Contains No CBI (1)

PHILLIPS PETROLEUM COMPANY
BARTLESVILLE, OKLAHOMA 74004 918 661-6600

HEALTH, ENVIRONMENT AND SAFETY

"Contains NO CBI"

(A)

August 24, 1992

Compliance Audit Program
CAP ID#: 8ECAP-0075

1992 SEP -2 PM 1:16
OTS CBIC

CERTIFIED MAIL - RETURN RECEIPT

8EHQ-92-12550
88920010734
INIT

Document Processing Center (TS-790)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street, SW
Washington, D. C. 20460

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 catagory 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, plese contact Fred Marashi at 918-661-8153.

Very truly yours,

Barbara J. Price

Barbara J. Price
Vice President
Health, Environment & Safety

Enclosure (Seven Boxes)

FFM/dh:29

RECEIVED
3/21/95

Phillips Petroleum Company

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

Contains No CBI

Title of Study: Salmonella Typhemurium/Mammalian Microsome Microfluctuation Assay with Compound Butadiene (LPG) and L5178Y Mouse Lymphoma Forward Mutation Assay with Butadiene and In Vitro Sister Chromatid Exchange in Chinese Hamster Ovary Cells Butadiene (LPG)

Name of Chemical: Butadiene

CAS#: 106-99-0

Summary: Butadiene was determined to produce positive mutagenic changes in the Salmonella typhimurium cell assay and the L5178Y Mouse Lymphoma Forward Mutation Assay and the Chinese Hamster Ovary Cell Assay.

Contact:

Fred Marashi
Phillips Petroleum Company
13 D2 PB
Bartlesville, OK 74004
Phone: 918/661-8153
Fax: 918/661-5664

HAZLETON
LABORATORIES AMERICA, INC
[illegible] FREDERICK TURNPIKE VIENNA VIRGINIA [illegible] U.S.A.

Salmonella typhimurium/Mammalian Microsome Microfluctuation Assay with Compound Butadiene (LPG)

FINAL REPORT

Submitted to

Phillips Petroleum Company
Bartlesville, Oklahoma

August 23, 1984

PHONE (703) 893-5400. TELEX 899436 (HAZLABS VINA) CABLE HAZLABS WASH DC

HAZLETON
LABORATORIES AMERICA, INC.

SUBJECT: Salmonella typhimurium Mammalian Microsome Microfluctuation Assay
Project No. 652-145

We, the undersigned, hereby declare that the work was performed under our supervision, according to the procedures herein described.

Study Director

Deborah H. Pence

DEBORAH H. PENCE, Ph.D.
Diplomate, American Board of Toxicology
Life Sciences Division

PROJECT PERSONNEL

Title	Name	Signature	Date
Study Manager and Report Preparation	Nancy E. McCarroll	Nancy E. McCarroll	07Z84

HAZLETON
LABORATORIES AMERICA, INC

FINAL REPORT

STUDY: Salmonella typhimurium Mammalian Microsome Microfluctuation Assay

COMPOUND INFORMATION:

1. Name - Butadiene (LPG)
2. Purity - Assumed 100%

EXPERIMENTAL DESIGN: See Project Sheet No. 3, Project No. 652-145

RESULTS:

1. Toxicity Evaluation

   The starting dose selected for the mutagenicity test was approximately 0.5% (volume/volume) because at the 10% and 1% levels the test material was toxic.

2. Mutagenicity Evaluation (Table 1 and 2).

   Exposure to five graded doses of Butadiene (LPG) in the absence of metabolic activation did not cause a significant increase in the reversion of histidine prototrophy of S. typhimurium strains TA1535, TA1537, TA98 or TA100. In the presence of metabolic activation, however, significant increases ($p < 0.05$) were seen following exposure to an atmosphere of 0.5% Butadiene with strains TA1535, TA98 and TA100. While the remaining percent atmospheres of Butadiene did not cause significant increases with strains TA1535, TA1537 and TA100, statistically significant increases in reversion of histidine prototrophy were noted at all test levels with strain TA98.

CONCLUSION:

Under these conditions, the experimental compound in the presence of metabolic activation induced statistically significant increases in the reversion of histidine prototrophy of S. typhimurium strain TA98 at all tested levels and is, therefore, considered to be mutagenic in this test system.

4395W/0156W

TABLE 1

Number of Positive Wells/48 Replicates Following Exposure to Graded Doses of Butadiene without Metabolic Activation

	STRAIN			
Agent	TA1535	TA1537	TA98	TA100
Air	1	1	1	2
2% Methylene Chloride	5	0	10*	31*
% Butadiene				
0.5	1	0	0	4
0.2	0	0	0	2
0.07	0	1	1	1
0.02	1	1	0	1
0.007	1	0	0	2

* = Statistically different from control ($p < 0.05$) CHI - Square Test

TABLE 2

Number of Positive Wells/48 Replicates Following Exposure to Graded Doses of Butadiene with Metabolic Activation

	STRAIN			
Agent	TA1535	TA1537	TA98	TA100
Air	1	2	3	7
3% Vinylidene Chloride	34*	4	37*	40*
% Butadiene				
0.5	8*	3	28*	19*
0.2	5	2	26*	9
0.07	1	1	21*	6
0.02	4	1	18*	4
0.007	1	1	13*	5

* = Statistically different from control ($p < 0.05$) CHI - Square Test

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE VIENNA, VIRGINIA 22182 U.S.A.

