

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 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). The water used in this medium and in all reagents came from a Polymetric Model 200-3 Water Purifier (Sunnyvale, CA). 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 assay. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound assay 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). Concurrent sterility and strain verification controls were run. 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 following tests were run to determine if:

- Lipopolysaccharide layer (LP) alteration causes growth inhibition in the presence of crystal violet.

- An ampicillin-resistant R factor has allowed growth in strains TA98 and TA100 in the presence of ampicillin impregnated disks.

- Absence of excision repair mechanism has inhibited growth in the presence of ultraviolet light.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. 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 the following criteria are met:

1. For strain TA98 and TA100, 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 strain. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

2. For strains TA1535, TA1537, and TA1538, a correlated dose response over three concentrations is achieved with at least one dose yielding a revertant colony count three times the spontaneous colony count for the strain.

RESULTS

On 3 October 1984, the toxicity level determination was performed on benzothiazole (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 2). At the highest dose of 5 μl per plate, no colony formation occurred. Toxicity was observed after exposure of the tester strain (TA100) to this maximum dose. The highest dose with no observed toxicity in the tester strain (1 μl/plate) was designated the high dose for the definitive assay. The remaining dose groups were obtained by diluting the highest dose level 1000-fold by using a sequential dilution factor of 5.

Normal results were obtained for all sterility, strain verification, positive and negative controls during the Ames Assay performed during the 3-day period, 10 to 12 October 1984 (Tables 3-4). Benzothiazole did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 5).
TABLE I

TOXICITY LEVEL DETERMINATION

Substance assayed: BENZOTHIAZOLE (TA037)  
Substance dissolved in: DMSO

Study Number: 84031  
Date: 5 OCT 1984  
Performed by: SANO

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>5 ul/plate</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NG</td>
</tr>
<tr>
<td>1 ul/plate</td>
<td>94</td>
<td>100</td>
<td>80</td>
<td>91</td>
<td>NL</td>
</tr>
<tr>
<td>0.2 ul/plate</td>
<td>114</td>
<td>104</td>
<td>112</td>
<td>110</td>
<td>NL</td>
</tr>
<tr>
<td>0.04 ul/plate</td>
<td>121</td>
<td>99</td>
<td>105</td>
<td>108</td>
<td>NL</td>
</tr>
<tr>
<td>0.008 ul/plate</td>
<td>125</td>
<td>123</td>
<td>109</td>
<td>119</td>
<td>NL</td>
</tr>
<tr>
<td>0.0016 ul/plate</td>
<td>113</td>
<td>115</td>
<td>121</td>
<td>116</td>
<td>NL</td>
</tr>
</tbody>
</table>

(1) NG = No Growth,  ST = Slight Growth,  NL = Normal Lawn
### TABLE 2

#### STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

<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>100</td>
<td>NG</td>
<td>G</td>
<td>NG</td>
<td>NG (16mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>Wild Type</td>
<td>NT</td>
<td>NT</td>
<td>G</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
</tr>
</tbody>
</table>

#### STERILITY CONTROL FOR TOXICITY LEVEL DETERMINATION

<table>
<thead>
<tr>
<th>His-Bio Mix</th>
<th>Initial:</th>
<th>End:</th>
<th>MGA Plate:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Agar</td>
<td>Initial:</td>
<td>End:</td>
<td></td>
</tr>
<tr>
<td>Diluent:</td>
<td>DMSO:NG</td>
<td>Nutrient Broth:</td>
<td>NG</td>
</tr>
<tr>
<td>Test Compound</td>
<td>(a) NG</td>
<td>(b) NG</td>
<td>(c) NG</td>
</tr>
</tbody>
</table>

