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MANAGER

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ATTN: Section 8(e) Coordinator  
Office of Toxic Substances  
US Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460



0010348395

RE: Follow-up Information on 8E HQ-0990-1061

Dear Sir:

Enclosed is a final report, "In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method".

Very truly yours,

*H. L. Hunter, Jr.*  
H. L. Hunter, Jr.

HLH:skm  
Attachment

E: HS031

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# **EXXON** BIOMEDICAL SCIENCES, INC.

FINAL REPORT

PROJECT NUMBER: 181430

TEST MATERIAL: MRD-90-814

IN VIVO MAMMALIAN BONE MARROW  
MICRONUCLEUS ASSAY - INHALATION DOSING METHOD

PERFORMED AT:

EXXON BIOMEDICAL SCIENCES, INC.  
Toxicology Laboratory  
Mettlers Road  
CN 2350  
East Millstone, New Jersey 08875-2350

COMPLETION DATE: January 10, 1991

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11-Jan-91  
Date

I hereby declare that to the best of my knowledge, this study was conducted in accordance with the EPA Good Laboratory Practice Regulations set forth in 40 CFR Part 792 except where noted within this report.

Robert T. Przygoda  
R. T. Przygoda, Ph.D.  
Study Director

11 JAN 91  
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EXXON BIOMEDICAL SCIENCES, INC.  
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**QUALITY ASSURANCE STATEMENT**

**NON-REGULATORY STUDY**

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**STUDY NUMBER: 181430**

**TEST SUBSTANCE/ARTICLE: MRD-90-814**

**STUDY SPONSOR: Exxon Corporation**

**All QA audits (including this final report) have been processed.**

  
\_\_\_\_\_  
R. T. Plutnick, B.A.  
Group Head, Quality Assurance

10-JAN-91  
Date

IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY:  
INHALATION DOSING METHOD: MRD-90-814, 181430

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**IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY:  
INHALATION DOSING METHOD: MRD-90-814, 181430**

**SUMMARY**

The micronucleus assay (see for example Schmid, Chemical Mutagens 1976, 4:31-53), is a means of evaluating the clastogenic (i.e., chromosome breaking) potential of test materials. Evidence of chromosome breakage or nondisjunction can be readily detected as micronuclei in enucleated erythrocytes. Positive test results are considered to provide presumptive evidence of mutagenic potential in mammals.

The objective of the current study was to determine whether MRD-90-814 was capable of inducing micronuclei formation in mouse bone marrow. The mice were exposed for six hours per day for two consecutive days. Target exposure concentrations were 500 and 1,000 ppm of test material in air. Actual mean exposure concentrations (overall mean of the individual samples) for each group were: 492 and 1,010 ppm, respectively. Bone marrow samples were collected and prepared for evaluation for micronucleus formation approximately 24 hours after dosing.

The study was initiated on July 25, 1990. The experimental start date was July 1, 1990 and the experimental termination date was August 15, 1990.

1,3 butadiene was used as the positive control. Target exposure concentration was 1,000 ppm in air and the actual mean exposure concentration was 1,081 ppm. 1,3 butadiene produced a statistically significant increase ( $P < 0.01$ ) in the number of micronuclei when compared to the negative control (air).

Based on the overall information obtained from the two statistical analyses, MRD-90-814 appears to induce a statistical increase ( $P = 0.05$ ) in micronucleus formation in the bone marrow of B6C3F1 male mice at 500 and 1,000 ppm in air. In addition, a dose response ( $P < 0.05$ ) was observed for the mean micronucleated polychromatic erythrocytes. A dose related statistically significant decrease ( $P < 0.01$ ) in the mean percent of polychromatic erythrocytes and a statistically significant difference ( $P < 0.01$ ) in the mean percent of polychromatic erythrocytes at 1,000 ppm in air when compared to the carrier control were also observed. This decrease is considered to be an indication of bone marrow cytotoxicity. Although the positive results are marginal, under the conditions of this test, MRD-90-814 is considered to be clastogenic in bone marrow of B6C3F1 male mice at 500 and 1,000 ppm in air when evaluated 24 hours after the last dosing.

IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY:  
INHALATION DOSING METHOD: MRD-90-814, 181430

INTRODUCTION

This study was conducted for Exxon Chemical Company, Baytown, Texas, in order to determine the potential of the test material, listed below, to cause an increase in the number of micronuclei observed in polychromatic erythrocytes of the bone marrow of B6C3F1 mice. Positive results in this assay, correlate well with the ability of certain test materials to cause neoplasia or genetic mutations in laboratory animals(1). Thus, positive results with a compound of unknown activity in animals, are considered presumptive evidence for mutagenic or neoplastic potential of that compound in animals.

