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HEALTH, ENVIRONMENT AND SAFETY

(A)

August 24, 1992

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Attn: Section 8(e) Coordinator
(CAP Agreement)

Gentlemen:

Phillips Petroleum Company is submitting the enclosed sixty (60) reports (two boxes, numbered 1 and 2) of toxicological studies pursuant to category II.B.2.b of the CAP Agreement 8ECAP-0075 Reports. Reports being submitted contain no confidential business information.

We are sending an additional five boxes (box numbers 3-7) of reports of studies that have, previously, been submitted to the FYI coordinator of the Office of Pollution Prevention and Toxics by the American Petroleum Institute (API). These are being provided solely for the Agency's convenience.

For questions concerning this correspondence, please contact Fred Marashi at 918-661-8153.

Very truly yours,

Barbara J. Price
Vice President
Health, Environment & Safety

Enclosure (Seven Boxes)

FFM/dh:29

mm
3/7/95



Phillips Petroleum Company

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CAP Identification Number: 8ECAP-0075
Pursuant to Category: II.B.2.b

Title of Study: Mutagenicity Evaluation of R-04

Name of Chemical: R-04

CAS#: 64741-79-3

Summary: The test material was mutagenic in the Ames assay.

The results of the mouse lymphoma assay, although not meeting all of the pre-established criteria for mutagenesis, indicated weak mutagenicity at the high end of the dose range employed.

Fiche # 2381

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MUTAGENICITY EVALUATION

OF

R-04

60-557

SUBMITTED TO

AMERICAN PETROLEUM INSTITUTE
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SUBMITTED BY

LITTON BIONETICS, INC.
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LBI PROJECT NO. 20725

JANUARY, 1978



BIONETICS

GENERAL INTERPRETATION AND CONCLUSIONS

1. INTRODUCTION AND BACKGROUND

The test chemical, R-04, was evaluated for its genetic properties in a battery of tests consisting of a test for mitotic gene conversion in yeast, gene mutation tests in bacteria, gene mutation tests in culture mammalian cells and in vivo chromosome analysis in rat bone marrow cells. This series of tests provides a sensitive screen for the detection of substances which produce mutagenic and/or clastogenic alterations. A high percentage of known mammalian mutagens and carcinogens demonstrate activity in one or more of the tests conducted in this evaluation, and positive results in one or more of these tests are an indication that the test substance has mutagenic and/or carcinogenic potential and that further analysis to define the limits of expression of this potential is warranted. Negative results in all of these tests suggest that the test material lacks genetic activity but are not sufficient for an unequivocal determination of genetic safety.

The assumptions of mutagenic and carcinogenic potential or lack of potential are based on correlations between extensive data bases.

2. MICROBIAL ASSAYS

The test chemical, R-04, produced point mutations in bacteria. The material reverted frameshift mutants TA-1537, TA-1538, and TA-98 in plate tests. Strain TA-98 was reverted in suspension tests. All responses were observed in activation (+S9) tests. The nonactivation results were considered negative.

3. MOUSE LYMPHOMA ASSAY

The results of this test, although not meeting all of the pre-established criteria for mutagenesis, indicated weak mutagenicity at the high end of the dose range employed. No clear dose response was observed, but the increased mutant frequencies were significant from the negative/solvent controls. The increases were observed in both nonactivation (-S9) and activation tests.



BIONETICS

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GENERAL INTERPRETATION AND CONCLUSIONS (Continued)

4. RAT BONE MARROW CYTOGENETIC ANALYSIS

The results of this assay were negative and the test chemical, R-04, was evaluated as nonclastogenic under the test conditions employed.

5. CONCLUSIONS

The test chemical, R-04, was mutagenic in bacteria-inducing frameshift mutations following incubation with rat liver S9. Results from the Mouse Lymphoma Assay data were suggestive of weak non dose-related mutagenic activity. The in vivo cytogenetic analysis in rats was negative.

R-04 is clearly a bacterial mutagen. The Mouse Lymphoma results must be considered suggestive of weak activity and also indicative that mammalian cells are capable of forming the active intermediate since positive findings were obtained in the nonactivation test. The cultured mouse cells appear from other studies to have certain metabolic capacities, especially for PNAs. No in vivo response was obtained and reduces the confidence in extrapolating in vitro data to the in vivo situation.

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BIONETICS

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EVALUATION SUMMARY

The test material, R-04, was evaluated in plate and suspension assays. The results from the plate assays were positive in activation (+S9) tests. Strains TA-1537, TA-1538, TA-98, and possibly TA-100 responded to R0-4 in these test. The suspension assays were positive with TA-98 and possibly TA-100 under activation (+S9) conditions.

SPONSOR: American Petroleum Institute

MATERIAL: R-04

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY AND SUSPENSION ASSAY

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: February 18, 1976
2. Description: Dark liquid

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

1. Yeast Strain: Saccharomyces cerevisiae, D4
2. Bacteria Strains: Salmonella typhimurium, strains
TA-1535 TA-98
TA-1537 TA-100
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	4 μ moles
2. Glucose-6-phosphate	5 μ moles
3. Sodium phosphate (dibasic)	100 μ moles
4. MgCl ₂	8 μ moles
5. KCl	33 μ moles
6. Homogenate	0.1-0.15 ml 9,000 x g supernatant of rat liver



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2. MATERIALS (Continued)

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from Sprague-Dawley rats pretreated with Aroclor 1254.

E. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL</u> ^a	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Ethylmethanesulfonate (EMS)	Water or Saline	BPS ^b
	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS ^b
	2-Nitrofluorene (NF)	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard (QM)	Water or saline	FS ^b
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide ^c	BPS ^b
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide ^c	FS ^b
	Dimethylnitrosamine (DMNA)	Water or Saline	BPS ^b

^aConcentrations given in Results section

^bBPS = Base-pair substitution

FS = Frameshift

^cPreviously shown to be nonmutagenic

F. Solvent

Either deionized water or Dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in the solvent vehicle. The specific solvent employed is recorded in the Results Section.



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3. EXPERIMENTAL DESIGN

A. Toxicity

The solubility, toxicity, and dose levels for the test compound were determined prior to screening.

The test compound was assayed for toxicity against bacteria and yeast indicator strains over a wide range of doses to determine the 50% killing dose (LD50). Bacteria were tested in phosphate buffer, pH 7.4, for 1 hour at 37C in a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for 4 hours at 30C in a shaker. The 50% killing dose and the 1/8, 1/4, and 1/2 50% killing doses were calculated from the toxicity assays. For most compounds, the toxicity test system employed a low amount of Dimethylsulfoxide (DMSO) to ensure solubility of the chemical at all screening levels.

If no toxicity was obtained for a chemical with indicator strains, a maximum dose of 5% was used as the high dose concentration.

The dose levels selected from preliminary toxicity tests were applied to both activation and nonactivation assays.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, a minimum of four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, 0.5 ml of $9,000 \times g$ tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. At least four dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for approximately 48 hours at 37C and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results section. Positive and solvent controls using both directly active mutagens and those that require metabolic activation were run with each assay.