C = Growth   NG = No Growth   NT = Not Tested   NA = Not Applicable

Spontaneous Revertants: TA 100, No S-9 (102,111, 90)101

(1) + = expected response   - = unexpected response

Study Number: 84031       Date: 4 OCT 84       By: SANO
### TABLE 3

**STRAIN VERIFICATION CONTROL FOR ASSAY**

<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 (17mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>NG</td>
<td>G</td>
<td>NG</td>
<td>NG (20mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>1535</td>
<td>NG</td>
<td>NT</td>
<td>NG</td>
<td>NG (18mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>1537</td>
<td>NG</td>
<td>NG (15mm)</td>
<td>NG</td>
<td>NG (17mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>1538</td>
<td>NG</td>
<td>NT</td>
<td>NG</td>
<td>NG (16mm)</td>
<td>NG</td>
<td>+</td>
</tr>
<tr>
<td>Wild Type</td>
<td>NT</td>
<td>NT</td>
<td>G</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
</tr>
</tbody>
</table>

#### STERILITY CONTROL FOR ASSAY

<table>
<thead>
<tr>
<th>His-Bio Mix</th>
<th>Initial:</th>
<th>End:</th>
<th>Diluent:</th>
<th>DMSO:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NG</td>
<td>NG</td>
<td></td>
<td>NG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Top Agar</th>
<th>Initial:</th>
<th>End:</th>
<th>NGA Plate:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S-9 Mix</th>
<th>Initial:</th>
<th>End:</th>
<th>Nutrient Broth:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
<th>(d)</th>
<th>(e)</th>
<th>(f)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
</tbody>
</table>

G = Growth   NG = No Growth   NT = Not Tested   NA = Not Applicable

Study Number: 86031   By: - SANO -   (1) + = expected response

Date: 11 OCT 84   = unexpected response
### TABLE 4

**POSITIVE AND NEGATIVE CONTROL TEST**

(Revertants/plate)

<table>
<thead>
<tr>
<th>STRAIN NUMBER</th>
<th>TA98</th>
<th>TA100</th>
<th>TA1535</th>
<th>TA1537</th>
<th>TA1538</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF 2 ug/plate</td>
<td>YES</td>
<td>772,823,982</td>
<td>1053,878,1216</td>
<td>(913,966,820)</td>
<td>900</td>
</tr>
<tr>
<td>BP 2 ug/plate</td>
<td>YES</td>
<td>230,175,387</td>
<td>335,332,302</td>
<td>(32, 25, 21)</td>
<td>(78, 46, 86)</td>
</tr>
<tr>
<td>AA 2 ug/plate</td>
<td>YES</td>
<td>1488,1613,1754</td>
<td>1725,1495,1994</td>
<td>(224,205,211)</td>
<td>(927,1073,1089)</td>
</tr>
<tr>
<td>MNNG 2 ug/plate</td>
<td>NO</td>
<td>(1935,1737,2129)</td>
<td>1934</td>
<td>(1852,1781,2053)</td>
<td>1896</td>
</tr>
</tbody>
</table>

**SPONTANEOUS REVERSION RATE (NEGATIVE CONTROL)**

<table>
<thead>
<tr>
<th>STRAIN NUMBER</th>
<th>TA98</th>
<th>TA100</th>
<th>TA1535</th>
<th>TA1537</th>
<th>TA1538</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Assay YES</td>
<td>15, 13, 15</td>
<td>89,102, 94</td>
<td>(15, 13, 12)</td>
<td>(5, 6, 1)</td>
<td>(12, 14, 16)</td>
</tr>
<tr>
<td>After Assay YES</td>
<td>27, 16, 16</td>
<td>(113,113,108)</td>
<td>(20, 15, 16)</td>
<td>(4, 3, 5)</td>
<td>(16, 8, 8)</td>
</tr>
<tr>
<td>Before Assay NO</td>
<td>13, 24, 18</td>
<td>86, 88, 87</td>
<td>(13, 13, 16)</td>
<td>(1, 4, 6)</td>
<td>(13, 11, 18)</td>
</tr>
<tr>
<td>After Assay NO</td>
<td>13, 17, 20</td>
<td>(99, 79,108)</td>
<td>(17, 15, 16)</td>
<td>(6, 4, 9)</td>
<td>(9, 15, 8)</td>
</tr>
</tbody>
</table>

