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THE MUTAGENIC POTENTIAL OF 4 NITROPHENYL BIS(2-THIENYL)-PHOSPHI--ETC(U)
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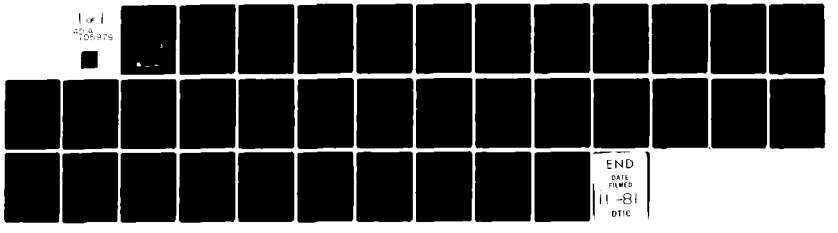

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THE MUTAGENIC POTENTIAL OF: 4-nitrophenyl bis(2-thienyl) phosphinate
4-nitrophenyl 2-furyl(methyl) phosphinate
4-cyanophenyl bis(2-furyl) phosphinate
4-nitrophenyl bis(2-furyl) phosphinate

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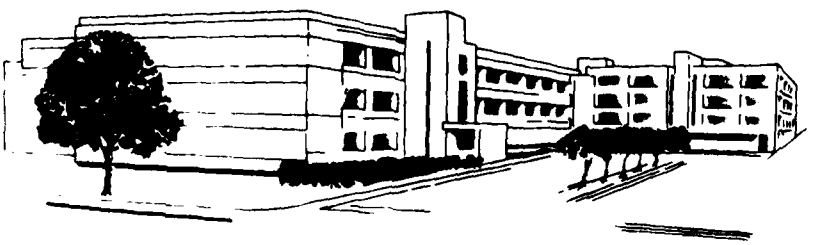
TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

SEPTEMBER 1981

Toxicology Series 17



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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*
The mutagenic potential of 4 nitrophenyl bis(2-thienyl)phosphinate (41*); 4-nitrophenyl 2-furyl(methyl)phosphinate (72*) 4 cyanophenyl bis(2-furyl)phosphinate (82*); 4-nitrophenyl bis(2-furyl)phosphinate (87*) 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 1 mg/plate to 3.2x10^{-4} mg/plate. It was determined that none of the tested substances had mutagenic potential. *Code number of compound.

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ABSTRACT

The mutagenic potential of 4 nitrophenyl bis(2-thienyl)phosphinate (41*); 4-nitrophenyl 2-furyl(methyl)phosphinate (72*); 4-cyanophenyl bis(2-furyl)phosphinate (82*); 4-nitrophenyl bis(2-furyl)phosphinate (87*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and 1538 were exposed to doses ranging from 1 mg/plate to 3.2 x 10-4 mg/plate. It was determined that none of the tested substances had mutagenic potential.

* Code number for compound.

PREFACE

AMES ASSAY REPORT:

SUBSTANCE	CODE NO.
4 nitrophenyl bis(2-thienyl)phosphinate	41
4-nitrophenyl 2-furyl(methyl)phosphinate	72
4-cyanophenyl bis(2-furyl)phosphinate	82
4-nitrophenyl bis(2-furyl)phosphinate	87

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

SPONSOR: Biomedical Laboratory, Aberdeen Proving Grounds
Aberdeen, MD 21005

PROJECT: Toxicity Testing of Phosphinate Compounds - 35162772A875

GLP STUDY NUMBER: 81013

STUDY DIRECTOR: LTC John T. Fruin D.V.M.,PhD.
CO-PRINCIPAL INVESTIGATORS: SSG Freddica R. Pulliam, B.S.
SP5 Leonard J. Sauers, B.A.

RAW DATA: A copy of the final report, study protocol and retired SOPs will be maintained in the LAIR archives. Test substances were provided by sponsor. Chemical, analytical, stability, purity, etc. data are available from the sponsor.

