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Texaco
EHS Division

February 26, 1996

Document Processing Center
Office of Toxic Substances
Room E-105
U.S. Environmental Protection Agency
401 M. Street, SW
Washington, DC 20460

ORIGINAL

Contains No CBI

Attention: TSCA 8(e) Coordinator

Reference: **TSCA Section 8(e) Notification**
Modified Ames Salmonella/Microsome Mutation Assay
Used Oil Vacuum Distillation Bottoms

Dear Sir/Madam:

Texaco Inc. is submitting, in accordance with Section 8(e) of the Toxic Substances Control Act (TSCA), draft results of a Modified Salmonella/Microsome Mutation Assay (Modified Ames) on a sample of Used Oil Vacuum Distillation Bottoms (CAS# 129893-17-0). This material is manufactured at the Marrero, LA used oil recycling facility operated by Texaco Fuel and Marine Marketing, Inc. and is currently sold as an asphalt extender. In a typical application the extender would comprise no more than 5% of the asphalt mixture.

Modified Ames testing of this sample (Sample #R950006606) indicated a maximum fold increase of 13.8, a Mutagenicity Index (MI) of 7.85, and a Mutagenic Potency Index (MPI) of 22.45. Based on the Texaco Inc. interpretation of TSCA 8(e) reporting requirements for *in vitro* genetic toxicity testing, these draft test results may be considered 8(e) reportable. For comparison, a sample of AC-20 road paving asphalt was also tested and showed a maximum fold increase of 3.74, a MI of 2.22, and a MPI of 5.25.

A copy of the draft report is enclosed. The MSDS for this material is being modified to reflect these test results.

Sincerely,

John A. Del Rey

88960000076

RECEIVED

3/28/96

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MUTAGENICITY TEST WITH

R950006606

IN THE MODIFIED *SALMONELLA*/MICROSOME MUTATION ASSAY FOR PETROLEUM SAMPLES

DRAFT REPORT

AUTHOR

Timothy E. Lawlor, M.A.

PERFORMING LABORATORY

Corning Hazleton Inc. (CHV)
9200 Leesburg Pike
Vienna, Virginia 22182

LABORATORY PROJECT ID

CHV Study No.: 17326-0-427

SUBMITTED TO

Texaco, Inc.
Old Glenham Road
Glenham, NY 12527

STUDY COMPLETION DATE

QUALITY ASSURANCE STATEMENT

PROJECT TITLE: Modified *Salmonella*/Microsome Mutation Assay for Petroleum Samples

STUDY NO.: 17326-0-427

PROTOCOL NO.: 427, Edition 4, Modified for Texaco

Quality Assurance Inspections of the study and review of the final report of the above referenced project were conducted according to the Standard Operating Procedures of the Quality Assurance Unit and according to the general requirements of the appropriate Good Laboratory Practice regulations. Findings from the inspections and final report review were reported to management and to the study director on the following dates:

Inspection - Date	Findings Reported	Auditor
Characterization of Tester Strains - 12/21/95	12/21/95	C. Orantes/C. Smith
Draft Report Review - 01/26/96	01/26/96	S. Ballenger

Quality Assurance Unit Date Released

COMPLIANCE AND CERTIFICATION STATEMENT

The described study was conducted in compliance with the Good Laboratory Practice regulations as set forth in the Code of Federal Regulations (21 CFR 58, EPA-TSCA 40 CFR 792, and 40 CFR 160) and the Organization for Economic Cooperation and Development (OECD), Principles of Good Laboratory Practice C(81)30 (Final) Annex 2, issued 1979-1980 (effective 1981). There were no significant deviations from the aforementioned regulations or the signed protocol that would affect the integrity of the study or the interpretation of the test results. The stability of the test article under the conditions of administration was the responsibility of the Sponsor. The raw data have been reviewed by the Study Director, who certifies that the evaluation of the test article as presented herein represents an appropriate conclusion within the context of the study design and evaluation criteria.

SUBMITTED BY:

Study Director:

Timothy E. Lawlor, M.A. Bacterial Mutagenesis Genetic and Cellular Toxicology	Study Completion Date

For the Sponsor:

	Date

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SECTION I. SUMMARY

SUMMARY

A. Introduction

At the request of Texaco, Inc., Corning Hazleton Inc. investigated a dimethylsulfoxide (DMSO) extract of R950006606 for mutagenic activity in the Modified *Salmonella*/Microsome Mutation Assay For Petroleum Samples. This assay evaluated an extract of the test article and/or its metabolites for their ability to induce reverse mutations at the histidine locus in the genome of *Salmonella typhimurium* tester strain TA98 in the presence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor induced hamster liver (S9).

The assay was conducted in triplicate, using eight doses of test article extract, 60.0, 50.0, 40.0, 30.0, 20.0, 15.0, 10.0, and 5.00 µl per plate, along with vehicle and positive controls, in the presence of S9 mix.

B. Results

The results of the Modified *Salmonella*/Microsome Mutation Assay For Petroleum Samples indicate that under the conditions of this study, Texaco, Inc.'s test article, R950006606, produced a maximum fold-increase of 13.8, a Mutagenicity Index (MI) of 7.85, and a Mutagenic Potency Index (MPI) of 22.45.

SECTION II. STUDY INFORMATION

STUDY INFORMATION

A. Sponsor: Texaco, Inc.

B. Test Article: R950006606

1. Date Received: 11/29/95

2. Physical Description of Test Article: Slightly viscous black liquid

3. Physical Description of Extract: clear brown liquid

C. Type of Assay: Modified *Salmonella*/Microsome Mutation Assay For Petroleum Samples

1. Protocol Number: CHV Protocol 427, Edition 4 Modified for Texaco

2. CHV Study Number: 17326-0-427

D. Study Dates

1. Study Initiation Date: 12/01/95

2. Experimental Start Date: 12/21/95

3. Experimental Termination Date: 12/27/95

E. Study Supervisory Personnel

Study Director: Timothy E. Lawlor, M.A.