In vitro Sister Chromatid Exchange in Chinese Hamster Ovary Cells

Butadiene (LPG)

FINAL REPORT

Submitted to

Phillips Petroleum Company
Bartlesville, Oklahoma

January 29, 1985

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE VIENNA, VIRGINIA 22180 U.S.A.

SUBJECT: In vitro Sister Chromatid Exchange in Chinese Hamster Ovary Cells
Project No. 652-147

We, the undersigned, hereby declare that the work was performed under our supervision, according to the procedures herein described.

Study Director:

Deborah H. Pence

DEBORAH H. PENCE, Ph.D.
Diplomate, American Board of Toxicology
Life Sciences Division

Laboratory Supervision and
Report Preparation:

Thomas A. Cortina

THOMAS A. CORTINA, B.S.
Research Associate
Genetic Toxicology Department

das

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE • VIENNA, VIRGINIA 22180 • U.S.A.

FINAL REPORT

STUDY: Sister Chromatid Exchange in Chinese Hamster Ovary Cells

COMPOUND INFORMATION:

1. Name: Butadiene (LPG)

2. Purity: Assumed 100%

EXPERIMENTAL DESIGN: See modified protocol for gases and vapors.

RESULTS:

1. Toxicity Evaluation

The maximum concentration selected for the mutagenicity test was 10% Butadiene in air because it exhibited growth inhibition.

2. Mutagenicity Evaluation (Table 1)

Following exposure to five graded concentrations of Butadiene, statistically significant increases in the number of SCE's per chromosome were seen at 10% Butadiene ($p = 0.0008$) without activation and at 10% ($p < 0.0001$) and 3% Butadiene ($p < 0.0001$) with metabolic activation. A significant linear trend (Cochran-Armitage Test) ($p < 0.0001$) was seen in the activation data.

CONCLUSION:

Under these conditions, the experimental compound Butadiene, did exhibit a positive response and is, therefore, considered to be mutagenic in this test system.

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE VIENNA VIRGINIA 22180 U.S.A.

STATISTICAL REFERENCES:

W. G. Cochran, Some methods for strengthening the common CHI-Square tests, Biometrics 1954, Vol. 10, pp. 417-451.

P. Armatige, Tests for linear trends in proportions and frequencies, Biometrics 1955, Vol. 11, pp. 375-386.

Table 1
Summary of Sister Chromatid Exchange Data
Butadiene (LPG)

Without Activation

Treatment	Percent Concentration in Air	Number of Cells Analyzed	Total Number of SCE's	Mean Number of SCE's/Cell	P Value	Fold Increase in SCE's/Cell	Number of Chromosomes Analyzed	Mean Number of SCE's/Chromosome	P Value	Fold Increase in SCE's/Chromosome
Air	-	50	516	10.32	-	-	970	0.53	-	-
MC	7	22	629	28.59	0.0000(S)	2.8	438	1.43	0.0000(S)	2.7
Butadiene	10	40	492	12.30	0.0011(S)	1.2	772	0.64	0.0008(S)	1.2
	3	50	594	11.88	0.0125(NS)	1.2	994	0.60	0.03(NS)	1.1
	1	50	602	12.04	0.0107(NS)	1.2	997	0.60	0.03(NS)	1.1
	0.3	50	559	11.18	0.0826(NS)	1.1	984	0.57	0.12(NS)	1.1
	0.1	50	540	10.80	0.2222(NS)	1.1	984	0.55	0.29(NS)	1.0

With Activation

Treatment	Percent Concentration in Air	Number of Cells Analyzed	Total Number of SCE's	Mean Number of SCE's/Cell	P Value	Fold Increase in SCE's/Cell	Number of Chromosomes Analyzed	Mean Number of SCE's/Chromosome	P Value	Fold Increase in SCE's/Chromosome
Air	-	50	603	12.06	-	-	976	0.62	-	-
VC	6	50	1023	20.46	0.0000(S)	1.7	998	1.02	0.0000(S)	1.7
Butadiene	10	50	823	16.46	0.0000(S)	1.4	991	0.84	0.0000(S)	1.4
	3	50	793	15.88	0.0000(S)	1.3	989	0.80	0.0000(S)	1.3
	1	50	650	13.00	0.0985(NS)	1.1	989	0.66	0.13(NS)	1.1
	0.3	50	656	13.12	0.1019(NS)	1.1	991	0.66	0.14(NS)	1.1
	0.1	50	641	12.82	0.1995(NS)	1.1	991	0.65	0.26(NS)	1.1

NS = Not significant
S = Significant
MC = Methylene Chloride
VC = Vinylidene Chloride

HAZLETON
BIOTECHNOLOGIES CORPORATION
9200 LEESBURG TURNPIKE VIENNA VIRGINIA [illegible] U.S.A.