3. EXPERIMENTAL DESIGN (Continued)

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures were grown in complete broth, washed, and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^8 cells/ml, respectively. These constituted the working stocks for evaluation of the test compound and the respective controls. Tests were conducted in 24-well, sterile plastic tissue culture plates (Linbro Chemical Company). Cells plus appropriate volume(s) of the test compound were added to the wells to give a final volume of 1.5 ml. An equal volume of solvent replaced the test compound in the negative controls. Treatment was at 30C for 4 hours for yeast tests, and at 37C for 1 hour for bacterial tests. All flasks were shaken on a rotary shaker during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4C), and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for approximately 48 hours at 37C. The yeast plates were incubated at 30C for 3 to 5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test compound and the control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture (Section 2,C). All flasks (bacteria and yeast) were incubated at 37C with shaking. The treatment times as well as the dilutions, the plating procedures and the scoring of the plates were the same as described for nonactivation tests.



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3. EXPERIMENTAL DESIGN (Continued)

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities of tissues) were killed by cranial blow, decapitated, and bled. Organs were immediately dissected from the animal, using aseptic techniques, and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4C. The organ homogenate obtained from this step was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80C.

E. Data Recording and Reporting

1. Plate Test Assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were transferred directly to the report form sheets and entered into a computer for analysis and printing.

2. Suspension Test Assays

Following the specified incubation periods, all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored, and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains, the number of colonies recorded from either the population or the selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program.



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4. RESULTS

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: R-04
2. Test solvent:* DMSO
3. Solubility of the test compound under treatment conditions:
Soluble
4. Additional comments: Dark liquid

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: Bacteria - March 10, 1977
Yeast - March 11, 1977
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Test Doses</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/8 50% Survival	0.00625	0.055
1/4 50% Survival	0.01250	0.110
1/2 50% Survival	0.02500	0.220
50% Survival	0.05000	0.440

*The concentration of solvent was equal to the highest volume of test material added.

4. RESULTS (Continued)

C. Summary of Plate Assay Results

The plate test results are summarized in the first two tables. The values presented in these tables are the number of revertants per plate.

D. Summary of Suspension Assay Results

The suspension assay results for the test compound are summarized in the two tables following the plate assay results. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table presents the results for the nonactivation assays and the second table presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided.



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3. SUMMARY OF PLATE TEST RESULTS

LITTON BIOMETICS, INC.

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: H-04
 B. SOLVENT: DMSO
 C. TEST DATE: MARCH 14, 1977
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	TISSUE	B.C.V.E.U.I.A.M.I.S. P.E.B. P.L.A.T.E.											
			IA-1535		IA-1537		IA-1538		IA-98		IA-100		0**	
			1	2	1	2	1	2	1	2	1	2	1	2
UNACTIVATION														
SOLVENT CONTROL	---	---	24	12	22	33	246	279						
POSITIVE CONTROL**	---	---	>1000	>1000	529	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
TEST COMPOUND														
0.00100 UL	---	---	27	14	25	34	255	138						
0.01000 UL	---	---	29	13	33	16	257	143						
0.10000 UL	---	---	27	17	26	43	371	100						
1.00000 UL	---	---	28	53	48	22	395	145						
5.00000 UL	---	---	16	42	29	17	313	111						
ACTIVATION														
SOLVENT CONTROL	RAT	LIVER	32	20	10	18	17	12	27	153	147	193		
POSITIVE CONTROL***	RAT	LIVER	254	262	605	473								
TEST COMPOUND														
0.00100 UL	RAT	LIVER	29	31	-	27	-	19	-	219	-	264		
0.01000 UL	RAT	LIVER	34	29	-	17	-	29	-	260	-	227		
0.10000 UL	RAT	LIVER	37	35	28	55	25	28	61	282	-	212		
1.00000 UL	RAT	LIVER	30	86	133	54	105	33	130	301	241	216		
5.00000 UL	RAT	LIVER	27	39	77	19	81	24	84	297	349	270		
10.00000 UL	RAT	LIVER		-	14	-	25	-	91	-	277			

* IBY* CONVERTANTS PER PLATE

- | | |
|-----------------------------|-------------------------------|
| ** TA-1535 MNNG 10 UG/PLATE | *** TA-1535 ANTH 100 UG/PLATE |
| TA-1537 QM 10 UG/PLATE | TA-1537 AMQ 100 UG/PLATE |
| TA-1538 NF 100 UG/PLATE | TA-1538 AAF 100 UG/PLATE |
| TA-98 NF 100 UG/PLATE | TA-98 AAF 100 UG/PLATE |
| TA-100 MNNG 10 UG/PLATE | TA-100 ANTH 100 UG/PLATE |
| D4 MNNG 10 UG/PLATE | D4 DMNA 100 MICROMOLES/PLATE |
| SOLVENT DMSO 2.5 %/PLATE | SOLVENT DMSO 2.5 %/PLATE |
- INDICATES TEST WAS NOT DONE

3. SUMMARY OF PLATE TEST RESULTS

LITTON BIOMETRICS, INC.

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: U-04

B. SOLVENT: DMSO

C. TEST INITIATION DATE: JUNE 7, 1977

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

INST	SPECIES	ISSUE	B. E. V. E. B. I. A. N. J. S. P. E. R. P. L. A. T. E.											
			TA-1535		TA-1537		TA-1538		TA-98		TA-100		O4	
			1	2	1	2	1	2	1	2	1	2	1	2
NONACTIVATION														
SOLVENT CONTROL	---	---	13	22	10	18	14	17	30	24	76	79	140	256
POSITIVE CONTROL**	---	---	>1000	>1000	225	200	>1000	>1000	>1000	>1000	536	661	690	807
TEST COMPOUND														
0.001000 UL	---	---	12	9	6	11	17	15	28	24	64	85	128	190
0.010000 UL	---	---	10	19	7	13	18	14	31	31	73	83	196	157
0.100000 UL	---	---	10	15	18	13	19	14	24	25	74	73	185	153
1.000000 UL	---	---	8	16	26	19	12	15	29	25	83	77	170	164
5.000000 UL	---	---	4	11	10	7	14	16	31	29	64	82	195	172
ACTIVATION														
SOLVENT CONTROL	HAT	LIVER	12	14	25	32	11	20	28	34	96	85	214	231
POSITIVE CONTROL***	RAT	LIVER	96	159	537	635	>1000	>1000	874	>1000	204	499	480	480
TEST COMPOUND														
0.001000 UL	RAT	LIVER	10	11	21	12	16	19	22	27	49	88	238	188
0.010000 UL	RAT	LIVER	7	9	14	20	14	17	28	26	90	84	210	177
0.100000 UL	RAT	LIVER	6	9	19	26	42	93	41	40	95	75	182	172
1.000000 UL	RAT	LIVER	10	11	167	142	76	87	61	85	90	85	177	198
5.000000 UL	RAT	LIVER	17	10	137	154	85	158	201	197	89	107	163	175
10.000000 UL	RAT	LIVER			48	71	49	76	75	82				

* TRY- CONVERTANTS PER PLATE

** TA-1535 MNMG 10 UG/PLATE
 TA-1537 DM 10 UG/PLATE
 TA-1538 NF 100 UG/PLATE
 TA-98 NF 100 UG/PLATE
 TA-100 MNMG 10 UG/PLATE
 O4 MNMG 10 UG/PLATE
 SOLVENT DMSO 50 UL/PLATE