Laboratory Supervisor: Michael S. Mecchi, B.S.

SECTION III. MATERIALS AND METHODS

MATERIALS AND METHODS

The experimental materials, methods and procedures are based on those described by Ames *et al* (1975) and Blackburn *et al* (1984).

MATERIALS

A. Tester Strain

The tester strain used was the *Salmonella typhimurium* histidine auxotroph TA98 as described by Ames *et al* (1975).

TABLE I. TESTER STRAIN GENOTYPES

Histidine Mutation	Additional Mutations		
	LPS	Repair	R Factor
*his*D3052			
TA98	*rfa*	*uvr*B	+R

In addition to a mutation in the histidine operon, tester strain TA98 contains two additional mutations which enhance its sensitivity to some mutagenic compounds. The *rfa* wall mutation results in the loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide barrier that forms the surface of the bacterial cell wall. The resulting cell wall deficiency increases permeability to certain classes of chemicals such as those containing large ring systems (e.g., benzo(a)pyrene) that would otherwise be excluded by a normal intact cell wall.

The second mutation, a deletion of the *uvr*B gene, results in a deficient DNA excision repair system which greatly enhances the sensitivity of this strain to some mutagens. Since the *uvr*B deletion extends through the *bio* gene, tester strains containing this deletion also require the vitamin biotin for growth.

Tester strain TA98 also contains the R-factor plasmid, pKM101, which further increases the sensitivity of this strain to some mutagens. The mechanisms by which this plasmid increases sensitivity to mutagens has been suggested to be by modifying an existing bacterial DNA repair polymerase complex involved with the mismatch-repair process.

Tester strain TA98 is reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frame shift mutagens.

1. Source of Tester Strain

Tester strain TA98 in use at CHV was received directly from Dr. Bruce Ames, Department of Biochemistry, University of California, Berkeley.

2. Storage of the Tester Strain

a. Frozen Permanent Stocks

Frozen permanent stocks were prepared by growing a fresh overnight culture, adding DMSO (0.09 ml/ml of culture) and freezing small aliquots (0.5 - 1.5 ml) at ≤ -65°C.

b. Master Plates

A master plate was prepared by streaking tester strain TA98 from a frozen permanent stock onto minimal agar supplemented with histidine (260 μM), biotin (3 μM), and ampicillin (25 μg/ml, for stable maintenance of the plasmid). Tester strain master plates were stored at 5 ± 3°C.

3. Preparation of Culture for Use in the Mutagenicity Assay

Initially, an overnight culture was prepared by transferring a colony from the master plate to a flask containing culture medium. The inoculated flask was incubated at 37 ± 2°C with shaking (shaking, 125 ± 25 rpm) for 16 ± 1 hr. Then, a 2.0 ml aliquot of the overnight (16 ± 1 hr.) culture was used to inoculate 8.0 ml of fresh culture medium. This inoculated flask was placed in a shaker/incubator for 3 ± 1 hr at 125 ± 25 rpm and 37 ± 2°C. The 3 ± 1 hr culture was held at 5 ± 3°C until used in the mutagenicity assay.

4. Confirmation of Tester Strain Genotypes

The tester strain culture was checked for the following genetic markers on the day of its use in the mutagenicity assay:

a. *rfa* Wall Mutation

The presence of the *rfa* wall mutation was confirmed by demonstration of the sensitivity of the culture to crystal violet.

b. pKM101 Plasmid

The presence of the pKM101 plasmid was confirmed for the culture of tester strain TA98 by demonstration of resistance to ampicillin.

c. Spontaneous Reversion

The mean number of spontaneous revertants per plate in the vehicle control that is characteristic of tester strain TA98 was demonstrated by plating aliquots of the culture along with the appropriate vehicle on selective medium.

5. Tester Strain Media

a. Culture Medium

The broth used to grow an overnight culture of the tester strain was Vogel-Bonner salt solution (Vogel and Bonner, 1956) supplemented with 2.5% (w/v) Oxoid Nutrient Broth #2 (dry powder).

b. Minimal Bottom Agar Plates

Bottom agar (25 ml per 15 x 100 mm petri dish) was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956) supplemented with 0.2% (w/v) glucose.

c. Top Agar for Selection of Histidine Revertants

Top (overlay) agar was prepared with 0.7% agar (w/v) and 0.5% NaC1 (w/v) and was supplemented with 10 ml of 0.5 mM histidine/biotin solution per 100 ml agar. A 2.0 ml aliquot of the supplemented top agar was used for the overlay.

B. Liver Microsomal Enzyme Reaction Mixture (S9 Mix)

1. S9 Homogenate

Liver microsomal enzymes (S9 homogenate) were purchased from Molecular Toxicology, Inc., Annapolis, MD 21401, Batch 0627, (42.5 mg of protein per ml). The homogenate was prepared from male Syrian Golden hamsters that had been injected (i.p.) with Aroclor 1254 (200 mg per ml in corn oil) at 500 mg/kg as described by Ames et *al* (1975).

2. S9 Mix

The S9 mix was prepared immediately prior to its use in any experimental procedure. The S9 mix contained the components indicated in Table II.

TABLE II. S9 MIX COMPONENTS	
1M NaH_2PO_4/Na_2HPO_4, pH 7.4	0.10 ml
0.25M Glucose-6-phosphate	0.02 ml
0.10M NADP	0.04 ml
0.825M KCl/0.2M $MgCl_2$	0.04 ml
S9 Homogenate	0.80 ml
	1.00 ml

C. **Controls**

1. Vehicle Controls

A dimethylsulfoxide (DMSO, CAS #67-68-5) vehicle control was plated for tester strain TA98 in the presence of S9 mix. The vehicle control was plated, using a 60 µl aliquot of vehicle (equal to the maximum aliquot of test article extract plated), along with a 100 µl aliquot of tester strain TA98 and a 500 µl aliquot of S9 mix, on selective agar.