PURPOSE: To determine the mutagenic potential of the above compounds using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were used.

ACKNOWLEDGMENTS

The authors wish to thank John Dacey and SP4 Larry Mullen, BS for their assistance in performing the research and for help in preparation of this report.

Signatures of Principal Scientists
Involved in the Study

We, the undersigned, believe the study, GLP number 81013, 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.

FREDDICA R. PULLIAM, BS Date
SSG
Co-Investigator

JOHN T. FRUIN, DVM, PhD Date
LTC, VC
Study Director

LEONARD J. SAUERS, BA Date
SP5
Co-Investigator

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

REPLY TO
ATTENTION OF:

SGRD-ULA-QA

21 July 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81013 the following inspection was made:

17 June 1981

Routine inspections with no adverse findings are reported quarterly, thus this inspection is also included in the July 1981 report to management.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

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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 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 microsome 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, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls 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 growth 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 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.

We compared 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) (2).

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 10^8 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, 0.1ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were 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 were used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000- 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 S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered 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, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS AND DISCUSSION

Throughout this report, each of the test substances will be referred to by the respective code number:

Substance	Code No.
4 nitrophenyl bis(2-thienyl)phosphinate	41
4-nitrophenyl 2-furyl(methyl)phosphinate	72
4-cyanophenyl bis(2-furyl)phosphinate	82
4-nitrophenyl bis(2-furyl)phosphinate	87

On 1 June 1981, the Toxicity Level Determination was performed on the 4 test chemicals. All positive, negative and sterility controls for this experiment were normal (Table 1). At the highest dose used, 1.0 mg/plate, no toxicity was observed (Table 2A-2D).

On 17 June 1981, the Ames Assay was performed using the 4 test substances. For this experiment, all sterility and strain verification controls were normal (Table 3). Expected responses were observed for all negative and positive controls (Table 4).

For all the chemicals tested, there were no incidence of mutagenicity (Table 5A-5D).

The MUTAR values listed in Tables 6A-6D were within the normal limits.

CONCLUSION

On the basis of the Ames Assay, test compounds 41, 72, 82, and 87 are not mutagenic at the levels tested.

RECOMMENDATION

We recommend that organophosphinate compounds 41, 72, 82, and 87 be tested by using other toxicological testing systems if efficacy tests show these chemicals to be promising antidotes.

REFERENCES

1. McCANN, J., E. CHOI, E. YAMASAKI, and B. N. AMES. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 72:5135-5139, 1975

2. AMES, B. N., J. McCANN and E. YAMASAKI. Methods for detection carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutation Res 31: 347-364, 1975

3. LAIR SOP OP-STX-1, Ames Salmonella/mammalian microsome mutagenicity test, 1 March 1981

4. VOGEL, H. J. and D. M. BONNER. Acetylornithinase of E. coli: Partial purification and same properties. J Biol Chem 218: 97-106, 1956

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

LIST OF TABLES

TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Strain No.	Histidine Requirements	Ampicillin Resistance	uvr-B Deletion	rfa Crystal Violet	Sterility Control	Response (a)
TA 100	NG	G	NG	15.46 mm	NG	+
TA 1537	NG	NG	NG	14.11 mm	NG	+
WT	G	NA	G	NA	NA	+
Diluent	NA	NA	NA	NA	NG	+
Positive Control - MNNG -		Average - 1612				
Test Compound (s)						
(a) 41	NA	NA	NA	NA	NG	+
(b) 72	NA	NA	NA	NA	NG	+
(c) 82	NA	NA	NA	NA	NG	+
(d) 87	NA	NA	NA	NA	NG	+
(e) NA	NA	NA	NA	NA	NA	NA

G = Growth; NG = No Growth; NT = Not Tested; NA = Not Applicable;
WT = Wild Type; (a) + = Expected Response; - = Unexpected Response