**Study Number:** B4031  **Date:** 12 Oct 84  **Performed by:** SANO & MARTIN

Compounds:  **AF** = 2-aminoflourene,  **BP** = Benzo(a) pyrene,  **AA** = 2-aminoanthracene,  **MNNG** = N-methyl-N'-nitro-N-nitrosoguanidine
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEVEL</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>TA037</td>
<td>1 ul/plate</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>TA317</td>
<td>0.2 ul/plate</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>TA037</td>
<td>0.04 ul/plate</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Study Number:** 84031  **Date:** 12 Oct 84  **Performed by:** SANO & MARTIN
### TABLE 5 (cont.)
**BENZOTHIAZOLE ASSAY**
*(Revertants/Plate)*
**Mean**

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>DOSE LEVEL</th>
<th>S-9 ADDED</th>
<th>TA98</th>
<th>TA100</th>
<th>STRAIN NUMBER</th>
<th>TA1535</th>
<th>TA1537</th>
<th>TA934</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA037</td>
<td>0.008 ul/plate</td>
<td>YES</td>
<td>(22, 22, 14)</td>
<td>(99, 89, 96)</td>
<td>(12, 11, 13)</td>
<td>(8, 7, 6)</td>
<td>(5, 7, 11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO</td>
<td>(14, 11, 12)</td>
<td>(100, 78, 105)</td>
<td>(20, 23, 23)</td>
<td>(5, 6, 6)</td>
<td>(5, 9, 6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0016 ul/plate</td>
<td>YES</td>
<td>(22, 17, 23)</td>
<td>(96, 81, 81)</td>
<td>(24, 21, 15)</td>
<td>(4, 7, 1)</td>
<td>(15, 14, 11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO</td>
<td>(14, 13, 17)</td>
<td>(110, 100, 104)</td>
<td>(10, 9, 17)</td>
<td>(7, 6, 7)</td>
<td>(13, 15, 19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00032 ul/plate</td>
<td>YES</td>
<td>(21, 19, 16)</td>
<td>(100, 101, 86)</td>
<td>(20, 21, 5)</td>
<td>(2, 2, 2)</td>
<td>(1, 1, 15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO</td>
<td>(13, 19, 20)</td>
<td>(88, 70, 100)</td>
<td>(14, 21, 23)</td>
<td>(1, 4, 3)</td>
<td>(2, 3, 9)</td>
<td></td>
</tr>
</tbody>
</table>

**Study Number:** 84031  **Date:** 12 Oct 84  **Performed by:** SANO & MARTIN
DISCUSSION

Certain test criteria must be satisfied before an Ames assay 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, LP layer alterations, and DNA excision repair deficiencies. Second, the Salmonella strains must be responsive to the mutagenic process by exposing the strains to 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 microcolony and microcolony formation. If these tests are performed and expected data are obtained, then the results of Ames assay can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, benzothiazole was evaluated in the Ames assay. Criteria for a positive response are a correlated dose-response relationship for the positive strains and a two-fold (strains TA98 or TA100) or three-fold (strains TA1535, TA1537, or TA1538) increase in revertant colony counts relative to the respective negative control counts (5). Benzothiazole 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 assay indicate that benzothiazole is not mutagenic when evaluated in the Ames assay.

CONCLUSION

Benzothiazole, both with and without metabolic activation, is not mutagenic in the Ames assay as conducted in this study.