2. Positive Controls

a. Positive Control Oil

Concurrently with the test article extract, an extract of a positive control oil was tested. The positive control oil extract was prepared from a petroleum sample which has been demonstrated to produce a positive response in this assay. The positive control oil was extracted and plated in the same manner as the test article.

b. Positive Control Article

The positive control article plated concurrently with the test article extract was benzo(a)pyrene at 10.0 µg per plate.

3. Sterility Controls

a. Test Article Extract

The most concentrated test article extract dilution was checked for sterility by plating an aliquot of the same volume used in the assay on selective agar.

b. S9 Mix

The S9 mix was checked for sterility by plating 0.5 ml on selective agar.

METHODS

A. Mutagenicity Assay Design

An extract of the test article was tested at eight doses along with vehicle and positive controls on tester strain TA98 in the presence of an Aroclor induced 80% hamster liver S9 mix, as described by Blackburn *et al* (1984).

1. Frequency and Route of Administration

The test system was exposed to the test article via the preincubation modification of the Ames Test originally described by Yahagi *et al* (1975) and Maron and Ames (1983), and further modified for petroleum samples by Blackburn *et al* (1984). All doses of test article extract, vehicle controls and positive controls were plated in triplicate.

2. Dose Selection

The doses used for the mutagenicity assay were 60.0, 50.0, 40.0, 30.0, 20.0, 15.0, 10.0, and 5.00 µl of test article extract per plate.

B. Procedures

1. Extraction Procedures

2.0 ml of the test article was added to a centrifuge tube containing 10 ml of DMSO. The extraction mixture was vortexed for 30 seconds every 5 minutes for a period of 30 minutes. The mixture was placed on an orbital shaker (room temperature, 150 rpm) between

vortex mixings. The mixture was then centrifuged for 10 minutes at 200 x g and the DMSO extract was removed and saved for testing. The extract was placed in an amber container and stored at 5 ±3 °C until used in the assay. The positive control oil was extracted in the same manner as the test article.

2. Mutagenicity Assay Plating Procedures

Each plate was labeled with a code which identified the test article, test phase, tester strain, and dose.

The test article extract was diluted and the doses of the extract (delivered in 60 µl aliquots) were added to glass test tubes at room temperature. Then, a 500 µl aliquot of S9 mix was added, followed by a 100 µl aliquot of a culture of tester strain TA98. Once all components had been added to the tubes, the tubes were incubated for 20 ± 2 minutes at 37 ± 2 °C with shaking. Following the incubation period, 2.0 ml of molten, supplemented top agar was added to each tube. The contents of the tube were then vortexed, and the mixture was overlaid onto the surface of 25 ml of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for 48 ± 8 hours at 37 ± 2 °C. The positive control oil extract was plated in the same manner as the test article extract. The positive control article was plated in the same manner as the test article except a 50 µl plating aliquot was used.

C. Scoring the Plates

Plates which were not evaluated immediately following the incubation period were held at 5 ± 3 °C until such time that colony counting and bacterial background lawn evaluation could take place.

1. Bacterial Background Lawn Evaluation

The condition of the bacterial background lawn was evaluated for evidence of test article extract toxicity and/or precipitate. Evidence of toxicity was scored relative to the vehicle control plate and recorded along with the revertant counts for all plates at that dose.

2. Counting Revertant Colonies

For the mutagenicity assay, revertant colonies from extract-treated and vehicle control-treated plates were counted entirely by hand. Positive control article-treated plates were counted either by hand or by automated colony counter.

3. Analysis of the Data

For all replicate platings, the mean revertants per plate and the standard deviation were calculated.

The data were analyzed using the robust nonlinear regression analysis published by Myers *et al* (1981) and used by Blackburn *et al* (1984) to assign a Mutagenicity Index (MI) to the observed response. This Mutagenicity Index is the slope value of the curve generated from the nonlinear regression analysis.

The data were also analyzed using linear regression analysis to assign a Mutagenicity Potency Index (MPI) to the observed response as described by Skisak *et al* (1987). This Mutagenicity Potency Index is the slope value derived from the linear regression analysis.

EVALUATION OF TEST RESULTS

Before assay data were evaluated, the criteria for a valid assay had to be met.

A. Criteria for a Valid Assay

The following criteria were used to determine a valid assay:

1. Tester Strain Integrity

a. *rfa* Wall Mutation

To demonstrate the presence of the *rfa* wall mutation, the tester strain culture exhibited sensitivity to crystal violet.

b. pKM101 Plasmid

To demonstrate the presence of the R-factor plasmid, pKM101, the tester strain culture exhibited resistance to ampicillin.

c. Characteristic Number of Spontaneous Revertants

To demonstrate the requirement for histidine, the tester strain culture exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. The acceptable range for the mean vehicle control was 20 - 55 revertants per plate.

d. Positive Control Values

To demonstrate that the S9 mix was capable of metabolizing a promutagen to its mutagenic form(s) and that the test system can detect a positive petroleum sample, the mean value of each positive control for tester strain TA98 in the presence of the S9 mix exhibited at least a three-fold increase over the mean value of the vehicle control.

2. Cytotoxicity

A minimum of three non-toxic doses were required to evaluate assay data.

RECORDS TO BE MAINTAINED

All raw data, documentation, records, the protocol, and the final report generated as a result of this study will be archived in the storage facilities of Corning Hazleton Inc., for at least one year following submission of the final report to the Sponsor. After the one year period, the Sponsor may elect to have the aforementioned materials retained in the storage facilities of Corning Hazleton Inc., for an additional period of time or sent to a storage facility designated by the Sponsor.

