AD-A164 696 MUTAGENIC POTENTIAL OF BENZOTHIAZOLE(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA S K SANO ET AL. AUG 85 LAIR-205 1/1

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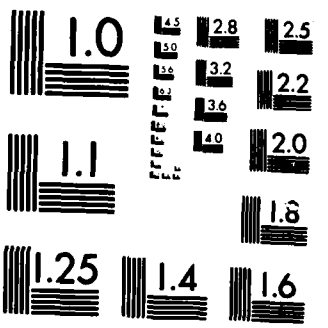

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INSTITUTE REPORT NO. 205

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

Toxicology Series 93

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

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

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 23 Aug '85

(Signature and date)

EDWIN S. BEATRICE, M.D.
Colonel, MC
Commanding, LAIR

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Mutagenicity, Genetic Toxicology, Ames Assay, Benzothiazole

Conc. .0032 Microliters

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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 ~~9.8 x 10⁻⁴~~ ul/plate. The test compound was not mutagenic under conditions of this assay. Keywords:

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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

GLP 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 in performing the research.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP study number 84031 was performed under our supervision, according to the procedures decribed herein, and that this report is an accurate record of the results obtained.

Don W. Korte 30 APR 85

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

Steven K. Sano 25APR85

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

CONRAD WHEELER, Ph.D. / DATE
DAC
Analytical Chemist

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

REPLY TO
ATTENTION OF

SGRD-ULZ-QA

18 August 1985

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, USA
Quality Assurance Unit

TABLE OF CONTENTS

Mutagenic Potential of: Benzothiazole--Sano and Korte

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 (1).

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.

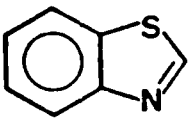
METHODS

Test Compound

Chemical name: Benzothiazole

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

Structural formula:



Empirical formula: C_7H_5NS

Storage: Ten millliliters 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 benzothiazole ranging from 1.6×10^{-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 ul 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 ul/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 1

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

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
5 ul/plate	0	0	0	0	NG
1 ul/plate	94	100	80	91	NL
0.2 ul/plate	114	104	112	110	NL
0.04 ul/plate	121	99	105	108	NL
0.008 ul/plate	125	123	109	119	NL
0.0016 ul/plate	113	115	121	116	NL

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

TABLE 2

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	NG (16mm)	NG	+
Wild Type	NT	NT	G	NT	NT	+

STERILITY CONTROL FOR TOXICITY LEVEL DETERMINATION

His-Bio Mix Initial: NG End: NG MGA Plate: NG

Top Agar Initial: NG End: NG

Diluent: DMSO:NG Nutrient Broth: NG

Test Compound TA037: (a) NG TA038: (b) NG TA039: (c) NG (d)_____ (e)_____

G = 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

Strains	Histidine Requirement	Ampicillin Resistance	UV Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG (17mm)	NG	+
100	NG	G	NG (20mm)	NG	+
1535	NG	NT	NG (18mm)	NG	+
1537	NG	NG (15mm)	NG (17mm)	NG	+
1538	NG	NT	NG (16mm)	NG	+
Wild Type	NT	NT	G NT	NT	+

STERILITY CONTROL FOR ASSAY

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

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

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

Test Compound TA037: (a) NG (b) TA038: NG (c) TA039: NG (d)_____ (e)_____ (f)_____

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

Study Number: 84031 By: SANO

Date: 11 OCT 84

(1) + = expected response
- = unexpected response

TABLE 4

POSITIVE AND NEGATIVE CONTROL TEST

(Revertants/plate)
Mean

COMPOUND	DOSE LEVEL	S-9 ADDED	STRAIN NUMBER TA98	TA100	TA1535	TA1537	TA1538
AF	2 ug/plate	YES	(772,825,982) 860	(1053,878,1216) 1049			(913,966,820) 900
BP	2 ug/plate	YES	(230,175,387) 264	(335,332,302) 323		(32, 25, 21) 26	(78, 46, 86) 70
AA	2 ug/plate	YES	(1488,1613,1754) 1618	(1725,1495,1994) 1738		(224,205,211) 213	(927,1073,1089) 1030
MNNG	2 ug/plate	NO		(1935,1737,2129) 1934			
	20 ug/plate	NO			(1852,1783,2053) 1896		
SPONTANEOUS REVERSION RATE (NEGATIVE CONTROL)							
Before Assay		YES	(15, 13, 15)	(89,102, 94)	(15, 13, 12)	(5, 6, 1)	(12, 14, 14)
After Assay		YES	(27, 16, 16) 17	(113,113,106) 103	(20, 15, 16) 15	(4, 3, 5) 4	(16, 8, 8) 12
Before Assay		NO	(13, 24, 18)	(86, 88, 87)	(13, 13, 16)	(1, 4, 6)	(13, 11, 18)
After Assay		NO	(13, 17, 20) 18	(99, 79,108) 91	(17, 15, 16) 15	(6, 4, 9) 5	(9, 15, 8) 12