*** TA-1535 ANTH 100 UG/PLATE
 TA-1537 AMQ 100 UG/PLATE
 TA-1538 AAF 100 UG/PLATE
 TA-98 AAF 100 UG/PLATE
 TA-100 ANTH 100 UG/PLATE
 O4 DMNA 100 MICROMOLES/PLATE
 SOLVENT DMSO 50 UL/PLATE

LITTON BIOGENETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR3A

COMPOUND FREQUENCY SUMMARY REPORT 09/14/77

NONACTIVATION COMPOUND R-04

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 THY EX-5	
NAN		43.74	5.28	15.35	20.29		10.22	23.74	27.17	17.21	CONTROLS
NAP		526.60	667.05	969.61	89.05		52.20	109.47	180.45	124.68	
<hr/>											
NA1		17.74	1.71	24.00	5.61		8.72	22.29	21.41	8.08	TEST DATA
NA2		21.49	1.53	19.14	4.53		10.80	25.43	15.80	6.45	
NA3		27.43	3.09	13.02	8.54		10.68	6.81	15.84	6.76	
NA4		26.45	4.92	13.09	12.44		18.66	14.77	20.03	5.87	
ACT	ALI						7.26				
ACT	LI4						13.71				

LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR36

COMPOUND FREQUENCY SUMMARY REPORT 09/14/77

SPECIES SPRAW/RAT COMPOUND R-04

TEST	ORG	TA100 HIS EX-A	TA1535 HIS EX-B	TA1537 HIS EX-B	TA1538 HIS EX-B	T498 HIS EX-B	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	60.67	3.17	4.32	117.78	10.70	12.95	1.50	NEGATIVE CONTROLS
ACT	A-C	47.96	1.98	3.15	4.44	13.81	10.60	1.31	
ACT	ALT	57.93	3.60	8.05	6.34	23.72	9.60	1.00	
ACT	PL1	349.02	74.39	41.15	335.01	533.71	55.24	50.83	POSITIVE CONTROLS
ACT	L11	84.77	3.19	7.67	15.71	80.48	9.21	2.71	TEST COMPOUND
ACT	L12	81.91	2.40	8.12	15.43	59.54	4.97	1.49	
ACT	L13	91.26	3.01	11.13	15.95	60.00	3.74	1.16	
ACT	L14	53.30	2.31	9.02	27.53	46.99	4.11	1.47	

DATA TABLE TERMS AND ABBREVIATIONS

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OR EXPLANATION</u>
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPI:	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (e.g., EP + 6 = $\times 10^6$).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (e.g., EP + 0 = $\times 10^0$). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.

DATA TABLE TERMS AND ABBREVIATIONS (Continued)

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OF EXPLANATION</u>
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.
AAF	2-Acetylaminofluorene
AMQ	8-Aminoquinoline
DMSO	Dimethylsulfoxide
DMNA	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
SPECIES	Animal Strains
SPRDAW	Sprague-Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (<u>Macaca mulatta</u>)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

5. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.



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5. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537 and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100 and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a



BIONETICS

5. EVALUATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g., TA-1537, responds to a mutagen in nonactivation tests, it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.

6. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or revertant counts, as well as calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment:

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (< 25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies, especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and to increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants, especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his⁻ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment that will be negative.



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6. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS (Continued)

C. Dose-Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct-acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contains the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cutoff points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run:

1. A solvent control minus the chemical and minus the activation system (A-C)
2. A control plus the positive control chemical minus the activation system (A+C), and
3. A control containing the activation system and the test chemical solvent (ALI or ALU).

All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control, but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

6. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS (Continued)

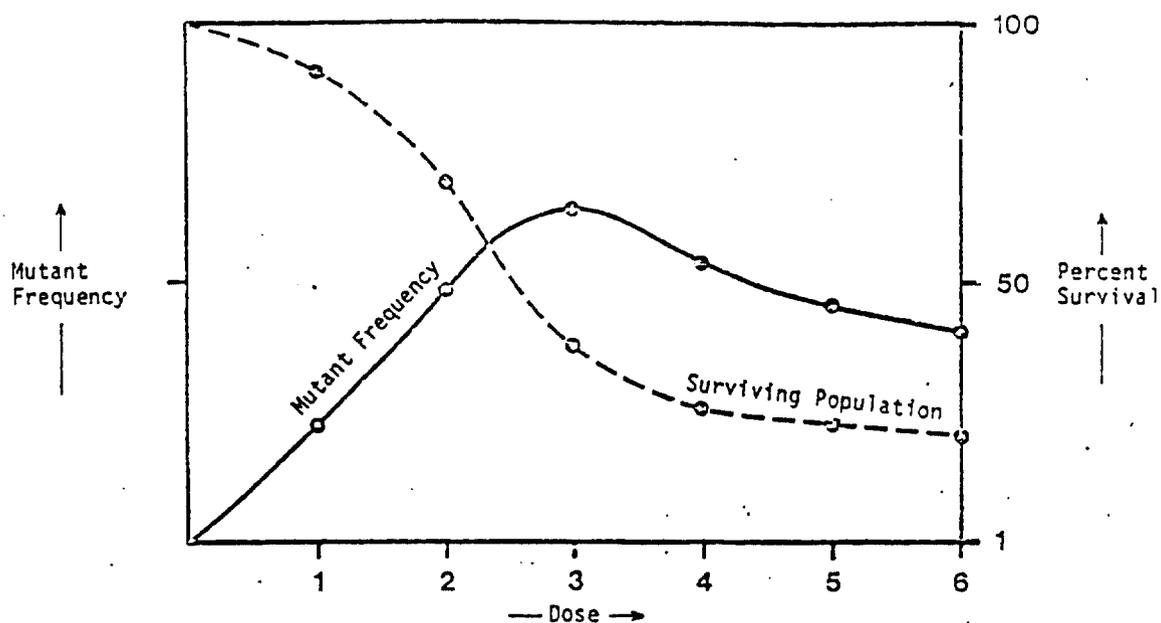
D. Control Tests

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical, but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens, and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.



HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT

- (1) Dose levels 1, 2 & 3 were used
- (2) Dose levels 2, 3 & 4 were used
- (3) Dose levels 3, 4 & 5 were used

OBSERVED DOSE RESPONSE

A typical positive dose response set of data would be obtained.

The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.

Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

TABULATION OF DATA

REPORT EXP33 LITTON BIOOPTICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	AMPETRINST	SPECIES		PROJECT 2525	DATE - 09/14/77	
709104	709104	DETECTOR TA100	POP1	MUT1	/		
COMPOUND	TEST	ORG ID	CONCENTRATION	EP+6	EP+0	FREQ1 EP-B	CONTAM
	NAN		SOLVENT	0900	0391	43.44	0
	HAP		EMS 0.066%	0703	3762	526.60	0
H-04	NA1		0005-2 PCT.	0988	0122	11.74	0
H-04	NA2		0025-3 PCT.	0847	0182	21.49	0
R-04	NA3		0125-4 PCT.	0915	0251	27.43	0
H-04	NA4		0625-5 PCT.	0881	0233	26.45	0

REPORT CAR 13 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT EXPERIMENT 709401		AMPETRINST DETECTOR TA1535	SPECIES		PROJECT 2525 /	DATE - 09/14/77	
COMPOUND	TEST	DMG 10	CONCENTRATION	POP EP+6	MUT EP+0	FREQ EP-8	CONTAM
	NAN		SOLVENT	0492	0026	5.28	0
	HAP		FMS 0.2%	0068	5790	667.05	0
R-04	NA1		0005-2 PCT.	0350	0006	1.71	0
R-04	NA2		0025-3 PCT.	0587	0009	1.53	0
R-04	NA3		0125-4 PCT.	0550	0017	3.09	0
R-04	NA4		0625-5 PCT.	0651	0032	4.92	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	AMPETREINST DETECTOR	TA1537	SPECIFS	PROJECT 2525 /	DATE - 09/14/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POP4 EP+6	MUT1 EP+0	FREQ1 EP-R	CONTAM
	NAN		SOLVENT	1029	0158	15.35	0
	NAP		DM 13 UG/ML	0031	0317	960.61	0
R-04	NA1		0005-2 PCT.	0250	0060	24.00	0
R-04	NA2		0025-3 PCT.	0397	0076	19.14	0
R-04	NA3		0125-4 PCT.	0599	0078	13.02	0
R-04	NA4		0625-5 PCT.	0703	0092	13.09	0

REPORT EXP33 LITTON BIOTETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	AMPE/INST	SPECIES		PROJECT	DATE	
704102	704102	TA1538	POPUL	MUT1	2525	09/14/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	EP+6	EP+0	FREQ1	CONTAM
				EP-8		EP-8	
	NA0		SOLVENT	0069	0014	20.29	1
	NA0		NF 667 UG/ML	0137	0122	89.05	0
R-04	NA1		0005-2 PCT.	0285	0016	5.61	0
R-04	NA2		0025-3 PCT.	0464	0021	4.53	1
R-04	NA3		0125-4 PCT.	0246	0021	8.54	0
R-04	NA4		0625-5 PCT.	0257	0033	12.04	0

REPORT EXR11 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	AMPETRINST DETECTOR	TALSIA	SPECIES	PROJECT 2525 /	DATE - 09/14/77	
COMPOUND	TEST	0-5 IP	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0702	0051	7.26	0
R-04	ACT	L14	0625-5 PCT.	0620	0085	13.71	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT		CONTRACT	AMPETRINST	SPECIFS		PROJECT 2525	DATE - 04/14/77
		707601	DETECTOR TA98			/	
COMPOUND	TEST	ORG ID	CONCENTRATION	POP1 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTA:
	HAN		SOLVENT	1047	0107	10.22	0
	NAP		NF 667 UG/ML	1436	0606	42.20	0
H-04	NA1		0005-2 PCT.	1399	0122	8.72	0
H-04	NA2		0025-3 PCT.	1287	0139	10.80	0
H-04	NA3		0125-4 PCT.	1573	0168	10.68	0
H-04	NA4		0625-5 PCT.	0793	0148	18.66	0

REPORT (XRAY) LITTON BIOGENETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	AMPETRINST DETECTOR	TA98	SPECIFS	PROJECT 2525 /	DATE - 09/14/77	
COMPOUND	TEST	OHG ID	CONCENTRATION	POP0 EP+6	MUT1 EP+0	FREQ1 EP-B	CONTAM
	NAN		SOLVENT	0973	0211	23.74	0
	NAP		NF 667 UG/ML	1214	1329	109.47	1
R-04	NA1		0005-2 PCT.	1144	0255	22.29	0
R-04	NA2		0025-3 PCT.	0928	0236	25.43	0
R-04	NA3		0125-4 PCT.	1057	0072	6.81	0
R-04	NA4		0625-5 PCT.	1090	0161	14.77	1

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

COMPOUND	TEST	ORG ID	CONCENTRATION	SPECIES			PROJECT 2525		DATE - 04/14/77
				POP FP+4	MUT1 FP+1	MUT2 FP+1	FREQ1 EP-5	FREQ2 EP-5	
	NAN		SOLVENT	1104	0300	0190	27.17	17.21	0
	NAP		FMS 1.0 %	0424	1126	0778	180.45	124.68	0
R-04	NA1		0044-2 PCT.	1238	0265	0100	21.41	8.08	0
R-04	NA2		0022-2 PCT.	1272	0201	0082	15.80	6.45	0
R-04	NA3		0011-2 PCT.	1376	0218	0093	15.84	6.76	0
R-04	NA4		0055-3 PCT.	1243	0249	0073	20.03	5.87	0

REPORT EXH33 LITTON HIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT 713A01	AMPETRINST DETECTOR TA100	SPECIES	PROJECT 2525 SPH/DAM/RAT	DATE - 09/14/77		
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1462	0887	60.67	0
	A-C		SOLVENT	1055	0506	47.96	0
	ALI		TISSUE	1160	0672	57.93	0
	ACP	L1	DMN 90 UM/ML	1379	4813	349.02	0
R-04	ACT	L11	0005-2 PCT.	1280	1085	84.77	0
R-04	ACT	L12	0025-3 PCT.	1327	1087	81.91	0
R-04	ACT	L13	0125-4 PCT.	1121	1023	91.26	0
R-04	ACT	L14	0625-5 PCT.	1289	0687	53.30	0

REPORT FR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	AMPETRINST DETECTOR	TA1535	SPECIES	PROJECT 2525 SPROAW/RAT	DATE - 09/14/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POP1 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		DMN 90 UM/ML	1073	0034	3.17	0
	A-C		SOLVENT	0962	0019	1.98	0
	ALT		TISSUE	0815	0030	3.68	0
	ACP	1.1	DMN 90 UM/ML	0652	0485	74.39	0
R-04	ACT	1.1	0005-2 PCT.	1600	0051	3.19	0
R-04	ACT	1.12	0025-3 PCT.	1961	0047	2.40	0
R-04	ACT	1.13	0125-4 PCT.	1596	0048	3.01	0
R-04	ACT	1.14	0625-5 PCT.	0693	0016	2.31	0

REPORT EXR13 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 713202		CONTRACT AMPETRINST DETECTOR TA1537	SPECIES SPRDAW/RAT		PROJECT 2525	DATE - 09/14/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POP1 EP+6	MUT1 EP+0	FREQ1 CP-R	CONTAM
	A+C		AMQ 333 UG/ML	2546	0110	4.32	0
	A-C		SOLVENT	2730	0086	3.15	0
	AL1		TISSUE	1243	0100	8.05	0
	ACP	L1	AMQ 333 UG/ML	1633	0672	41.15	0
R-04	ACT	L11	0005-2 PCT.	1434	0110	7.67	0
R-04	ACT	L12	0025-3 PCT.	1379	0112	8.12	0
R-04	ACT	L13	0125-4 PCT.	1186	0132	11.13	0
R-04	ACT	L14	0625-5 PCT.	1175	0106	9.02	0