REFERENCES

Ames, B.N., J. McCann, and E. Yamasaki (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Research 31:347-364.

Blackburn, G.R., R.A. Deitch, C.A. Schreiner, M.A. Mehlman, and C.R. Mackerer (1984). Estimation of the dermal carcinogenic activity of petroleum fractions using a modified Ames assay. Cell Biology and Toxicology 1:40-48.

Maron, D.M., and B. Ames (1983). Revised methods for the *Salmonella* mutagenicity test. Mutation Research 113:173-215.

Myers, L.E., N.H. Sexton, L.I. Southerland, and T.J.Wolff (1981). Regression analysis of Ames test data. Environmental Mutagenesis 3:575-586.

Skisak, C.M., C. B. Venier, and D.O. Baker (1987). Ames tests of lubricating oil products: the mutagenic potency index. In Vitro Toxicology 1:263-276.

Yahagi, T., M. Degawa, Y. Seino, T. Matsushima, M. Nagao, T. Sugimura, and Y. Hashimoto (1975). Mutagenicity of carcinogenic azo dyes and their derivatives. Cancer Letters 1:91-96.

Vogel, H. J. and D. M. Bonner (1956). Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol Chem. 218:97-106.

BACTERIAL BACKGROUND LAWN EVALUATION CODE

The condition of the background bacterial lawn is evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate as follows:

CODE	DEFINITION	CHARACTERISTICS OF BACKGROUND LAWN
1	Normal	A healthy microcolony lawn.
2	Slightly Reduced	A noticeable thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	A marked thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	An extreme thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
5	Absent	A complete lack of any microcolony lawn.
6	Obscured by Precipitate	The background bacterial lawn cannot be accurately evaluated due to microscopic and/or macroscopic test article precipitate.

Evidence of macroscopic test article precipitate on the plates is recorded by addition of the following precipitate code to the code number used to evaluate the condition of the background bacterial lawn.

sp	Slight Precipitate	Noticeable macroscopic precipitate on the plate, however, the precipitate does not influence automated counting of the plate.
mp	Moderate Precipitate	The amount of macroscopic precipitate on the plate would interfere with automated counting, thus requiring the plate to be hand counted.
hp	Heavy Precipitate	The large amount of macroscopic precipitate on the plate makes the required hand counting difficult.

Example: 4mp would indicate a plate observed to have an extremely reduced background lawn which had to be counted manually due to the marked amount of macroscopic test article precipitate.

SECTION IV. RESULTS

RESULTS

A. Mutagenicity Assay

The test article was extracted with dimethylsulfoxide (DMSO, CAS# 67-68-5, Aldrich Chemical Company, Lot 10039KN, 99.9%). The test article extract was tested at eight doses, 60.0, 50.0, 40.0, 30.0, 20.0, 15.0, 10.0 and 5.00 µl per plate, three plates per dose, in the presence of hamster liver microsomal enzymes (S9 mix), along with the vehicle and positive controls. The mutagenicity assay results for R950006606 are presented in Table 1. The mutagenicity assay results for the positive control oil are presented in Table 2. These data were generated in Experiment 17326-B1. The data are presented as mean revertants per plate with standard deviation for each treatment and control group and as individual plate counts.

In Experiment 17326-B1, all data were acceptable and the maximum fold-increase in the number of TA98 revertants per plate was 13.8-fold. A Mutagenicity Index (MI) of 7.85 and a Mutagenic Potency Index (MPI) of 24.45 were calculated for this response.

For positive control oil, a maximum fold-increase of 4.19 was observed producing an MI value of 4.52 and an MPI value of 9.91.

All criteria for a valid study were met.

B. Results

The results of the Modified *Salmonella*/Microsome Mutation Assay For Petroleum Samples indicate that under the conditions of this study, Texaco, Inc.'s test article, R950006606, produced a maximum fold-increase of 13.8, a Mutagenicity Index (MI) of 7.85, and a Mutagenic Potency Index (MPI) of 22.45.

SECTION V. DATA TABLES

TABLE 1

TEST RESULTS

MODIFIED *SALMONELLA*/MICROSOME MUTATION ASSAY

EXPERIMENT ID: 17326-B1

TEST ARTICLE ID: R950006606

DATE PLATED: 21-Dec-95

VEHICLE: DMSO

DATE COUNTED: 27-Dec-95

PLATING ALIQUOT: 60 µl

DOSE/PLATE		REVERTANTS PER PLATE TA98			MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION TA98		BACKGROUND LAWN*
		1	2	3	MEAN	S.D.	
MICROSOMES: Hamster Liver							
VEHICLE CONTROL		29	30	47	35	10	1
TEST ARTICLE	5.0 µl	71	75	83	76	6	1
	10 µl	110	114	128	117	9	1
	15 µl	108	133	143	128	18	1
	20 µl	156	197	206	186	27	1
	30 µl	243	277	288	269	23	1
	40 µl	375	377	396	383	12	1
	50 µl	397	445	457	433	32	1
	60 µl	440	481	530	484	45	1
POSITIVE CONTROL		503	537	565	535	31	1