L5178Y MOUSE LYMPHOMA FORWARD MUTATION ASSAY

with Butadiene

FINAL REPORT

Submitted to:

Phillips Petroleum Company
Bartlesville, Oklahoma

March 7, 1985

PHONE (703) 893-5400. TELEX 899436 (HAZLABS VINA). CABLE HAZLABS WASH DC

HAZLETON
BIOTECHNOLOGIES CORPORATION

SPONSOR: Phillips Petroleum Company

SUBJECT: FINAL REPORT
L5178Y Mouse Lymphoma Forward Mutation Assay

Test Material:	Butadiene
Laboratory Number:	546
HBC Project Number:	652-146
LH Number:	20,240
Receipt Date:	July 1, 1982
Assay Completed:	February 11, 1985 (plate counts)

PROJECT PERSONNEL

Title	Name	Signature	Date
Study Director	Deborah Pence, Ph.D.	Deborah M. Pence	3/6/85
Research Associate and Report Preparation	Russell C. Sernau	Russell C. Sernau	2/25/85

RAW DATA STORAGE

At the completion of this study, the original copy of all raw data and the final report were sent to the Archives of Hazleton Laboratories America, Inc., Vienna, VA.

SUMMARY

In the presence and absence of S-9 metabolic activation, L5178Y TK +/- mouse lymphoma cells were treated with Butadiene using four duplicate dose levels ranging from 100 to 20 percent (v/v). The highest dose level (100%) was too toxic for analysis and was consequently discarded.

Of the three remaining dose levels in the absence of activation (60, 40 and 20 percent v/v), none induced a mutation frequency greater than two-fold above the solvent control. In the presence of activation, however, a greater than two-fold increase was induced at every dose level selected. This positive effect was confirmed by statistical analysis using Student's t-test ($p < .01$).

Under the conditions of this assay, Butadiene elicited a positive response in the presence of metabolic activation in the L5178Y Mouse Lymphoma Forward Mutation Assay and is, therefore, considered to be mutagenic.

OBJECTIVE

The purpose of this study was to evaluate Butadiene for the potential to induce forward mutations at the thymidine kinase (TK) locus in L5178Y TK +/- mouse lymphoma cells when tested with and without metabolic activation.

INTRODUCTION

The Mouse Lymphoma Assay is a short-term test for screening compounds for potential genetic activity. This test utilizes a mammalian cell line as a target to measure forward mutations. This system has been shown to be sensitive and capable of detecting the activity of a wide range of chemical classes, some of which are not detected in the Ames Test.

The L5178Y mouse lymphoma cell line is presumed to be diploid and three TK phenotypes have been recognized: TK+/+, TK+/-, and TK-/-. The TK+/+ and TK+/- cells are sensitive to trifluorothymidine (TFT) and resistant to methotrexate prepared in a solution with thymidine, hypoxanthine and glycine (THMG). The TK-/- phenotype exhibits reverse sensitivity and resistance patterns. The heterozygous TK+/- phenotype is used as the target cell in this test system.

When TK+/- cells are exposed to agents that can alter DNA, one of the possible consequences of this alteration is the induction of forward mutations which result in a change from TK+/- to the TK-/- phenotypes. This assay measures the induction of the TK-/- phenotype as its endpoint.

MATERIALS

A. Test Materials

1. Storage of Test Compound

A gas cylinder containing a compound designated Butadiene was submitted by the sponsor on July 1, 1982 and stored at room temperature.

Upon receipt in the laboratory, the compound was assigned laboratory number 546.

Information on the method of synthesis, stability and composition of the test material reside with the sponsor. It should be noted that for the purpose of this study, the test material was assumed stable and 100% active ingredient.

B. Control Articles

1. Positive Controls

Ethyl methanesulfonate (EMS), a positive control not requiring activation, was dissolved in culture medium and used at a final treatment concentration of 300 ug/ml. 3-Methylcholanthrene (MCA), a positive control requiring metabolic activation, was dissolved in DMSO and used at a final treatment concentration of 3 ug/ml. Both positive controls were assumed stable and 100% active ingredient under the conditions of this assay.

2. Media Control

Serum-free culture medium was used as the media control.

Control Articles	Supplier	Lot Number
Ethyl Methanesulfonate (EMS)	Kodak	A1A
3-Methylcholanthrene (MCA)	Kodak	A0A
DMSO	Fisher	730913

C. Cells

The cell culture used for this assay was L5178Y TK+/- mouse lymphoma cells, subline 3.7.2 C. The cells originated with Dr. Donald Clive, Research Triangle Park, North Carolina. Stocks of these cells were frozen and stored in liquid nitrogen until used. Prior to use in the assay, cells were cleansed of background mutants with an overnight treatment using methotrexate prepared in a solution of thymidine, hypoxanthine and glycine. The culture used for the mutation assay was thawed on September 24, 1984 and cleansed on November 8, 1984.