REPORT EXH 11 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 711203		CONTRACT	AMPETRIINST	SPECIES		PROJECT 2525	DATE - 09/14/77
		DETECTOR	TA1538	SPROAM/RAT			
COMPOUND	TEST	ORG ID	CONCENTRATION	POP1 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0793	0934	117.78	2
	A-C		SOLVENT	1486	0066	4.44	2
	ALI		TISSUE	1072	0068	6.34	2
	ACP	L1	ANTH 67 UG/ML	0697	2335	335.01	0
R-04	ACT	L11	0005-2 PCT.	0751	0118	15.71	2
R-04	ACT	L12	0025-3 PCT.	0635	0098	15.43	2
R-04	ACT	L13	0125-4 PCT.	0796	0127	15.95	2
R-04	ACT	L14	0625-5 PCT.	0534	0147	27.52	2

REPORT EXR13 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT		CONTRACT	AMPETRINST	PROJECT 2525			DATE - 09/14/77
713399			DETECTOR TA9M	SPECIES	SPRDAM/RAT		
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	COUNTS
	A+C		ANTH 67 UG/ML	1533	0164	10.70	0
	A-C		SOLVENT	1144	0150	13.01	0
	ALI		TISSUE	0624	0140	23.72	0
	ACP	L1	ANTH 67 UG/ML	0531	2034	533.71	0
R-04	ACT	L11	0005-2 PCT.	0456	0367	80.48	0
R-04	ACT	L12	0025-3 PCT.	0600	0362	59.54	0
R-04	ACT	L13	0125-4 PCT.	0455	0273	66.00	0
R-04	ACT	L14	0625-5 PCT.	0432	0203	46.99	0

REPORT EXR13 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	AMPESTRINST	DETECTOR	000004	SPECIES	SPRDAM/HAT	PROJECT	2525	DATE	09/14/77
COMPOUND	TEST	ORG ID	CONCENTRATION	POP4	MUT1	MUT2	FREQ1	FREQ2	CONTAM	
				EP-4	EP-1	EP-1	EP-5	EP-5		
	A-C		DMN 90 UM/ML	0865	0112	0013	12.95	1.50	0	
	A-C		SOLVENT	0940	0089	0011	10.60	1.31	0	
	ALI		TISSUE	0802	0077	0008	9.60	1.00	0	
	ACP	LI	DMN 90 UM/ML	1021	0564	0519	55.24	50.83	0	
R-04	ACT	LI1	0044-2 PCT.	0923	0085	0025	9.21	2.71	0	
R-04	ACT	LI2	0022-2 PCT.	1207	0060	0018	4.97	1.49	0	
R-04	ACT	LI3	0011-2 PCT.	1633	0061	0019	3.74	1.16	0	
R-04	ACT	LI4	0055-3 PCT.	1705	0070	0025	4.11	1.47	0	

EVALUATION SUMMARY

The test results with R-04 in the Mouse Lymphoma Assay indicated increased mutation frequencies in the high dose range for both nonactivation (-S9) and activation (+S9) test conditions. The increases appeared to plateau and not demonstrate a clear dose response curve. Thus, the results do not exactly fit our established criteria for mutagenesis.



Litton BIONETICS

SPONSOR: American Petroleum Institute

MATERIAL: R-04

SUBJECT: FINAL REPORT L5178Y MOUSE LYMPHOMA MUTAGENICITY ASSAY

1. OBJECTIVE

The objective of this study was to evaluate R-04 for specific locus forward mutation induction in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay.

2. MATERIALS

A. Test Compound

1. Date Received: February 18, 1976
2. Description: Dark liquid

B. Indicator Cells

The Fischer mouse lymphoma cell line used in this study was derived from L5178Y. The cells are heterozygous for a specific autosomal mutation at the TK locus and are bromodeoxyuridine (BUdR) sensitive. Scoring for mutation was based on selecting cells that have undergone forward mutation from a TK+/- to a TK-/- genotype by cloning them in soft agar with BUdR.

C. Media

The cells were maintained in Fischer's Medium for Leukemic Cells of Mice with 10% horse serum and sodium pyruvate. Cloning medium consisted of Fischer's medium with 20% horse serum, sodium pyruvate, and 0.37% agar. Selection medium was made from cloning medium by the addition of 5.0 mg of BUdR to 100 ml of cloning medium.

D. Control Compounds

1. Negative Control

The solvent in which the test compound was dissolved was used as a negative control and is designated as solvent control in the data table. The actual solvent is listed in the Results Section.



BIONETICS

2. MATERIALS (Continued)

D. Control Compounds

2. Positive Controls

Ethylmethanesulfonate (EMS), which induces mutation by base-pair substitution, was dissolved in culture medium and used as a positive control for the nonactivation studies at a final concentration of .3 μ l/ml.

Dimethylnitrosamine (DMN), which requires metabolic biotransformation by microsomal enzymes, was used as a positive control substance for the activation studies at a final concentration of .5 μ l/ml.

3. EXPERIMENTAL DESIGN

A. Toxicity

The solubility, toxicity, and doses for all chemicals were determined prior to screening. The effect of each chemical on the survival of the indicator cells was determined by exposing the cells to a wide range of chemical concentrations in complete growth medium. Toxicity was measured as loss in growth potential of the cells induced by a five-hour exposure to the chemical followed by a 24-hour expression period in growth medium. A minimum of four doses was selected from the range of concentration by using the highest dose that showed no loss in growth potential as the penultimate dose and by bracketing this with one higher dose and at least two lower doses. Toxicity produced by chemical treatment was monitored during the experiment.

B. Assays

1. Nonactivation Assay

The procedure used is a modification of that reported by Clive and Spector (Mutation Research, 31:17-29, 1975). Prior to each treatment, cells were cleansed of spontaneous TK-/- by growing them in a medium containing thymidine, hypoxanthine, methotrexate, and glycine (THMG). This medium permits the survival of only those cells that produce the enzyme thymidine kinase, and can therefore utilize the exogenous thymidine from the medium. The test compound was added to the cleansed cells in growth medium at the predetermined doses for five hours. The mutagenized cells were washed, fed, and allowed to express in growth medium for three days. At the end of this expression period, TK-/- mutants were detected by cloning the cells in the selection medium for ten days. Surviving cell populations were determined by plating diluted aliquots in nonselective growth medium.



BIONETICS

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3. EXPERIMENTAL DESIGN (Continued)

B. Assays

2. Activation Assay

The activation assay differs from the nonactivation assay in the following manner only. Two milliliters of the reaction mixture were added to 10 ml of growth medium. The desired number of cleansed cells was added to this mixture, and the flask was incubated on a rotary shaker for five hours. The incubation period was terminated by washing the cells twice with growth medium. The washed mutagenized cells were then allowed to express for three days and were cloned as indicated for the nonactivated cells.