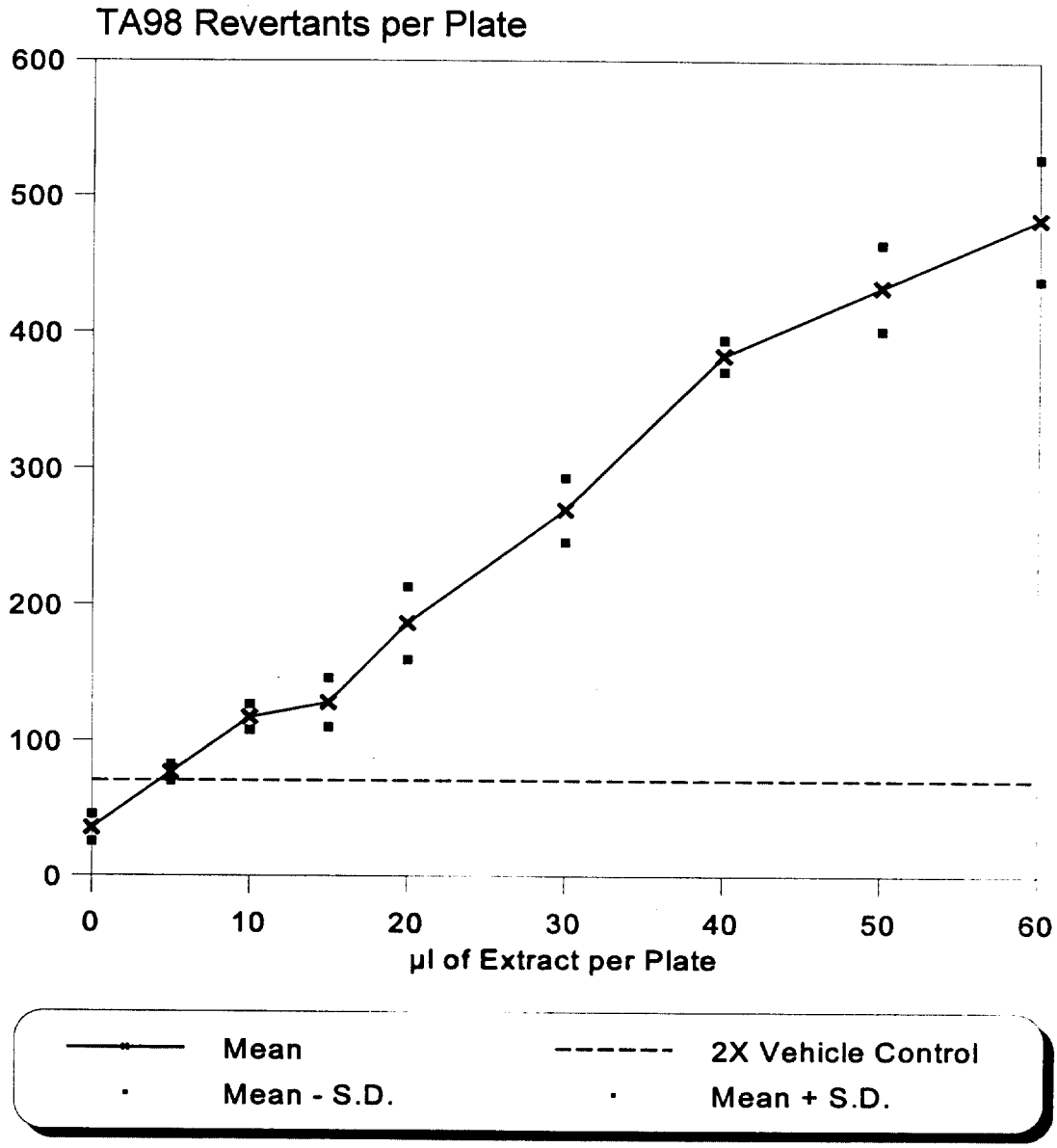
** TA98 benzo[a]pyrene 10 µg/plate

* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

Results Summary

Maximum Fold-Increase: 13.80
Mutagenicity Index (MI): 7.85
Mutagenic Potency Index (MPI): 24.24