RECOMMENDATION

Benzothiazole should be tested with other toxicological assays in accordance with the Toxic Substance Control Act.
REFERENCES


Chemical name: Benzothiazole

Chemical Abstracts Service Registry No.: 74-46-1

Chemical structure:

\[ \text{Chemical formula: } C_7H_{11}NS \]

Molecular weight: 135.18

Physical state: Brown liquid

Compound density: \( d^2_{20} 1.246^a \)

Source: Aldrich Chemical Co.
Milwaukee, WI

Lot number: 1723LX

Analytical data: Compound described as 99% pure by source. Analysis provided by sponsor demonstrated a purity of 99.99%. IR and NMR analysis performed on receipt of compound provided the following data: IR (KBr): 3060, 1670, 1545, 1455, 1290, 875, 800, 760, 730 cm\(^{-1}\). NMR (80 MHz, \( d_6 \)-DMSO): \$3.45 (singlet, 1 H, S-CH=N), complex multiplet centered at 8.18 (2H, aromatic protons), complex multiplet centered at 7.56 (2H, aromatic protons). The IR spectrum was identical to the Sadtler standard spectrum.\(^7\)

Stability: No decomposition was observed by NMR after 66 h in DMSO.


\(^b\)Rosencrance AH. [Memorandum for Dr. Reddy]. SUBJECT: Results from the chemical analysis of three compounds slated for toxicity testing (24 July 1984). Frederick, Maryland: USAMBRIL.

\(^c\)Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p2. Letterman Army Institute of Research, Presidio of San Francisco, CA.

\(^d\)Ibid. p3.

ANALYTICAL DATA

Date June 10, 1984

Our: 10133-8 Benzothiazole, 99%

Batch No.: 17231X

Analytical Results:

Appearance: Dark gold liquid

mp: 114°

b.p.: 129°

nD 1.6423

[a]D 0

Spectral Data:


U.V.

N.M.R.

Assay:

V.P.C. 99+%

Titration

Other:

DS/Kb

M. Majickowski

APPENDIX A (cont.)
MEMORANDUM FOR DR. REDDY

SUBJECT: Results from the Chemical Analysis of Three Compounds Slated for Toxicity Testing

Benzothiazole, 1,4-thioxane and 1,4-dithiane were given by Dr. Reddy for analysis on 15 June 84. The following is a summary of the results from those analysis:

<table>
<thead>
<tr>
<th>% of Total Formula</th>
<th>Compound</th>
<th>Other Possibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzothiazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>98.38 C₆H₄NS</td>
<td>Benzothiazole</td>
<td></td>
</tr>
<tr>
<td>0.61 C₆H₄NS</td>
<td>2-Methylbenzothiazole (isomers)</td>
<td></td>
</tr>
<tr>
<td>0.26 C₆H₈N</td>
<td>Aniline</td>
<td>3 or 4-Cyanopyrazole</td>
</tr>
<tr>
<td>0.12 C₄H₈S₂</td>
<td>Diphenyldisulfide</td>
<td></td>
</tr>
<tr>
<td>0.11 C₇H₄N</td>
<td>Toluidine (isomers)</td>
<td>Benzylamine, N-Methylaniline</td>
</tr>
<tr>
<td>0.03 C₈H₈NS</td>
<td>Methylbenzothiazole (isomers)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,4-Thioxane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99.93 C₄H₈O₂</td>
<td>1,4-Thioxane</td>
<td></td>
</tr>
<tr>
<td>1.06 C₄H₈S₂</td>
<td>1,4-Dithiane</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,4-Dithiane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99.92 C₄H₈O₂</td>
<td>1,4-Dithiane</td>
<td></td>
</tr>
<tr>
<td>0.08 C₄H₈S₃</td>
<td>Methyltrithiane</td>
<td></td>
</tr>
</tbody>
</table>

APPENDIX A (concluded)
OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
and 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 (I&LS), Room 3D129
The Pentagon
Washington, DC 20301-3080

The Surgeon General
ATTN: DASG-TLO
Washington, DC 20310

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 1221
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 & Development Laboratory
ATTN: SGRD-UBG-M
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 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

Commander
USAFSAM/TSZ
Brooks Air Force Base, TX 78235-5000

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

FILMED

END