D. Test Chamber

The test chambers for this assay consisted of an individual 150 cc capacity serum bottle for each dose level of the test compound and control. Each serum bottle cap contained a rubber septum through which the test compound was introduced via a gas sampling syringe equipped with a 23 gage needle. Immediately following the introduction of the test gas, the cap of the serum bottle, was wrapped with Parafilm to reduce the possibility of leakage.

E. Toxicity Test

Using a method similar to that described under Forward Mutation Assay, a toxicity test was performed. Dose levels for the toxicity test ranged from 100 to 20% v/v in the presence and absence of metabolic activation.

F. Forward Mutation Assay

Based upon the toxicity test, four dose levels of the test gas were used in the mutation assay. Duplicate cultures for each dose level of the test gas and triplicate cultures for each positive and medium control were dosed according to the following protocol:

Six million cells, previously cleansed of spontaneous background mutations, were suspended in 6 ml of cell culture medium and added to each serum bottle. An additional four ml of culture medium were added to the non-activated series and four ml of S-9 mix were added to the activation series. The bottles were gassed with 5% CO_2 in air and tightly stoppered.

In order for the bottles to accommodate the test gas, an equivalent volume was withdrawn from the bottle with a gas syringe prior to dosing. The gas was then introduced and the caps of the serum bottles were wrapped with Parafilm to prevent the escape of test gas.

The positive, solvent and media controls were also dosed in serum bottles, but with a micropipetter rather than a gas syringe.

The treatment bottles were placed in a 37C tissue culture incubator on a rotary shaker set at approximately 100 rpm for a four hour treatment.

At the end of the treatment period, each cell suspension was transferred from the bottle to a 50 mm tube, centrifuged at 200xg for ten minutes and the supernate discarded. The cells were then rinsed twice with culture medium with intervening centrifugations at 200xg. Finally, the treated cells were suspended in 20 ml of complete growth medium. The cultures were gassed with 5% CO_2 and reincubated for a two-day expression time. Growth of the cells was monitored at one day postexposure and the cultures adjusted to $.3 \times 10^6$ cells/ml. At the end of the two day expression period, the cultures to be cloned were prepared at 1.0×10^6 cells/ml.

Three dose levels (60%, 40% and 20% v/v) of the test compound with and without metabolic activation were cloned; the high dose (100% v/v)

was discarded due to toxicity. For each cloned dose, 1.0×10^6 cells were plated in each of three selection plates containing trifluorothymidine (TFT), and 200 cells were plated in each of three nonselective (viability) plates for each test and control tube. After 11 days of incubation, colonies in the selection and viability plates were counted.

RESULTS AND DISCUSSION

The toxicity at the 100% dose both in the presence and absence of S-9 activation resulted in an insufficient number of cells; the dose was consequently discarded.

For the three remaining doses (60%, 40% and 20% v/v) the suspension growth results and cloning results are presented in Tables 1 and 2, respectively. In Table 3 the percentage total survival, induced mutation frequency and statistical significance are presented for each dose. As shown, the induced mutation frequencies without activation were not significantly different from the media control. In the presence of S-9 metabolic activation, however, all three doses selected produced a significant increase ($p < .01$) in the mutation frequencies compared to the media control. Significance was calculated for each dose using Student's t-test at a 99% confidence level. As shown in Figure 1, this increase in mutation frequency was dose related.

The positive controls induced a significant increase in the mutation frequency confirming the sensitivity of the test system.

CONCLUSION

Under the conditions of this assay, Butadiene was negative in the absence of metabolic activation and positive in the presence of metabolic activation in the Mouse Lymphoma Forward Mutation Assay and is consequently considered to be mutagenic.

5914W/2016W

ABBREVIATIONS

CON	Contaminated
H_2O	Deionized glass distilled water
DMSO	Dimethylsulfoxide
EMS	Ethyl Methanesulfonate
FOP	Fischer's Medium with Antibiotics, Pluronic F68 and Sodium Pyruvate
F1OP	FOP with 10% Horse Serum
MCA	3-Methylcholanthrene
MF	Mutation Frequency
NR	No Result
T	Toxic dose
TE	Technical Error
TFT	Trifluorothymidine
NA	Not Applicable

All abbreviations may not appear in the text or Tables 1, 2 and 3.

Key to Tables 1, 2 and 3

[a]Total Suspension Growth = Day 1 Counts x $\frac{\text{Day 2 Counts}}{3 \times 10^5}$

Relative Suspension Growth (%) = $\frac{\text{Total Suspension Growth (Treated)}}{\text{Total Suspension Growth (Solvent)}^{b}} \times 100$

Percent Cloning Efficiency = $\frac{\text{Mean No. NS colonies in Treated Dishes}}{\text{Mean No. NS Colonies in Solvent}} \times 100$

Percentage Total Survival =

Relative Suspension Growth X $\frac{\text{Mean No. Colonies NS Treated}}{\text{Mean No. Colonies NS Solvent}}$

Mutation Frequency = $\frac{\text{Mean No. Colonies S Plates}}{\text{Mean No. Colonies NS Plates } (5 \times 10^3)}$

Fold Increase = $\frac{\text{Treated Mutation Frequency}}{\text{Solvent Control Mutation Frequency}}$

[a]When Day 1 cell counts are equal to or less than $.3 \times 10^6$, then the total suspension growth will be the Day 2 cell counts.