The following compound was tested and is reported:

MRD-90-814

The animals used in this study were housed in Animal Room No. 408, and the exposure procedure was performed in Room Nos. 413 and 414 of the Toxicology Laboratory, Exxon Biomedical Sciences Inc., (an AAALAC accredited facility), Mettlers Road, CN2350, East Millstone, New Jersey 08875-2350. The study protocol, slides of bone marrow smears, all raw data, computer generated listings of raw data, study information and a copy of the final report are maintained on file in the Archives of the Laboratory.

Heddle, J.A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J., Newell, G.W., Salamone M.F. "The induction of micronuclei as a measure of genotoxicity - A Report of the U.S. Environmental Protection Agency Gene-Tox Program". Mutation Research 123:61-118 (1983).

IN VIVO MICELIAR BONE MARROW MICRONUCLEUS ASSAY:  
IRRADIATION DOSING METHOD: MRD-90-814, 181430

MATERIALS AND METHODS

Test Material

Material identification

MRD-90-814

Description

Batch No.	I
Physical description	Colorless Liquid
Strength/purity	Mixture, considered 100% pure for test purposes
Date received	June 21, 1990
Date expired	June 30, 1995
Storage conditions	Room Temperature

Data on the stability, strength, purity (other than that the material was to be considered approximately 100% pure), composition and characterization are the responsibility of the sponsor and are located at Basic Chemicals Technology, Baytown, Texas.

Solubility

Not applicable.

Sample retention

Archival retention samples of the carrier mixture of MRD-90-814 and 1,3 butadiene were not taken as this would represent a safety hazard.

Carrier

Air

Vehicle

Not applicable.

IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY:  
INHALATION DOSING METHOD: MRD-90-814, 181430

MATERIALS AND METHODS

Test Material (continued)

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Control Materials

1,3 butadiene  
(MRD-89-362)  
Batch No. I

Purity: Considered to be 100% for testing  
purposes  
Expires: 01-Nov-94

The positive control material was considered to have been stable for the duration of the assay since it produced the expected level of mutagenic activity and since no changes in the chromatograms were observed over the duration of the study. Data on the strength, purity (other than that the material was to be considered 100% pure for testing purposes), composition and characterization are documented and are located at Matheson Gas Products, 932 Paterson Plank Road, East Rutherford, N.J. 07073.

## MATERIALS AND METHODS

### Test System

#### Test Animal

Mouse

#### Strain/Stock

B6C3F1

#### Number and Sex

40 males

#### Age at Dosing

Approximately 8 to 9 weeks of age  
Weight between 24 and 30 grams one day prior to experimental  
start date.

#### Supplier

Charles River Portage  
Shaver Road  
Portage, Michigan 49081

#### Quarantine and Acclimation Period

Animals were received on July 03, 1990, (Purchase Order Number  
XEM313112D, Freight Reference Number 39031658), held in  
acclimation and observed for 29 days.

#### Observations During Acclimation

All animals were checked for viability once daily during the  
acclimation period.

#### Animal Identification

Monel ear tags and corresponding cage identification.

#### Justification

This assay is designed to be a primary means of determining  
the capacity of the test material to produce cytogenetic  
abnormalities in mammals by analysis of bone marrow cells of  
treated animals for the presence of elevated levels of  
micronucleated polychromatic erythrocytes.

MATERIALS AND METHODS

Test System (continued)

Selection

More animals than required for the conduct of the study were purchased and acclimated. Animals determined to be unsuitable for inclusion in this study because of poor health, outlying body weights, or other abnormalities were excluded from selection by the technical staff and were also examined by R. L. Harris prior to selection. Study animals were selected from the remaining animals using a computer generated, body weight sorting program.

Husbandry

Housing (for both non-exposure and exposure periods)

After a week of group housing all animals were then single housed.

Caging (for both non-exposure and exposure periods)

Suspended stainless steel

Bedding (for both non-exposure and exposure periods)

Indirect except during exposure periods when there was no bedding.

Feed (available ad libitum during non-exposure periods)

Purina Certified Rodent 5002 Chow (pellets) ad libitum

Manufacturer: Ralston Purina Company

Analysis: To be provided by the manufacturer.

Contaminants: This feed has had extensive use and has not, to the best of our knowledge, adversely affected studies of this nature.

Water (available ad libitum during non-exposure periods)

Automatic watering system, ad libitum

Supplier: Elizabethtown Water Company

Analysis: Provided periodically by the supplier.

Contaminants: There are no known contaminants believed to be present in the water at levels that may interfere with this study.

## MATERIALS AND METHODS

### Husbandry (continued)

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Animals were housed in appropriate caging, and the environment, including temperature, humidity, water pressure and diurnal cycle were maintained and monitored in accordance with the Animal Welfare Act (2) and the Guide for the Care and Use of Laboratory Animals (3).