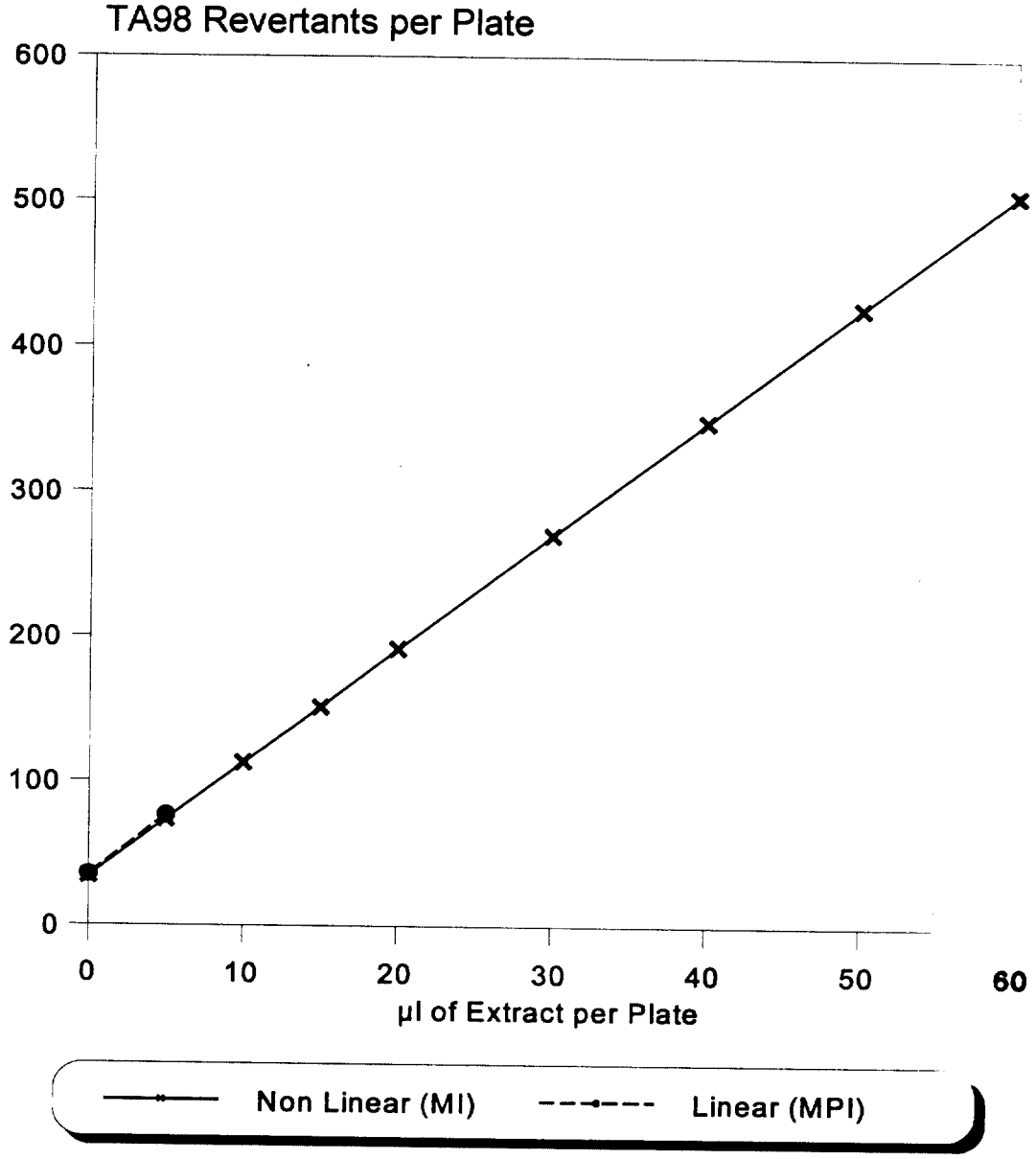
RESULTS OF THE MODIFIED SALMONELLA ASSAY
R950006606
DMSO EXTRACT



RESULTS OF THE MODIFIED SALMONELLA ASSAY

R950006606

PREDICTED VALUES (yhat)



TEST ARTICLE: R950006606 Experiment 17326-B1

Non-Linear Least Squares Iterative Phase
Dependent Variable Y Method: Gauss-Newton

Iter	B	S	T	Weighted SS
0	50.000000	2.000000	0.001000	354.685460
1	49.214594	2.275185	0	303.519172
2	35.824387	7.448345	0	24.421254
3	34.531313	7.824294	0	20.870876
4	34.196407	7.850116	0	20.776974
5	34.114363	7.853699	0	20.769958
6	34.094279	7.854457	0	20.768956
7	34.089369	7.854636	0	20.768747
8	34.088170	7.854680	0	20.768697
9	34.087877	7.854691	0	20.768685
10	34.087805	7.854693	0	20.768682
11	34.087788	7.854694	0	20.768681
12	34.087783	7.854694	0	20.768681

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable Y

Source	DF	Weighted SS	Weighted MS
Regression	3	1804.2003066	601.4001022
Residual	24	20.7686812	0.8653617
Uncorrected Total	27	1824.9689878	
(Corrected Total)	26	956.8676764	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
B	34.08778348	3.0892957378	27.711841792	40.463725162
**S	7.85469415	0.6390011876	6.535871140	9.173517164
T	0.00000000	0.0016649307	-0.003436220	0.003436220

Asymptotic Correlation Matrix

Corr	B	S	T
B	1	-0.52840589	-0.391688749
S	-0.52840589	1	0.9275349028
T	-0.391688749	0.9275349028	1

**S = Slope = Mutagenicity Index

TEST ARTICLE: R950006606

Experiment 17326-B1

Non-Linear Predicted Values

OBS	X	Y	YHAT
1	0	29	34.088
2	0	30	34.088
3	0	47	34.088
4	5	71	73.361
5	5	75	73.361
6	5	83	73.361
7	10	110	112.635
8	10	114	112.635
9	10	128	112.635
10	15	108	151.908
11	15	133	151.908
12	15	143	151.908
13	20	156	191.182
14	20	197	191.182
15	20	206	191.182
16	30	243	269.729
17	30	277	269.729
18	30	288	269.729
19	40	375	348.276
20	40	377	348.276
21	40	396	348.276
22	50	397	426.822
23	50	445	426.822
24	50	457	426.822
25	60	440	505.369
26	60	481	505.369
27	60	530	505.369

TEST ARTICLE: R950006606 Experiment 17326-B1

Linear Regression Model
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	2521.50000	2521.50000	36.107	0.0039
Error	4	279.33333	69.83333		
C Total	5	2800.83333			

Root MSE	8.35663	R-square	0.9003
Dep Mean	55.83333	Adj R-sq	0.8753
C.V.	14.96711		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEPT	1	35.333333	4.82470494	7.323	0.0018
SLOPE	1	8.200000	1.36463263	6.009	0.0039

OBS	X	Y	ZHAT
1	0	29	35.3333
2	0	30	35.3333
3	0	47	35.3333
4	5	71	76.3333
5	5	75	76.3333
6	5	83	76.3333

TABLE 2

TEST RESULTS

MODIFIED *SALMONELLA*/MICROSOME MUTATION ASSAY

EXPERIMENT ID: 17326-B1

DATE PLATED: 21-Dec-95

DATE COUNTED: 26-Dec-95

TEST ARTICLE ID: Positive Control Oil

VEHICLE: DMSO

PLATING ALIQUOT: 60 µl

DOSE/PLATE		REVERTANTS PER PLATE TA98			MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION TA98		BACKGROUND LAWN*
		1	2	3	MEAN	S.D.	
MICROSOMES: Hamster Liver							
VEHICLE CONTROL		37	37	35	36	1	1
TEST ARTICLE	5.0 µl	48	64	66	59	10	1
	10 µl	92	66	90	83	14	1
	15 µl	84	91	76	84	8	1
	20 µl	98	127	84	103	22	1
	30 µl	111	103	118	111	8	1
	40 µl	152	130	119	134	17	1
	50 µl	166	126	156	149	21	1
	60 µl	155	152	146	151	5	1
POSITIVE CONTROL		503	436	529	489	48	1