[b]Control tubes are calculated individually; mean value of each control is then calculated from these individual values.

REFERENCES

Clive, D., K. O. Johnson, J. F. S. Spector, A. G. Batson, and M. M. M. Brown (1979) Validation and Characterization of the L5178Y/TK+/- Mouse Lymphoma Mutagen Assay System. Mutation Res. 59: 61-108.

Table 1

MOUSE LYMPHOMA ASSAY
Suspension Growth Results
Butadiene

Treatment (% v/v)	S-9	Cell Counts ($\times 10^6$ per/ml) Day 1	Day 2	Total Suspension Growth		Relative Suspension Growth (%)
Cell Culture Medium	-	.84	1.13	3.16	3.47	100
Cell Culture Medium	-	1.00	1.12	3.73		
Cell Culture Medium	-	.96	1.10	3.52		
100.0	-	.24	.21	.21		6.1
100.0	-	.12	.05	.05		1.4
60.0	-	.71	1.03	2.44		70.3
60.0	-	.75	1.00	2.50		72.0
40.0	-	.89	.99	2.94		84.7
40.0	-	.89	.91	2.70		77.8
20.0	-	.92	1.06	3.25		93.7
20.0	-	.88	1.13	3.31		95.4
Cell Culture Medium	+	.68	1.08	2.45	2.33	NA
Cell Culture Medium	+	.66	.98	2.16		
Cell Culture Medium	+	.71	1.01	2.39		
100.0	+	.23	.19	.19		8.2
100.0	+	.15	.16	.16		6.9
60.0	+	.27	.46	.46		19.7
60.0	+	.29	.50	.50		21.5
40.0	+	.45	1.15	1.73		74.2
40.0	+	.41	.87	1.19		51.1
20.0	+	.48	1.11	1.78		76.4
20.0	+	.54	1.13	2.03		87.1

Table 1 (continued)

MOUSE LYMPHOMA ASSAY
Suspension Growth Results
Butadiene

Treatment	S-9	Cell Counts ($\times 10^6$ per/ml) Day 1	Day 2	Total Suspension Growth		Relative Suspension Growth (%)
Positive Controls						
EMS (300 ug/ml)[a]	-	.73	.98	2.38		68.6
EMS (300 ug/ml)	-	.73	.93	2.26		65.1
EMS (300 ug/ml)	-	.74	1.05	2.59		74.6
MCA (3 ug/ml)[b]	+	.37	.83	1.02		43.8
MCA (3 ug/ml)	+	.43	.67	.96		41.2
MCA (3 ug/ml)	+	.28	.73	.73		31.3
Medium Control						
Cell Culture Medium	-	.84	1.13	3.16		
Cell Culture Medium	-	1.00	1.12	3.73	3.47	100
Cell Culture Medium	-	.96	1.10	3.52		
Cell Culture Medium	+	.68	1.08	2.45		
Cell Culture Medium	+	.66	.98	2.16	2.33	NA
Cell Culture Medium	+	.71	1.01	2.39		
Solvent Control For MCA						
DMSO	+	.52	.89	1.54		
DMSO	+	.70	1.08	2.52	2.20	100
DMSO	+	.60	1.27	2.54		

[a]Compared with cell culture medium - S-9
[b]Compared with DMSO + S-9

Table 2

MOUSE LYMPHOMA ASSAY
Cloning Results
Butadiene

Treatment (% v/v)	S-9	Number Colonies Selective Plates (1)	(2)	(3)	Mean	Number Colonies Nonselective Plates (1)	(2)	(3)	Mean		% Cloning Efficiency
Cell Culture Medium	-	48	47	43	46	183	213	266	221		
Cell Culture Medium	-	48	35	47	43	231	224	185	213	218	100
Cell Culture Medium	-	47	53	58	53	198	234	224	219		
100.0	-	. . . . .				Not Cloned, Too Toxic					
100.0	-	. . . . .				Not Cloned, Too Toxic					
60.0	-	57	58	65	60	213	202	202	206		94
60.0	-	44	52	56	51	227	251	215	231		106
40.0	-	47	54	60	54	242	252	229	241		111
40.0	-	54	56	43	51	241	264	267	257		118
20.0	-	48	48	41	46	262	262	255	260		119
20.0	-	48	53	40	47	190	171	188	183		84
Cell Culture Medium	+	34	56	47	46	354	383	365	367		
Cell Culture Medium	+	55	54	52	54	338	319	312	323	326	100
Cell Culture Medium	+	53	55	47	52	247	304	315	289		
100.0	+	. . . . .				Not Cloned, Too Toxic					
100.0	+	. . . . .				Not Cloned, Too Toxic					
60.0	+	110	125	133	123	158	118	112	129		40
60.0	+	96	112	105	104	137	137	122	132		40
40.0	+	109	110	87	102	223	228	200	217		67
40.0	+	94	100	110	101	237	256	254	249		76
20.0	+	99	85	109	98	196	251	217	221		68
20.0	+	83	86	83	84	215	251	242	236		72