C. Preparation of 9,000 x g Supernatant

Male random bred mice were killed by cranial blow, decapitated, and bled. The liver was immediately dissected from the animal using aseptic technique and placed in ice-cold 0.25M sucrose buffered with Tris buffer at a pH of 7.4. When an adequate number of livers had been collected, they were washed twice with fresh buffered sucrose and completely homogenized. The homogenate was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from this centrifuged sample was retained and frozen at -80C until used in the activation system. This microsome preparation was added to a "core" reaction mixture to form the activation system described below:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	6 μ moles
2. Isocitric acid	35 μ moles
3. Tris buffer, pH 7.4	28 μ moles
4. MgCl ₂	2 μ moles
5. Homogenate fraction equivalent to 25 mg of wet tissue	

D. Screening

A mutation index was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR. The ratio was then compared to that obtained from other dose levels and from positive and negative controls. Colonies were counted on an electronic colony counter that resolves all colonies greater than 200 microns in diameter.



BIONETICS

4. RESULTS

The data presented in the following table show the concentrations of the test compound employed, the number of mutant clones obtained, the surviving populations after the expression period, and the calculated mutation frequencies.

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: H-04

B. SOLVENT: DMSO

C. TEST DATE: APRIL 12, 1977

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) OR NANOLITERS (NL) PER MILLILITER.

ILSI	S-9		DAILY COUNTS			RELATIVE SUSPENSION GROWTH (% OF CONTROL)	MUTANT CLONES	VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH	MUTANT FREQUENCY (10 ⁻⁶)
	ISSUE	SOURCE	CELLS/ML	1	2						
NONACTIVATION											
SOLVENT CONTROL	---	---	7.2	20.2	10.8	100.0	25.0	134.0	100.0	100.0	0.1866
NEGATIVE CONTROL	---	---	12.0	11.2	19.4	166.0	12.0	125.0	93.3	154.8	0.0960
EMS .3 UL/ML	---	---	5.4	14.4	10.8	53.5	113.0	114.0	85.1	45.5	0.9912
TEST COMPOUND											
0.01000 UL/ML	---	---	9.4	14.2	13.8	117.3	29.0	156.0	116.4	136.5	0.1059
0.02000 UL/ML	---	---	10.6	12.2	14.2	116.9	10.0	85.0	63.4	74.2	0.1176
0.04000 UL/ML	---	---	8.0	12.6	14.2	91.1	33.0	154.0	114.9	104.7	0.2143
0.08000 UL/ML	---	---	8.2	15.0	14.2	111.2	69.0	101.0	75.4	83.8	0.6832
0.16000 UL/ML	---	---	7.4	7.4	17.8	62.1	46.0	129.0	96.3	59.7	0.1566
ACTIVATION											
SOLVENT CONTROL	HOUSE	LIVER	6.0	19.6	12.0	100.0	21.0	162.0	100.0	100.0	0.1276
DMN .5 UL/ML	HOUSE	LIVER	9.3	6.3	6.5	27.0	102.0	25.0	15.4	4.2	4.0800
TEST COMPOUND											
0.02000 UL/ML	HOUSE	LIVER	7.4	13.0	14.2	96.8	19.0	165.0	101.9	98.6	0.1152
0.04000 UL/ML	HOUSE	LIVER	10.6	10.8	13.0	105.5	25.0	150.0	92.6	97.6	0.1667
0.08000 UL/ML	HOUSE	LIVER	4.4	23.2	14.4	104.2	61.0	188.0	116.0	120.9	0.3245
0.16000 UL/ML	HOUSE	LIVER	9.0	10.4	13.4	88.9	38.0	168.0	103.7	92.2	0.2262
0.32000 UL/ML	HOUSE	LIVER	5.2	3.6	18.0	23.9	79.0	216.0	133.3	31.8	0.3657

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
 ** (MUTANT CLONES / VIABLE CLONES) X 10⁻⁶

5. CRITERIA USED IN THE EVALUATION

Several criteria have been established which, if met, provide a basis for declaring a material genetically active in the Mouse Lymphoma Assay. These criteria are derived from a historical data base and are helpful in maintaining uniformity in evaluations from material to material and run to run. While these criteria are reasonably objective, a certain amount of flexibility may be required in making the final evaluation since absolute criteria may not be applicable to all biological data.

A compound is considered mutagenic in the Mouse Lymphoma Assay if:

- a. A dose response relationship is observed over three of the four dose levels employed.
- b. The minimum increase at the high level of the dose response curve is at least 2.5 times greater than the solvent control value.
- c. The solvent control data are within the normal range of the spontaneous background for the TK locus.

All evaluations of mutagenic activity are based on the concurrent solvent control value run with the experiment in question. Positive control values are not used as reference points, but are included to ensure the current cell population responds to direct and promutagens under the appropriate treatment conditions.

Occasionally, a single point within a concentration range will show an increase 2.5 times greater than the spontaneous background. If the increase is at the high dose, is reproducible, and if an additional higher dose level is not feasible because of toxicity, the chemical can be considered mutagenic. If the increase is internal within the dose range and is not reproducible, the increase will normally be considered aberrant. If the internal increase is reproducible, several doses clustered around the positive concentration will be examined to either confirm or reject the reliability of the effect.

As the data base on the assay increases, the evaluation criteria can be expected to become more firmly established.



EVALUATION SUMMARY

The data from the Rat Bone Marrow Cytogenetic Analysis of R-04 were all within the normal range and the test was considered negative.

SPONSOR: American Petroleum Institute
MATERIAL: R-04
SUBJECT: FINAL REPORT
Rat Bone Marrow Cytogenetic Analysis

1. OBJECTIVE

The purpose of this study was to evaluate the chromosomes of bone marrow cells from R-04 treated rats for genetic aberrations.

2. MATERIALS

A. Test Compound

1. Date Received: February 18, 1977
2. Description: Dark liquid

3. OVERVIEW AND RATIONALE

One means of detecting chemically-induced in vivo genetic activity is to examine mitotically active cells that have been arrested at metaphase for structural changes and rearrangements of their chromosomes. The occurrence of such chromosome aberrations correlates well with the administration of known chemical mutagens to the animals, and thus can serve as an indicator for possible mutational events and the compounds that induce them. Rats were treated with one of three dosage levels of the test compound or a positive or negative control substance both on an acute and a subchronic schedule. Acute study rats were killed 6, 24, and 48 hours after treatment, and bone marrow spreads prepared. Subchronic study rats were killed six hours after the last dose was administered and the bone marrow removed and processed like the acute dose.

4. EXPERIMENTAL DESIGN

A. Animal Husbandry

Male rats, ages 10 to 12 weeks old, from a random bred closed colony were purchased from Charles River Laboratories and used in the cytogenetic experiments. These rats were housed five to a cage and offered a commercial laboratory chow (pellet) diet (Purina) and water ad libitum. These animals were quarantined and observed for 10 days prior to being used in the experiments.

Personnel handling the animals or working within the animal facility wear suitable protective clothing, gloves, and face masks. When appropriate, individuals with respiratory or other overt infections were excluded from the animal facilities.

B. Acute Studies

Figure 1 shows the design of the test. Forty-five animals were dosed acutely with the three levels of the compound. The remaining 20 animals were used as controls.