Study Number: 84031 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 5

BENZOTHIAZOLE ASSAY

(Revertants/Plate)
Mean

COMPOUND	DOSE LEVEL	S-9 ADDED	STRAIN NUMBER TA98	TA100	TA1535	TA1537	TA1538
TA037	1 ul/plate	YES	(12, 15, 15) 14	(73,125, 57) 85	(13, 11, 10) 11	(2, 2, 6) 3	(8, 8, 16) 11
		NO	(14, 13, 15) 14	(58, 68, 64) 63	(17, 13, 11) 14	(5, 7, 6) 6	(5, 6, 4) 5
TA037	0.2 ul/plate	YES	(18, 15, 15) 16	(87, 96,109) 97	(14, 11, 12) 12	(8, 4, 5) 6	(6, 7, 6) 6
		NO	(10, 13, 13) 12	(76, 82, 78) 79	(16, 10, 14) 13	(3, 3, 4) 3	(9, 7, 5) 7
TA037	0.04 ul/plate	YES	(20, 18, 19) 19	(100, 89,101) 97	(14, 14, 18) 15	(10, 5, 5) 7	(12, 12, 17) 14
		NO	(8, 12, 15) 12	(104, 99,103) 102	(20, 19, 18) 19	(7, 9, 3) 6	(13, 9, 11) 11

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

TABLE 5 (cont.)

BENZOTHIAZOLE ASSAY

(Revertants/Plate)
Mean

COMPOUND	DOSE LEVEL	S-9 ADDED	STRAIN NUMBER TA98	TA100	TA1535	TA1537	TA1538
TA037	0.008 ul/plate	YES	(22, 22, 14) 19	(99, 89, 96) 95	(12, 13, 13) 13	(8, 2, 5) 5	(5, 7, 14) 13
		NO	(14, 11, 12) 12	(100, 78,105) 94	(20, 23, 23) 22	(6, 6, 6) 6	(5, 9, 6) 7
TA037	0.0016 ul/plate	YES	(22, 17, 23) 21	(96, 81, 81) 86	(24, 21, 15) 20	(4, 7, 1) 4	(15, 13, 11) 13
		NO	(14, 13, 17) 15	(110,100,104) 105	(14, 9, 17) 13	(7, 6, 7) 7	(14, 15, 19) 16
TA037	0.00032 ul/plate	YES	(21, 19, 16) 19	(100,101, 86) 96	(20, 21, 5) 15	(2, 2, 2) 2	(12, 11, 15) 13
		NO	(13, 19, 20) 17	(88, 70,100) 86	(14, 21, 23) 19	(1, 4, 3) 3	(21, 15, 9) 15

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 macrocolony 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

1. McCann JE, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 1975;72:5135-5139.

2. Ames Salmonella/Mammalian Microsome Mutagenicity Assay. LAIR Standard Operating Procedure OP-STX-1, Letterman Army Institute of Research, Presidio of San Francisco, California, 15 November 1983.

3. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with Salmonella/Mammalian microsome mutagenicity test. Mutation 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.

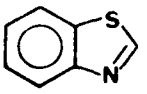
APPENDIX

CHEMICAL DATA

Chemical name: Benzothiazole

Chemical Abstracts Service Registry [illegible]

Chemical structure:



Molecular formula: C_7H_5NS

Molecular weight: 135.18

Physical state: Brown liquid

Compound density: d_4^{20} 1.246*

Source: Aldrich Chemical Co.
Milwaukee, WI

Lot number: 1723LK

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, 1470, 1455, 1445, 1315, 1290, 875, 800, 760, 730 cm^{-1}.‡ NMR (80 MHz, d_6-DMSO): δ 9.45 (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.¶

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

---

*Windholz M., ed. The Merck index. 9th ed. Rahway, New Jersey: Merck and Co., Inc., 1976: Monograph number 1118.

†Rosencrance AB. [Memorandum for Dr. Reddy]. SUBJECT: Results from the chemical analysis of three compounds slated for toxicity testing (24 July 1984). Frederick, Maryland: USAMBRDL.

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

§Ibid. p3.

¶Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #7752.

APPENDIX A

Sano--16

Sano--20

Chemists Helping Chemists in Research and Industry

aldrich chemical company, inc.

ANALYTICAL DATA

Date June 18, 1984

Our: 10133-8 Benzothiazole, 99%

Batch No.: 1723LK

Analytical Results:

Appearance Dark gold liquid

m.p. b.p.

n^{25} 1.6423 $[\alpha]_D$

Spectral Data:

I.R. Conforms to structure and standard as illustrated on page 1278 D of Edition III, of "The Aldrich Library of Infrared Spectra".

U.V.

N.M.R.

Assay:

V.P.C. 99+%

Titration

Other:

DS/kb

J Majchrowski

Anna Majchrowski, Manager
Quality Control/Quality Assurance

APPENDIX A (cont.)

SGRD-UBG-L 24 July 84

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:

% of Total	Formula	Compound	Other Possibilities
Benzothiazole			
98.88	C_7H_5NS	Benzothiazole	
0.61	C_8H_7NS	2-Methylbenzothiazole	(isomers)
0.26	$C_4H_3N_3$	Aniline	3 or 4-Cyanopyrazole
0.12	$C_{10}H_{10}S_2$	Diphenyldisulfide	
0.11	C_7H_9N	Toluidine (isomers)	Benzylamine, N-Methylaniline
0.03	C_8H_7NS	Methylbenzothiazole	(isomers)
1,4-Thioxane			
98.93	C_4H_8OS	1,4-Thioxane	
1.06	$C_4H_8S_2$	1,4-Dithiane	
1,4-Dithiane			
99.92	$C_4H_8S_2$	1,4-Dithiane	
0.08	$C_4H_8S_3$	Methyltrithiane	

Alan B Rosencrance

ALAN B. ROSENCRANCE
Research Chemist

CF:
Dr. Kulkarni
Dr. Rosenblatt

APPENDIX A (concluded)

Sano--18

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