Table 2 (continued)

MOUSE LYMPHOMA ASSAY
Cloning Results
Butadiene

Treatment	S-9	Number Colonies Selective Plates (1)	(2)	(3)	Mean	Number Colonies Nonselective Plates (1)	(2)	(3)	Mean		% Cloning Efficiency
Positive Controls											
EMS (300 ug/ml)[a]	-	392	366	365	374	165	160	174	166		76
EMS (300 ug/ml)	-	359	341	356	352	150	144	159	151		69
EMS (300 ug/ml)	-	318	319	334	324	143	163	176	161		74
MCA (3 ug/ml)[b]	+	211	228	238	226	139	113	107	120		46
MCA (3 ug/ml)	+	229	196	230	218	96	56	84	79		30
MCA (3 ug/ml)	+	248	249	253	250	110	131	139	127		49
Media Control											
Cell Culture Medium	-	48	47	43	46	183	213	266	221		
Cell Culture Medium	-	48	35	47	43	231	224	185	213	218	100
Cell Culture Medium	-	47	53	58	53	198	234	224	219		
Cell Culture Medium	+	34	56	47	46	354	383	365	367		
Cell Culture Medium	+	55	54	52	54	338	319	312	323	326	100
Cell Culture Medium	+	53	55	47	52	247	304	315	289		
Solvent Control for MCA											
DMSO	+	59	46	47	51	265	306	302	291		
DMSO	+	59	55	45	53	170	221	273	221	221	100
DMSO	+	45	49	54	51	289	275	251	272		

[a] Compared with cell culture medium - S9
[b] Compared with DMSO + S9

Table 3

MOUSE LYMPHOMA ASSAY
Summary of Mutation Frequencies
Butadiene

Treatment (% v/v)	S-9	Percentage Total Survival	Mutation Frequency ($\times 10^{-5}$)		Fold Increase	Significance[a]
Solvent Control (FOP)	-		4.2			
Solvent Control (FOP)	-	100.0	4.0	4.3	1.0	NA
Solvent Control (FOP)	-		4.8			
60.0	-	66.1	5.8		1.3	
60.0	-	76.3	4.4		1.0	NS
40.0	-	94.0	4.5		1.0	
40.0	-	91.8	4.0		.9	NS
20.0	-	111.5	3.5		.8	
20.0	-	80.4	5.1		1.2	NS
Solvent Control (FOP)	+		2.5			
Solvent Control (FOP)	+	100.0	3.3	3.1	1.0	NA
Solvent Control (FOP)	+		3.6			
60.0	+	7.9	19.1		6.2	S
60.0	+	8.6	15.8		5.1	p=.0001
40.0	+	49.4	9.4		3.0	S
40.0	+	38.8	8.1		2.6	p=.0008
20.0	+	52.0	8.9		2.9	S
20.0	+	62.7	7.1		2.3	p=.0013

[a]S = Significant; $p < .01$; calculations are performed with transformed data using Student's t-test; p-value is recorded if significance is achieved.

NS = Not Significant

Table 3 (continued)

MOUSE LYMPHOMA ASSAY
Summary of Mutation Frequencies
Butadiene

Treatment	S-9	Percentage Total Survival	Mutation Frequency		Fold Increase	Significance
Positive Controls						
EMS (300 ug/ml)[a]	-	52.4	45.1		10.5	
EMS (300 ug/ml)	-	44.9	46.6		10.8	S
EMS (300 ug/ml)	-	55.2	40.2		9.3	p<.0001
MCA (3 ug/ml)[b]	+	21.3	37.7		9.4	
MCA (3 ug/ml)	+	13.1	55.2		13.8	S
MCA (3 ug/ml)	+	16.3	39.4		9.9	p=.0001
Medium Control						
Cell Culture Medium	-		4.2			
Cell Culture Medium	-	100.0	4.0	4.3	1.0	NA
Cell Culture Medium	-		1.6			
Cell Culture Medium	+	NA	2.5		NA	
Cell Culture Medium	+	NA	3.3		NA	
Cell Culture Medium	+	NA	3.6		NA	NA
Solvent Control For MCA						
DMSO	+		3.5			
DMSO	+	100.0	4.8	4.0	1.0	NA
DMSO	+		3.6			

[a]Compared with cell culture medium - S-9
[b]Compared with DMSO + S-9

Figure 1a

PROGRAM VERSION 3 REV. 4 RUN DATE: / / 0

MOUSE LYMPHOMA;CMPD 546 ; WITH ACTIVATION @
STATISTICAL ANALYSIS OF MUTAGENICITY

DOSE	AVERAGE	T	PROB	MUTATION FREQUENCY
.000	3.4330			31.0
20.000	4.3757	6.48	.0013	79.5
40.000	4.4689	7.12	.0008	87.3
60.000	5.1574	11.85	.0001	173.7

T IS STUDENT'S T-STATISTIC FOR THE COMPARISON OF EACH DOSE LEVEL TO THE NEGATIVE CONTROL (DOSE = 0).