Two hours prior to being killed, the animals were injected with 4 mg/kg of colchicine to arrest dividing cells in metaphase. These animals were killed with CO₂ 6, 24, and 48 hours after the compound was administered and the bone marrow aspirated from the canals of the femurs and tibias of the lower limbs. The marrow plug was transferred to Hank's balanced salt solution and the cells pelleted by centrifugation. The marrow button was resuspended in 0.075 M KCl by repeated pipetting during a 10-minute incubation at 37C. Centrifugation was repeated and the marrow button resuspended in Carnoy's fixative. Fixative was changed after one-half hour, and the cells were left in fixative overnight at 4C.

Slides were prepared by dropping cells from the fixative onto a glass slide and air drying the slide. Spreads were stained with 5% Giemsa at pH 6.8.

Slides were coded and searched for metaphases according to a standardized pattern. Fifty spreads were located for each animal and when of suitable quality, the chromosomes were counted and evaluated for the presence of abnormalities.

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FIGURE 1

RAT BONE MARROW CYTOGENETIC ANALYSIS
NUMBER OF ANIMALS USED

ACUTE STUDY

<u>TREATMENT</u>	<u>NUMBER OF ANIMALS KILLED</u> <u>6, 24, AND 48 HOURS AFTER DOSING</u>		
	<u>6 Hours</u>	<u>24 Hours</u>	<u>48 Hours</u>
	High Level	5	5
Intermediate Level	5	5	5
Usage Level	5	5	5
Positive Controls	-	5	-
Negative Controls	3	3	3

SUBCHRONIC STUDIES

5 EXPOSURES - 24 HOURS APART

ANIMALS KILLED 6 HOURS AFTER LAST EXPOSURE

<u>TREATMENT</u>	<u>NUMBER OF ANIMALS KILLED</u> <u>AT TERMINATION</u>
High Level	5
Intermediate Level	5
Usage Level	5
*Positive Controls	5
Negative Controls	3

* Positive controls will not be utilized in subchronic studies if acute studies are conducted since the positive controls are administered on an acute one-time dosage basis only. TEM (0.3 mg/kg) is used as a positive control substance.

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4. EXPERIMENTAL DESIGN (Continued)

C. Subchronic Studies

Figure 1 also shows the overall scheme of the subchronic studies. Twenty-five animals were used in this study. They were dosed with the three levels of the test compound and the control compounds as indicated, once each day for five days. All animals were killed six hours after administration of the last dose. Two hours prior to being killed, the animals were injected with 4 mg/kg of colchicine to arrest dividing cells in metaphase.

The bone marrow was removed from the animals and processed exactly as indicated for the acute study.

D. Control Compounds

The negative control compound consisted of the solvent vehicle for the test compound. It was administered to the animals in 0.5 ml doses which were equivalent to the maximum amount given the experimental animals.

Triethylene melamine (TEM) at 0.3 mg/kg was used as the positive control substance. It was given in an acute dosage for 24 hours prior to killing the animals.

E. Dosage Levels

R-04 was administered to rats by oral gavage. The material was administered in pure form. The dose levels were established following a preliminary dose range study. The final doses were 5.0, 1.7, and .5 ml/kg. There was very little toxicity associated with exposure to this material.

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5. DATA COLLECTION

The test compound was evaluated in the rat bone marrow cytogenetic assay under both acute and subchronic schedules. Bone marrow cells from animals treated with the test and control substances were evaluated for the presence of chromosome aberrations. Breaks, gaps, fragments, and chromosome rearrangement figures were all recorded on the standard record sheet along with the number of centromeres scored in each spread and the coordinates of the microscope stage which identify the position of the aberration. These records are attached in the Appendix. Fifty cells were located and scored with a 100X objective for each dose level. In the event that 50 suitable spreads could not be found on one slide then additional slides labeled B, C, D, etc. were prepared from the original fixed marrow material and these were searched. In some cases, the material dosed to the animals appeared sufficiently toxic to the cells to prevent scoring 250 cells per animal and in other cases the colchicine appeared to be ineffective in arresting the cells. These slides/animals are identified in the slide code/animals list and on the raw data sheets appended.

6. RESULTS

The data have been collected from the raw data sheets and tabulated in summary form in Table 1.

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TABLE 1

A SUMMARY OF THE CYTOGENETIC ANALYSIS OF R-04 IN RAT BONE MARROW

Treatment	Dose (mg/kg)	Kill Time (hrs) ^a	No. of Animals	Unique Animal No. ^b	Total No. of Cells	Type and Frequency of Aberrations		No. of Cells With One or More Aberrations	No. of Animals Without Aberrations	Mitotic Index ^c
						Structural	Numerical			
Negative Control	Corn Oil	A 6 hr	5(0)	16-20	250	1cb,1ab	-	2 (0.8%)	4	4.5
		A 24 hr	5(0)	36-40	212	1cb	4pp	5 (2.4%)	2	5.0
		A 48 hr	4(1)	61-65	154	1f,1tb	1pp	3 (1.9%)	2	6.2
		SC 6 hr	5(0)	81-85	223	-	-	0 (0.0%)	5	5.0
Triethylene Melamine	0.5 mg/kg	A 24 hr	5(0)	41-45	182	15tb,1ab 11f,9af, 4t,5>,2qr 2pu,4td,1d 2f	-	54 (29.7%)	1	1.5
R-04	0.5 ml/kg	A 6 hr	5(0)	156-160	250	-	-	0 (0.0%)	5	3.4
		A 24 hr	5(0)	171-175	207	1f,1cb	-	2 (1.0%)	3	5.0
		A 48 hr	5(0)	186-190	250	1f,1cb	-	2 (0.8%)	3	4.8
		SC 6 hr	5(0)	201-205	208	1f	-	1 (0.5%)	4	4.1
R-04	1.7 ml/kg	A 6 hr	5(0)	151-155	243	-	-	0 (0.0%)	5	4.7
		A 24 hr	5(0)	166-170	212	-	-	0 (0.0%)	5	5.1
		A 48 hr	5(0)	181-185	250	-	-	0 (0.0%)	5	4.9
		SC 6 hr	5(0)	196-200	250	1cb	-	1 (0.4%)	4	4.0
R-04	5.0 ml/kg	A 6 hr	5(0)	146-150	214	-	-	0 (0.0%)	5	3.9
		A 24 hr	5(0)	161-165	250	1td,1f	-	2 (0.8%)	3	5.0
		A 48 hr	5(0)	176-180	250	1tb	1pp	2 (0.8%)	3	3.4
		SC 6 hr	0(5)	191-195	0	-	-	0 (0.0%)	-	-

FOOTNOTES AND SYMBOLS FOR TABLE 1

^aTime of death after dosing.

^bRefers to the 1, 2, or 3 digits following the hyphen in the Cytogenetics Exp. No. on the raw data sheets in Appendix B.

^cBased on a count of at least 500 cells per animal.

^dNumbers in () are animals for which less than 5 metaphase spreads were found.