** TA98 benzo[a]pyrene 10 µg/plate

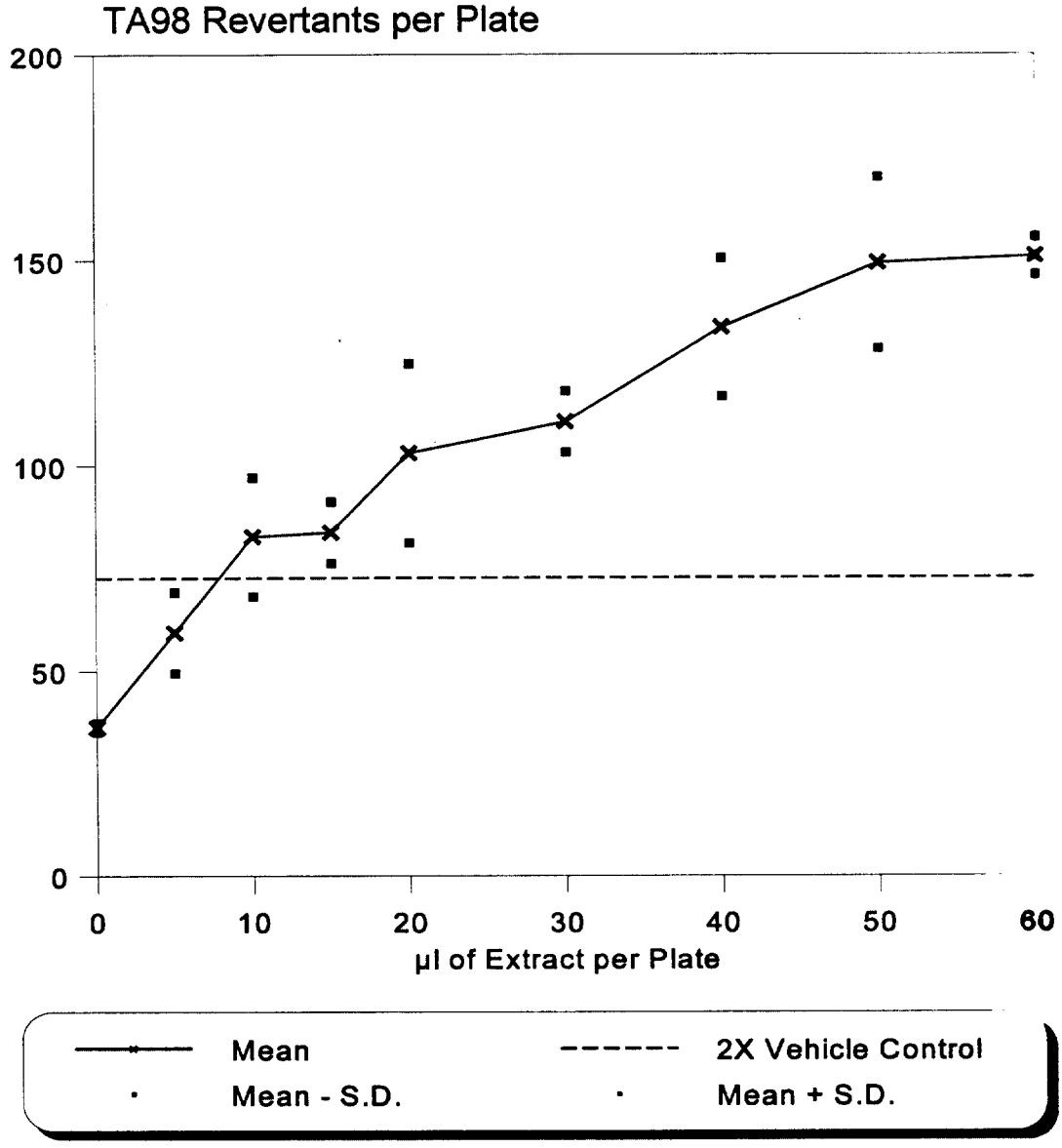
* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