DOSE-RESPONSE ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F RATIO	PROB
TOTAL	8	3.9037			
TRIAL	0	.0000			
DOSE	3	3.7766			
LINEAR	1	3.5219	3.5219	138.51	.0001
QUADRATIC	1	.0424	.0424	1.67	.2530
HIGHER ORDER	1	.2123	.2123	8.35	.0342
RESIDUAL	5	.1271	.0254		

DOSE x TRIAL INTERACTION

F RATIO	DEGREES OF FREEDOM	PROBABILITY
	0 AND 5	

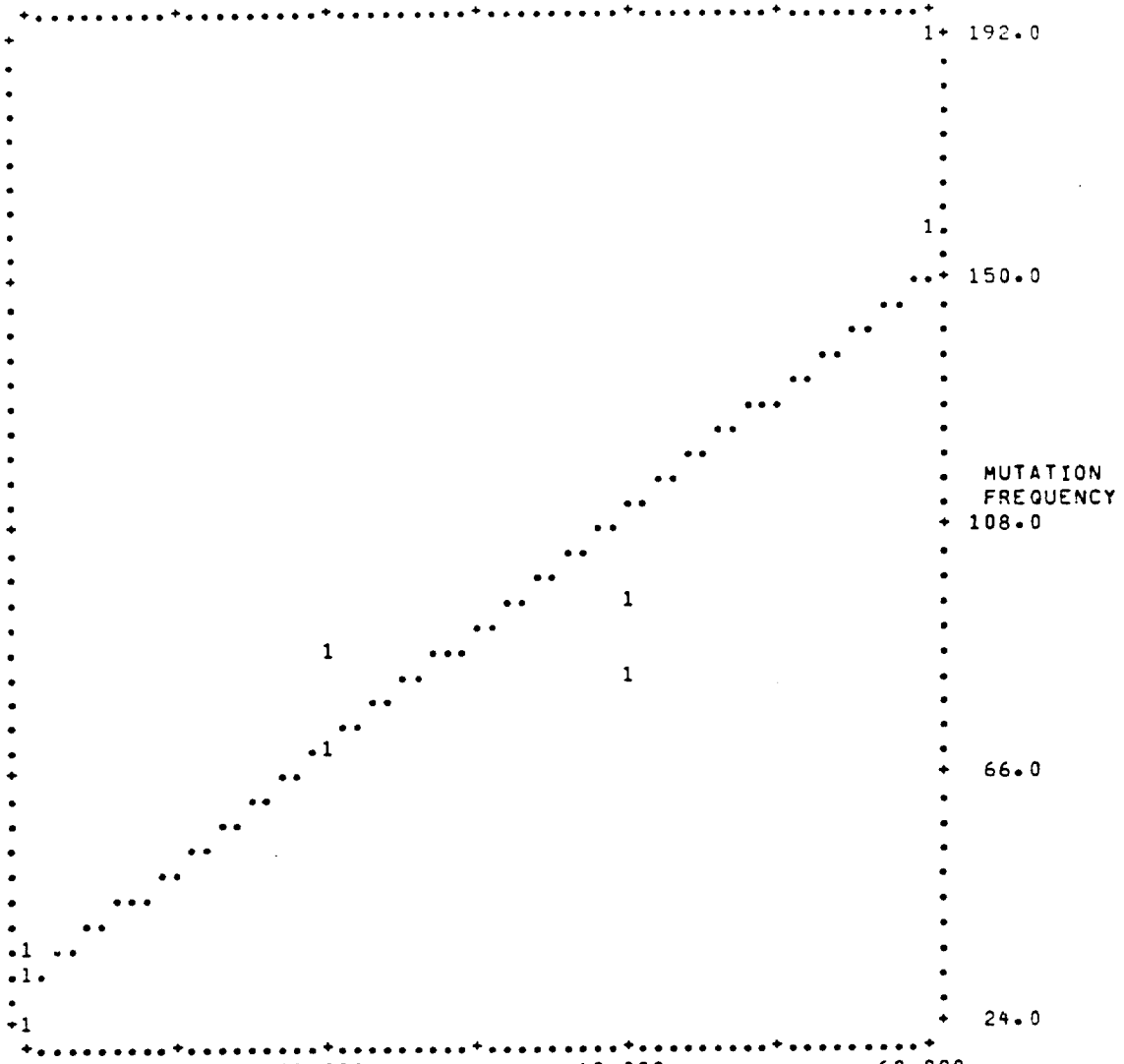
A SIGNIFICANT DOSE-TRIAL INTERACTION INDICATES THAT THE DOSE-RESPONSE RELATIONSHIP IS DIFFERENT IN THE DIFFERENT TRIALS . THE DOSE-RESPONSE ANOVA ABOVE ASSUMES THAT THIS INTERACTION IS NOT STATISTICALLY SIGNIFICANT.