Any of the following abbreviations may be used in the summary tables:

f = fragments
af = acentric fragment (2 tid)
lm = large metacentric
pp = polyploid
tb = chromatid break
sb = chromosome break
d = dicentric
r = ring
t = translocation
qr = quadriradial
pu = pulverized chromosome
cr = complex rearrangement
> = greater than 10 aberrations
tr = triradial
td = chromatid deletion
sd = chromosome deletion
cf = centric fusion
sl = slide lost or broken
min = minute chromosome

SC = subchronic
A = acute

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TABLE 2
 RAT-BONE MARROW
 CYTOGENETIC
 HISTORIC NEGATIVE CONTROL DATA^a

TYPE OF ABERRATION	6 HR. KILL		ACUTE 24 HR. KILL		48 HR. KILL		SUBACUTE 6 HR. KILL	
	No.	%	No.	%	No.	%	No.	%
Chromatid Gaps	53	0.92	13	0.22	33	0.50	53	0.82
Chromatid Breaks	31	0.54	45	0.75	47	0.75	61	0.95
Chromosome Gaps	4	0.07	3	0.05	3	0.05	5	0.08
Chromosome Breaks	0	0.0	1	0.02	4	0.06	2	0.03
Fragments	4	0.07	1	0.02	2	0.03	4	0.06
Acentric Fragments	1	0.02	4	0.07	1	0.02	0	0.00
Other Aberrations	1	0.02	6	0.10	1	0.02	4	0.06
<hr/>								
Percent Cells With Aberrations	94	1.62	73	1.21	91	1.46	129	2.00
	37	0.64	57	0.95	55	0.88	71	1.10
Total Cells Scored	5785		6010		6227		6436	
Approximate Number of Animals Scored	122		122		130		131	

^aCompiled from several studies using Sprague-Dawley Rats with various solvents. No significant difference was noted among solvents and the data were pooled. These data were collected over the period 1972 - 1977.

^bGaps included.

^cGaps excluded. This is the value normally employed in evaluation decisions. Gaps are not considered as hard evidence of clastogenic effects unless found in extremely high percentages.

7. GUIDELINES USED IN EVALUATING CYTOGENETIC DATA

A number of general guidelines has been established to serve as an aid in determining the meaning of bone marrow chromosomal aberrations.

A. General

Basically, we were trying to establish whether a substance or its metabolites can interact with chromosomes to produce gross lesions or changes in chromosome numbers, and whether these were of a type which can survive more than one mitotic cycle of the cell. The assay design was such that bone marrow samples were taken at 6, 24, and 48 hours after an acute administration of the compound. Since the cell transit time for bone marrow was 20 to 24 hours, one can, based on the time of kill obtain an indication of when in the cell cycle a compound may be active.

One would anticipate that many of the cells bearing breaks or reunion figures would be eliminated during their first mitotic division, and as a corollary, that those cells which survived the first anaphase would primarily bear balanced lesions. The detection of these lesions; and hence, a complete risk evaluation must usually rely on additional testing, but in general a cell bearing configurations such as small deletions or translocations not involving duplication of the centromere may be perpetuated and, therefore, constitute a greater risk to the individual than simple breaks.

B. Aberrations/Records

All aberration figures detected by this assay result from breaks in the chromatin which have either failed to repair or have repaired in atypical combinations. We scored and recorded on standard forms gaps, breaks, fragments, and reunion figures which involved a single chromatid or both chromatids of a single chromosome. The number and type of aberration for each cell was recorded as was the number of chromosomes for every cell located and scored. Up to 50 cells were scored on each slide. Depending upon the suitability of the material, it could have been necessary to prepare additional slides from the original fixed material. The location of cells bearing aberrations was identified by the use of coordinates on the mechanical stage. A mitotic index based on at least 500 cells counted was also recorded.



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7. GUIDELINES USED IN EVALUATING CYTOGENETIC DATA (Continued)

C. Data Interpretation

Data were summarized in tabular form and evaluated. Gaps were not counted as significant aberrations unless they were present in a higher than usual frequency. Open breaks were considered as indicators of genetic damage as were configurations resulting from the repair of breaks. The latter would include deletions, translocations, multiradials, rings, multicentrics, etc. Reunion figures such as these were weighted slightly higher than one break and may lead to stable configurations.

The number of aberrations per cell was also considered to be significant and frequent; cells with more than one aberration would be considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number was also considered in the evaluation of mutagenic potential.

Frequently, one was unable to locate 50 suitable metaphase spreads for each animal even after preparing additional material. Possible causes for this appear to relate to colchicine failure or to cytotoxic effects which altered the duration of the cell cycle, killed the cell or caused clumping of the chromosomes. Additional information can be gained from the mitotic index which also appeared to reflect cytotoxic effects. The type and extent of this damage must be evaluated in terms of dose response, the ability to determine anything about the integrity of the chromatin and the ability of the cell to recover and divide.

Comparison with a concurrent negative control which lacks aberrations can suggest statistical significance; therefore, treatment data were also considered against historical control data. In either event, the type of aberration, its frequency, and its correlation to dose trends within a given time period, were all considered in evaluating a compound as being mutagenically positive or negative.



BIONETICS



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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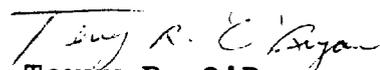
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TYPE: INT. SUPP FLWP

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SUB. DATE: 08/24/92 OTS DATE: 09/02/92 CSRAD DATE: 03/07/95

CHEMICAL NAME: _____ CAS# 64741-79-3

INFORMATION REQUESTED: FLWP DATE: _____
 0501 NO INFO REQUESTED
 0502 INFO REQUESTED (TECH)
 0503 INFO REQUESTED (VOL ACTIONS)
 0504 INFO REQUESTED (REPORTING RATIONALE)
 DISPOSITION:
 0639 REFER TO CHEMICAL SCREENING
 0678 CAP NOTICE

VOLUNTARY ACTIONS:
 0401 NO ACTION REPORTED
 0402 STUDIES PLANNED/IN PROGRESS
 0403 NOTIFICATION OF WORKING RATIONALE
 0404 LABEL/MSDS CHANGES
 0405 PROCESS/HANDLING CHANGES
 0406 APP/USE DISCONTINUED
 0407 PRODUCTION DISCONTINUED
 0408 CONFIDENTIAL

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	0216 EPI/CLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEM/PHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCC/REL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQEST DELAY	01 02 04	0248 PROD/USE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/CHEM ID	01 02 04	0251 MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	0299 OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0239 METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAB/PHARMACO (HUMAN)	01 02 04		

TRIAGE DATA NON-CBI INVENTORY ONGOING REVIEW SPECIES TOXICOLOGICAL CONCERN: USE: PRODUCTION:
 YES YES (DROP/REFER) RAT LOW
 NO NO (CONTINUE) In Vitro MED
 IN PROGRESS REFERR HIGH

COMMENTS

27)

8EHQ-92-12572: Rank - medium.

Chemical: R-04 (CAS# 64741-79-3).

Mutagenicity evaluation of R-04, Litton Bionetics. Inc., Kensington MD, dated January 1987: Positive for gene mutations in the Salmonella typhimurium/mammalian microsomal (Ames) assay in strains TA98, TA1537 and TA1538 with but not without metabolic activation, negative in strains TA100 and TA1535 both without and with activation.

Positive (weakly) for gene mutations in the L5178Y TK⁺ mouse lymphoma gene mutation assay in vitro both without and with metabolic activation.

Negative for chromosome mutations (aberrations) in the bone marrow of rats exposed in vivo by oral gavage.