Spontaneous Revertants

Strain	Time				Average
TA 100	Beginning	146	148	155	140
TA 100	End	122	118	149	

Test Inculated By: Sauers, Pulliam, Dacey, Mullen Date 1 June 1981

Test Read By: Sauers, Pulliam Date 3 June 1981

TABLE 2A

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #41 (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/pl	135	105	138	126	NL
10^{-1}	125	150	106	127	NL
10^{-2}	121	124	117	121	NL
10^{-3}	138	132	126	132	NL
10^{-4}	128	118	132	126	NL
10^{-5}	134	125	152	137	NL
10^{-6}	154	139	123	139	NL
10^{-7}	139	160	179	159	NL

TABLE 2B

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #72 (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 June 1981 Performed by: Pulliam, Sauers, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	*Background* Lawn
1.0 mg/pl	180	194	166	180	NL
10^{-1}	193	232	228	218	NL
10^{-2}	206	193	181	193	NL
10^{-3}	194	193	190	192	NL
10^{-4}	130	135	113	126	NL
10^{-5}	125	116	89	110	NL
10^{-6}	108	113	142	121	NL
10^{-7}	159	140	146	148	NL

TABLE 2C

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #82 (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/pl	141	148	108	132	NL
10^{-1}	108	134	154	132	NL
10^{-2}	132	102	142	125	NL
10^{-3}	133	148	144	142	NL
10^{-4}	130	143	164	146	NL
10^{-5}	154	125	123	134	NL
10^{-6}	136	123	109	123	NL
10^{-7}	139	132	139	137	NL

TABLE 2D

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #87 (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/pl	125	103	121	116	NL
10^{-1}	167	158	128	151	NL
10^{-2}	140	123	132	132	NL
10^{-3}	140	158	131	143	NL
10^{-4}	141	119	136	132	NL
10^{-5}	139	97	123	120	NL
10^{-6}	139	165	116	140	NL
10^{-7}	122	140	182	148	NL

TABLE 3

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV Sensitivity to Crystal Violet		Sterility Control	Response (1)
98	NG	G	NG	14.15 mm	NG	+
100	NG	G	NG	14.51 mm	NG	+
1535	NG	NA	NG	13.89 mm	NG	+
1537	NG	NG	NG	14.75 mm	NG	+
1538	NG	NA	NG	15.11 mm	NG	+
WT	G	NA	G	NA	NA	+

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) 41-NG (b) 72-NG (c) 82-NG (d) 87-NG (e) NA (f) NA

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

Study Number: 81013 By: Sauers, Pulliam, Dacey, Mullen

Date: 17 June 1981

(1) + = expected response
- = unexpected response

TABLE 4

SPONTANEOUS REVERTANT RATE AND POSITIVE CONTROL REVERTANT RATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
AF	2 ug/plate	yes	(372,593,430) (465)	(347,366,471) (395)			(212,452,469) (378)
BF	2 ug/plate	yes	(88,91,153) (111)	(186,207,306) (233)		(63,28,60) (50)	(85,82,64) (77)
DMBA	20 ug/plate	yes	(70,39,50) (53)	(266,207,249) (241)		(36,24,40) (33)	(36,37,13) (29)
MNNG	2 ug/plate	no		(369,312,425) (369)			
	20 ug/plate	no			(87,102,NG) (95)		

Strain Performance

Spontaneous Revertants						
before	no	(23,15,17)	(99,86,82)	(7,12,8)	(4,7,7)	(9,9,10)
after		(18,18,18) (17)	(85,69,92) (86)	(15,21,9) (12)	(6,13,7) (7)	(15,15,18) (13)
before	yes	(18,17,20)	(87,101,105)	(10,12,9)	(4,4,6)	(6,7,9)
after		(15,12,11) (16)	(75,109,85) (94)	(17,15,10) (13)	(4,7,9) (6)	(20,11,10) (11)