Figure 1b

PROGRAM VERSION 3 REV. 4 RUN DATE: / / 0

MOUSE LYMPHOMA;CMPD 546 ; WITH ACTIVATION @

NPTS= 9 R= .9383



DOSE-RESPONSE RELATIONSHIP		SLOPE	CONFIDENCE LIMITS	
			LOWER	UPPER
INTERCEPT = 31.10				
SLOPE = 1.9971				
LACK OF FIT	LINEAR		1.5040	2.4901
	NONLINEAR		1.3723	2.8009

F= 3.55 PROBABILITY= .1097

DEGREES OF FREEDOM = 2, 5

CONVERGENCE GAMMA-EPSILON TEST 3 ITERATIONS

Triage of 8(e) Submissions

Date sent to triage: 2/5/96

NON-CAP CAP

Submission number: 12550 A

TSCA Inventory Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only

entire document: 0 1 2 **pages** 1 **pages** 1,2, tabs

Notes:

Contractor reviewer : LPS **Date:** 5/11/95

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 0992 -12550 SEQ. A

TYPE: (INT.) SUPP FLWP

SUBMITTER NAME: Phillips Petroleum Company

SUB. DATE: 08/24/92 OTS DATE: 09/02/92 CSRAD DATE: 03/21/95

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/UNDERWAY
0403 NOTIFICATION OF WORKERS/USERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

CHEMICAL NAME:

CAS#
106-99-0

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
(0204)	MUTA (IN VITRO)	01 (02) 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
0243	CHEM/PHYS PROP	01 02 04
(0244)	CLASTO (IN VITRO)	01 (02) 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA:

NON-CBI INVENTORY
(YES)
CAS SR NO
IN TERMINI

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER

SPECIES
In Vitro

TOXICOLOGICAL CONCERN:
LOW
(MED)
HIGH

USE:

PRODUCTION:

COMMENTS

9) 8EHQ-92-12550: Rank - medium.

Chemical: butadiene (CAS# 106-99-0).

Salmonella typhimurium/Mammalian Microsomal Microfluctuation Assay with Compound Butadiene (LPG), Hazelton Laboratories America, Inc., Vienna VA, dated August 23, 1984: Negative for gene mutations in Salmonella typhimurium in strain TA98 with but not without metabolic activation, negative in strains TA100, TA1535 and TA1537 both without and with activation.

L5178Y mouse lymphoma forward mutation assay, Hazelton Laboratories America, Inc., Vienna VA, dated March 7, 1985: Positive for gene mutations in L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay in vitro with but not without metabolic activation.

In vitro Sister Chromatid Exchange in Chinese Hamster Ovary Cells, Hazelton Laboratories America, Inc., Vienna VA, dated January 29, 1985: Induces DNA effects in the form of sister chromatid exchanges (SCEs), with dose response, in CHO cells in vitro both without and with metabolic activation.

Correction for 8e 12550 (2 copies)

June 13, 1996

MEMORANDUM

SUBJECT: Mutagenicity Triage of 8(e) Submissions (#92): 8889, 8892, and 9377, and; 12282, 12402, 12527, 12533, 12542, 12550, 12567, 12598 and 13141

FROM: Michael C. Cimino, Ph.D.
Biologist
Metabolism and
Carcinogenesis Section
Health Effects Branch
Health and Environmental
Review Division (7403)

TO: Terry R. O'Bryan
Risk Analysis Branch
Chemical Screening and
Risk Assessment Division (7402)

THRU: Angela E. Auletta, Pd.D.
Branch Chief
Health Effects Section
Health and Environmental
Review Division (7403)

Mutagenicity studies for twelve chemicals in twelve 8e's were subjected to triage evaluation and ranked. The 8e's are reported on the following pages in numerical order.

THE REPORT DATA WERE NOT SUBJECTED TO DETAILED REVIEW.

Section 8(e) Notice-Triage/Review Priority Decision Tracking Sheets were appropriately annotated and remain attached to the original 8e's.

9) 8EHQ-92-12550: Rank - medium.

Chemical: butadiene (CAS# 106-99-0).

Salmonella typhimurium/Mammalian Microsomal Microfluctuation Assay with Compound Butadiene (LPG), Hazelton Laboratories America, Inc., Vienna VA, dated August 23, 1984: Positive for gene mutations in Salmonella typhimurium in strain TA98 with but not without metabolic activation, negative in strains TA100, TA1535 and TA1537 both without and with activation.

L5178Y mouse lymphoma forward mutation assay, Hazelton Laboratories America, Inc., Vienna VA, dated March 7, 1985: Positive for gene mutations in L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay in vitro with but not without metabolic activation.

In vitro Sister Chromatid Exchange in Chinese Hamster Ovary Cells, Hazelton Laboratories America, Inc., Vienna VA, dated January 29, 1985: Induces DNA effects in the form of sister chromatid exchanges (SCEs), with dose response, in CHO cells in vitro both without and with metabolic activation.