Results Summary

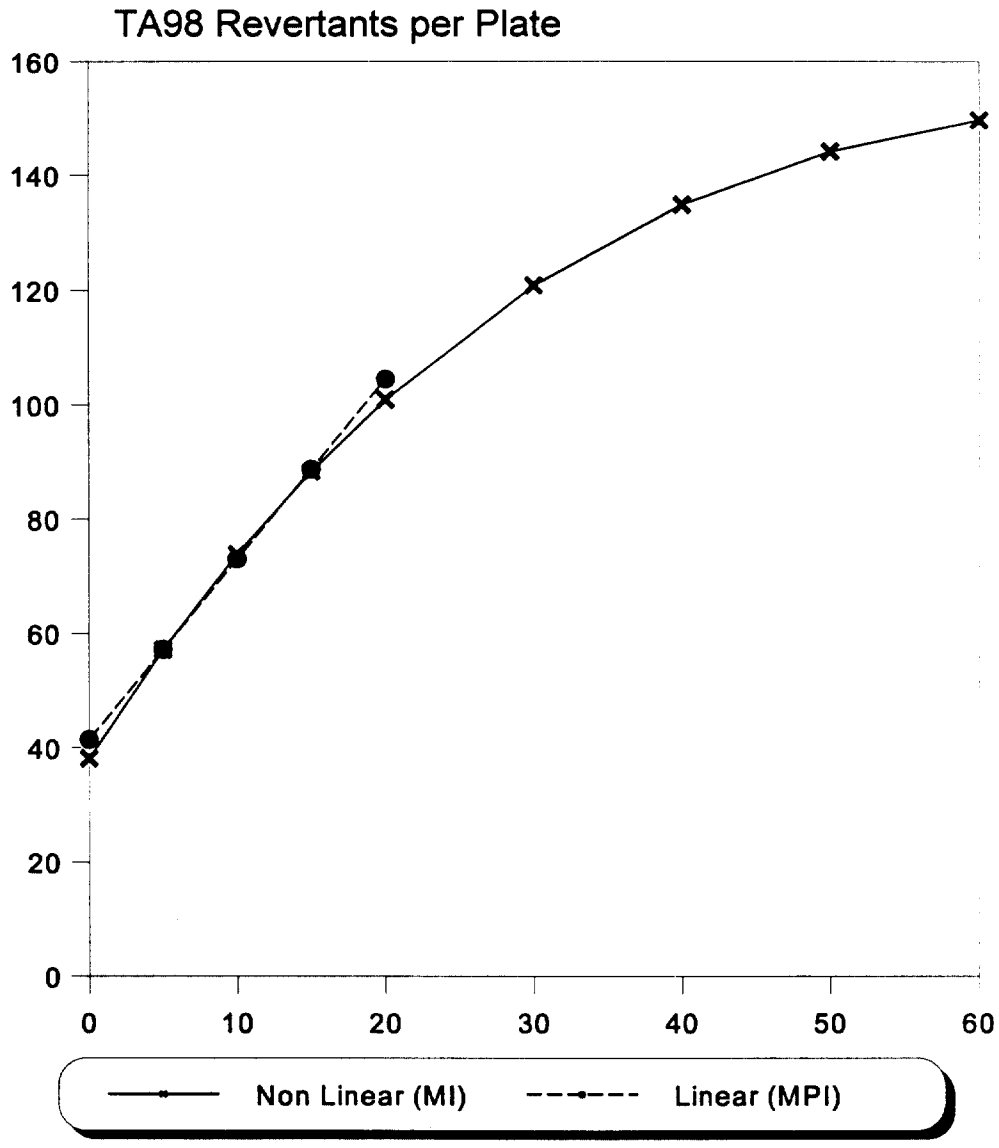
Maximum Fold-Increase: 4.19
Mutagenicity Index (MI): 4.52
Mutagenic Potency Index (MPI): 9.91

RESULTS OF THE MODIFIED SALMONELLA ASSAY

DMSO EXTRACT OF THE POSITIVE CONTROL OIL



RESULTS OF THE MODIFIED SALMONELLA ASSAY DMSO EXTRACT OF THE POSITIVE CONTROL OIL PREDICTED VALUES (yhat)



TEST ARTICLE: Positive Control Oil Experiment 17326-B1

Non-Linear Least Squares Iterative Phase
Dependent Variable Y Method: Gauss-Newton

Iter	B	S	T	Weighted SS
0	50.000000	2.000000	0.001000	30.459327
1	40.217956	4.119956	0.013526	31.320390
2	38.411078	4.347134	0.011500	20.013471
3	38.177687	4.480289	0.011962	20.055407
4	38.141504	4.512216	0.012075	20.080591
5	38.134150	4.520194	0.012104	20.081421
6	38.131703	4.521681	0.012110	20.081640
7	38.131201	4.521951	0.012111	20.081684
8	38.131107	4.521999	0.012111	20.081693
9	38.131090	4.522008	0.012111	20.081694
10	38.131087	4.522010	0.012111	20.081694
11	38.131087	4.522010	0.012111	20.081694

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable Y

Source	DF	Weighted SS	Weighted MS
Regression	3	1375.1230446	458.3743482
Residual	24	20.0816945	0.8367373
Uncorrected Total	27	1395.2047391	
(Corrected Total)	26	291.8616777	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
B	38.13108682	3.0445406790	31.847514291	44.414659345
**S	4.52200998	0.5645265562	3.356893815	5.687126145
T	0.01211088	0.0022983200	0.007367417	0.016854339

Asymptotic Correlation Matrix

Corr	B	S	T
B	1	-0.596087965	-0.457285711
S	-0.596087965	1	0.9419423261
T	-0.457285711	0.9419423261	1

**S = Slope = Mutagenicity Index

TEST ARTICLE: Positive Control Oil

Experiment 17326-B1

Non-Linear Predicted Values

OBS	X	Y	YHAT
1	0	37	38.131
2	0	37	38.131
3	0	35	38.131
4	5	48	57.172
5	5	64	57.172
6	5	66	57.172
7	10	92	73.844
8	10	66	73.844
9	10	90	73.844
10	15	84	88.359
11	15	91	88.359
12	15	76	88.359
13	20	98	100.914
14	20	127	100.914
15	20	84	100.914
16	30	111	120.847
17	30	103	120.847
18	30	118	120.847
19	40	152	134.921
20	40	130	134.921
21	40	119	134.921
22	50	166	144.212
23	50	126	144.212
24	50	156	144.212
25	60	155	149.628
26	60	152	149.628
27	60	146	149.628

TEST ARTICLE: Positive Control Oil Experiment 17326-B1

Linear Regression Model

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	7457.63333	7457.63333	45.127	0.0001
Error	13	2148.36667	165.25897		
C Total	14	9606.00000			

Root MSE	12.85531	R-square	0.7764
Dep Mean	73.00000	Adj R-sq	0.7591
C.V.	17.61001		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEPT	1	41.466667	5.74906904	7.213	0.0001
SLOPE	1	3.153333	0.46940952	6.718	0.0001

OBS	X	Y	ZHAT
1	0	37	41.467
2	0	37	41.467
3	0	35	41.467
4	5	48	57.233
5	5	64	57.233
6	5	66	57.233
7	10	92	73.000
8	10	66	73.000
9	10	90	73.000
10	15	84	88.767
11	15	91	88.767
12	15	76	88.767
13	20	98	104.533
14	20	127	104.533
15	20	84	104.533