Study Number: 81013

Date: 17 Jun 31 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5A

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #41	1 mg/plate	no	(Toxic) (Toxic)	(Toxic) (Toxic)	(Toxic,Toxic,Toxic) (Toxic)	(Toxic,Toxic,Toxic) (Toxic)	(Toxic,Toxic,Toxic) (Toxic)
		yes	(18,32,21) (24)	(125,97,140) (121)	(9,10,9) (9)	(7,5,4) (5)	(20,17,20) (19)
Code #41	0.2 mg/plate	no	(18,15,10) (14)	(85,100,88) (91)	(14,12,9) (12)	(3,4,5) (4)	(10,8,13) (10)
		yes	(18,28,27) (24)	(123,144,129) (132)	(10,12,12) (11)	(3,6,7) (5)	(22,18,23) (21)
Code #41	0.04 mg/plate	no	(14,14,14) (14)	(98,71,95) (88)	(12,13,16) (14)	(6,8,5) (6)	(11,9,17) (12)
		yes	(18,19,28) (22)	(117,110,110) (112)	(7,9,10) (9)	(6,6,10) (7)	(20,8,19) (16)

-continued

Study Number: 81013 Date: 17 June 1981 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5A, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #41	0.008 mg/plate	no	(20,23,24) (22)	(117,126,135) (126)	(17,13,17) (16)	(3,8,8) (6)	(20,15,14) (16)
		yes	(20,14,11) (15)	(93,90,113) (99)	(12,10,8) (18)	(6,5,10) (7)	(10,8,8) (9)
Code #41	0.0016 mg/plate	no	(11,11,21) (14)	(97,84,93) (91)	(12,11,6) (10)	(4,9,5) (6)	(6,6,11) (8)
		yes	(29,17,17) (21)	(133,120,126) (126)	(11,7,10) (9)	(7,6,6) (6)	(20,17,22) (20)
Code #41	0.00032 mg/plate	no	(12,12,21) (15)	(98,101,87) (95)	(17,20,14) (17)	(5,9,9) (8)	(15,15,13) (14)
		yes	(25,16,37) (26)	(105,80,119) (101)	(20,18,10) (16)	(11,8,7) (9)	(18,12,23) (18)

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TABLE 5B

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #72	1 mg/plate	no	(7,9,15) (10)	(82,91,72) (82)	(6,8,14) (9)	(6,9,7) (7)	(12,9,6) (9)
		yes	(23,17,18) (19)	(107,111,108) (109)	(10,7,8) (8)	(6,7,14) (9)	(14,8,9) (10)
Code #72	0.2 mg/plate	no	(13,14,18) (15)	(68,62,65) (65)	(11,12,12) (12)	(4,5,5) (5)	(7,5,7) (6)
		yes	(19,18,12) (16)	(124,138,110) (124)	(8,12,5) (8)	(8,6,5) (6)	(17,28,72) (19)
Code #72	0.04 mg/plate	no	(19,11,14) (15)	(79,86,71) (80)	(17,11,17) (15)	(7,5,6) (6)	(12,11,7) (10)
		yes	(25,24,26) (25)	(101,108,101) (103)	(12,7,12) (10)	(7,5,8) (7)	(13,25,21) (20)

-continued

Study Number: 81013 Date: 17 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

TABEL 5B, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #72	0.008 mg/plate	no	(15,10,14) (13)	(72,53,83) (69)	(9,9,12) (10)	(4,6,7) (6)	(7,4,12) (8)
		yes	(25,10,10) (15)	(106,115,116) (112)	(8,7,8) (8)	(4,4,12) (7)	(14,24,11) (16)
Code #72	0.0016 mg/plate	no	(21,12,18) (17)	(73,65,82) (73)	(12,14,12) (13)	(6,3,3) (4)	(12,9,8) (10)
		yes	(16,29,20) (22)	(101,85,92) (93)	(15,7,16) (13)	(4,5,10) (6)	(25,10,14) (16)
Code #72	0.00032 mg/plate	no	(11,15,17) (14)	(97,68,95) (87)	(15,13,21) (16)	(8,7,4) (6)	(17,12,12) (14)
		yes	(27,23,10) (20)	(83,130,115) (109)	(7,8,11) (9)	(5,9,5) (6)	(12,17,23) (17)

Study Number: 81013 Date: 17 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5C

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #82	1 mg/plate	no	(10,14,10) (11)	(74,70,70) (71)	(10,11,15) (12)	(4,4,7) (5)	(17,10,18) (15)
		yes	(27,16,13) (19)	(91,105,109) (102)	(6,7,11) (8)	(8,4,7) (6)	(14,12,20) (15)
Code #82	0.2 mg/plate	no	(19,17,10) (15)	(86,77,54) (72)	(12,6,9) (9)	(5,9,7) (7)	(15,10,17) (14)
		yes	(21,30,30) (27)	(125,109,107) (114)	(11,11,9) (10)	(14,7,4) (8)	(16,19,11) (15)
Code #82	0.04 mg.plate	no	(12,10,12) (11)	(77,64,56) (66)	(14,25,10) (16)	(3,4,7) (5)	(15,7,10) (11)
		yes	(18,14,15) (16)	(101,79,90) (90)	(9,12,8) (10)	(3,7,7) (6)	(17,20,11) (16)

-continued

Study Number: 81013 Date: 17 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5C, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #82	0.008 mg/plate	no	(11,17,12) (13)	(82,75,64) (74)	(12,15,12) (13)	(9,5,8) (7)	(8,9,13) (10)
		yes	(23,14,17) (18)	(126,122,108) (119)	(8,6,8) (7)	(10,9,6) (8)	(13,9,19) (14)
Code #82	0.0016 mg/plate	no	(10,19,15) (15)	(89,72,71) (77)	(12,12,9) (11)	(4,7,5) (5)	(7,11,6) (8)
		yes	(25,20,20) (22)	(77,83,113) (91)	(9,8,18) (12)	(6,10,5) (7)	(18,14,9) (14)
Code #82	0.00032 mg/plate	no	(14,9,11) (11)	(96,71,111) (93)	(12,17,12) (14)	(5,6,6) (6)	(10,8,11) (10)
		yes	(30,29,14) (24)	(80,99,94) (91)	(11,6,8) (8)	(4,6,5) (5)	(10,19,14) (14)

Study Number: 81013 Date: 17 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5D

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #87	1 mg/plate	no	(10,8,11) (10)	(78,64,98) (80)	(9,11,6) (9)	(6,6,5) (6)	(6,9,7) (7)
		yes	(35,21,19) (25)	(102,100,134) (112)	(14,15,7) (12)	(10,9,5) (8)	(7,10,18) (12)
Code #87	0.2 mg/plate	no	(14,10,10) (11)	(73,95,74) (81)	(9,21,10) (13)	(7,6,10) (8)	(14,14,9) (12)
		yes	(21,21,18) (20)	(135,91,92) (106)	(17,8,7) (11)	(9,7,10) (9)	(15,26,14) (18)
Code #87	0.04 mg/plate	no	(8,12,13) (11)	(83,91,77) (84)	(21,13,20) (18)	(5,4,5) (5)	(9,10,9) (9)
		yes	(20,18,22) (20)	(79,89,134) (101)	(7,12,12) (10)	(6,12,7) (8)	(21,14,10) (15)

-continued

Study Number: 81013 Date: 17 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5D, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #87	0.008 mg/plate	no	(12,12,19) (14)	(101,93,78) (91)	(9,16,16) (14)	(4,5,2) (4)	(6,7,10) (8)
		yes	(23,24,25) (24)	(105,92,98) (98)	(9,19,14) (14)	(9,6,18) (11)	(18,15,18) (17)
Code #87	0.0016 mg/plate	no	(12,11,16) (13)	(93,86,74) (84)	(13,4,9) (9)	(4,5,7) (5)	(10,9,12) (10)
		yes	(19,22,16) (19)	(115,92,113) (107)	(12,12,9) (11)	(5,5,7) (6)	(20,11,7) (13)
Code #87	0.00032 mg/plate	no	(16,17,12) (15)	(75,86,89) (83)	(6,14,10) (10)	(4,8,5) (6)	(6,9,11) (9)
		yes	(10,10,19) (13)	(112,78,75) (88)	(7,9,8) (8)	(10,7,7) (8)	(6,17,10) (11)

Study Number: 81013 Date: 17 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 6A

MUTAGENIC ACTIVITY RATIO

Substance Assayed: Code #41 Dissolved in: DMSO

Study Number: 81013 Date: 15 July 1981 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1.0 mg/plate	TA 98	0.32	*	0.008 mg/plate	TA 1535	*	0.26
0.2 mg/plate	TA 98	0.32	*	0.0016 mg/pl.	TA 1535	*	*
0.04 mg/plate	TA 98	0.24	*	0.00032 mg/pl.	TA 1535	0.27	0.32
0.008 mg/plate	TA 98	*	0.24				
0.0016 mg/pl.	TA 98	0.2	*	1.0 mg/plate	TA 1537	*	*
0.00032 mg/pl.	TA 98	0.4	*	0.2 mg/plate	TA 1537	*	*
				0.04 mg/plate	TA 1537	0.15	*
1.0 mg/plate	TA 100	0.25	*	0.008 mg/plate	TA 1537	0.15	*
0.2 mg/plate	TA 100	0.35	0.05	0.0016 mg/pl.	TA 1537	*	*
0.04 mg/plate	TA 100	0.17	0.02	0.00032 mg/pl.	TA 1537	0.46	0.15
0.008 mg/plate	TA 100	0.05	0.42				
0.0016 mg/pl.	TA 100	0.29	0.05	1.0 mg/plate	TA 1538	0.43	*
0.00032 mg/pl.	TA 100	0.06	0.1	0.2 mg/plate	TA 1538	0.53	*
				0.04 mg/plate	TA 1538	0.27	*
1.0 mg/plate	TA 1535	*	*	0.008 mg/plate	TA 1538	*	0.21
0.2 mg/plate	TA 1535	*	*	0.0016 mg/pl.	TA 1538	0.48	*
0.04 mg/plate	TA 1535	*	0.13	0.00032 mg/pl.	TA 1538	0.37	0.07

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 6B

MUTAGENIC ACTIVITY RATIO

Substance Assayed: Code #72 Dissolved in: DMSO

Study Number: 81013 Date: 15 July 1981 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1.0 mg/plate	TA 98	0.12	*	0.008 mg/plate	TA 1535	*	*
0.2 mg/plate	TA 98	*	*	0.0016 mg/pl.	TA 1535	*	0.06
0.04 mg/plate	TA 98	9.36	*	0.00032 mg/pl.	TA 1535	*	0.26
0.008 mg/plate	TA 98	*	*				
0.0016 mg/pl.	TA 98	0.24	*	1.0 mg/plate	TA 1537	0.46	*
0.00032 mg/pl.	TA 98	0.16	*	0.2 mg/plate	TA 1537	*	*
				0.04 mg/plate	TA 1537	0.15	*
1.0 mg/plate	TA 100	0.14	*	0.008 mg/plate	TA 1537	0.15	*
0.2 mg/plate	TA 100	0.28	*	0.0016 mg/pl.	TA 1537	*	*
0.04 mg/plate	TA 100	0.08	*	0.00032 mg/pl.	TA 1537	*	*
0.008 mg/plate	TA 100	0.17	*				
0.0016 mg/pl.	TA 100	*	*	1.0 mg/plate	TA 1538	*	*
0.00032 mg/pl.	TA 100	0.14	0.01	0.2 mg/plate	TA 1538	0.43	*
				0.04 mg/plate	TA 1538	0.48	*
1.0 mg/plate	TA 1535	*	*	0.008 mg/plate	TA 1538	0.27	*
0.2 mg/plate	TA 1535	*	*	0.0016 mg/pl.	TA 1538	0.27	*
0.04 mg/plate	TA 1535	*	0.19	0.00032 mg/pl.	TA 1538	0.32	0.07

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 6C

MUTAGENIC ACTIVITY RATIO

Substance Assayed: Code #82 Dissolved in: DMSO

Study Number: 81013 Date: 15 July 1981 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1.0 mg/plate	TA 98	0.12	*	0.008 mg/plate	TA 1535	*	0.06
0.2 mg/plate	TA 98	0.44	*	0.0016 mg/pl.	TA 1535	*	*
0.04 mg/plate	TA 98	*	*	0.00032 mg/pl.	TA 1535	*	0.13
0.008 mg/plate	TA 98	0.08	*				
0.0016 mg/pl.	TA 98	0.24	*	1.0 mg/plate	TA 1537	*	*
0.00032 mg/pl.	TA 98	0.32	*	0.2 mg/plate	TA 1537	0.31	*
				0.04 mg/plate	TA 1537	*	*
1.0 mg/plate	TA 100	0.07	*	0.008 mg/plate	TA 1537	0.31	*
0.2 mg/plate	TA 100	0.18	*	0.0016 mg/pl.	TA 1537	0.15	*
0.04 mg/plate	TA 100	*	*	0.00032 mg/pl.	TA 1537	*	*
0.008 mg/plate	TA 100	0.23	*				
0.0016 mg/pl.	TA 100	*	*	1.0 mg/plate	TA 1538	0.21	0.14
0.00032 mg/pl.	TA 100	*	0.07	0.2 mg/plate	TA 1538	0.21	0.07
				0.04 mg/plate	TA 1538	0.27	*
1.0 mg/plate	TA 1535	*	*	0.008 mg/plate	TA 1538	0.16	*
0.2 mg/plate	TA 1535	*	*	0.0016 mg/pl.	TA 1538	0.16	*
0.04 mg/plate	TA 1535	*	0.26	0.00032 mg/pl.	TA 1538	0.16	*

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 6D

MUTAGENIC ACTIVITY RATIO

Substance Assayed: Code #87 Dissolved in: DMSO

Study Number: 81013 Date: 15 July 1981 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1.0 mg/plate	TA 98	0.36	*	0.008 mg/plate	TA 1535	0.09	0.13
0.2 mg/plate	TA 98	0.16	*	0.0016 mg/pl.	TA 1535	*	*
0.04 mg/plate	TA 98	0.16	*	0.00032 mg/pl.	TA 1535	*	*
0.008 mg/plate	TA 98	0.32	*				
0.0016 mg/pl.	TA 98	0.12	*	1.0 mg/plate	TA 1537	0.31	*
0.00032 mg/pl.	TA 98	*	*	0.2 mg/plate	TA 1537	0.46	0.15
				0.04 mg/plate	TA 1537	0.31	*
1.0 mg/plate	TA 100	0.17	*	0.008 mg/plate	TA 1537	0.77	*
0.2 mg/plate	TA 100	0.11	*	0.0016 mg/pl.	TA 1537	*	*
0.04 mg/plate	TA 100	0.06	*	0.00032 mg/pl.	TA 1537	0.31	*
0.008 mg/plate	TA 100	0.04	0.05				
0.0016 mg/pl.	TA 100	0.12	*	1.0 mg/plate	TA 1538	0.05	*
0.00032 mg/pl.	TA 100	*	*	0.2 mg/plate	TA 1538	0.37	*
				0.04 mg/plate	TA 1538	0.21	*
1.0 mg/plate	TA 1535	*	*	0.008 mg/plate	TA 1538	0.32	*
0.2 mg/plate	TA 1535	*	0.06	0.0016 mg/pl.	TA 1538	0.11	*
0.04 mg/plate	TA 1535	*	0.39	0.00032 mg/pl.	TA 1538	*	*

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

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