#### Animal Room

The temperature was maintained between 68 and 76 degrees Fahrenheit. Water pressure was maintained between 5-9 psi except on July 20, 27, 30 and 31, 1990. This is not believed to have had an adverse effect on the study. Humidity was maintained between 40 and 70% relative humidity except on July 21, 1990. This is not believed to have had an adverse effect on the study.

Information concerning temperature, humidity and animal observations during exposure can be found in Appendix C.

A diurnal cycle of approximately 12 hours light and 12 hours dark was maintained by automatic timer.

#### Human Treatment

All animals were treated humanely as described in the Guide for the Care and Use of Laboratory Animals (3). The study design and personnel training were sufficient to minimize animal suffering within the confines of the study objectives.

(2) CFR, Title 9 [Animals and Animal Products], Subchapter A-Animal Welfare Parts 1, 2, and 3. Animal Welfare Act of 1966 (P.L. 89-544), as amended by the Animal Welfare Act of 1970 (P.L. 91-579), and by the 1976 Amendments to the Animal Welfare Act (P.L. 94-279).

(3) Guide for the Care and Use of Laboratory Animals, U.S. Department of Health and Human Services, Public Health Services, National Institutes of Health, NIH Publication No. 85-23, Revised 1985.

**IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY:  
INHALATION DOSING METHOD: MRD-90-814, 181430**

**EXPERIMENTAL DESIGN**

**Preparation of Test Material**

The test material was administered as received from the sponsor.

**Preparation of Animals**

All animals were weighed the day prior to dosing and sorted into groups. Target doses were 500 and 1,000 ppm of test material in air. Air was the negative control. The target dose for the 1,3 butadiene, the positive control, was 1,000 ppm in air.

**Group Designation and Exposure Levels**

Upon completion of the quarantine period, healthy mice were randomly selected and placed into the following groups:

Group	Description	Material Number (Exxon Code)	Number of Animals (male)	Target Exposure Levels (ppm)
I	Control	Air	10 mice	0
II	Low"	MRD-90-814	10 mice	500
III	High"	MRD-90-814	10 mice	1,000
V	Positive Control	1, 3 butadiene	10 mice	1,000

" Doses based upon results of a rangefinding assay.

The experimental design and the exposure level for 1, 3 butadiene were based on the study reported by Cunningham et al(4). However, the method of dosing was modified from using nose-only to using whole body.

(4)Cunningham, M. J., Choy, W.M., Arce, G.T., M.J., Rickard, L.B., Vlachos, D. A., Kinney, L.A. and Sarrif, A.M. "In vivo sister chromatid exchange and micronucleus induction studies with 1,3 butadiene in B6C3F1 mice and Sprague-Dawley rats." Mutagenesis, 1: 449-452 (1986).

EXPERIMENTAL DESIGN

5  
1

Dosing Procedures

Route of Administration

Intubation

Information concerning administration of test material, chamber, in-chamber animal observations, test atmosphere and analytical procedures can be found in Appendix C.

Sacrifices

Animals from the appropriate groups were sacrificed by carbon dioxide asphyxiation approximately 24 hours after last dosing.

Viability Checks

The animals were examined at least once daily.

**IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY:  
INHALATION DOSING METHOD: MRD-90-814, 181430**

**EXPERIMENTAL EVALUATION**

**TOXICITY OF TEST MATERIAL**

Two range finding studies were performed. The first range finding study was performed using target doses of 1,000, 3,260 and 10,000 ppm of test material in air. Four male mice were used for each dose group. Only the 4 mice comprising the low dose (1,000 ppm) survived 24 hours after the last dosing.

The second range finding study (181430A) was performed using test doses of 0.15 ml/kg, 0.75 ml/kg and 1.5 ml/kg administered by oral gavage. Four male mice were used for each dose group. The 4 top dose (1.5 ml/kg) mice, 1 mid dose (0.75 ml/kg) and 2 low dose (0.15 ml/kg) animals succumbed before 72 hours after dosing.

Target dose concentrations for the mutation assay were changed to 500 and 1,000 ppm of test material in air by request of the study monitor.

**MICRONUCLEUS ASSAY**

Immediately following the sacrifice of the animals, both femurs were removed. The bone marrow was then removed and suspended in fetal bovine serum. After the suspension was centrifuged, the pellet was resuspended and smears were prepared (two slides per animal). Slides were labeled with animal identification number and study number. Since animals are assigned to each dose group by body weight, the animal numbers are not sequential for each group and constitute a blind coding.

Prior to microscopic evaluation, slides were stained using acridine orange. Polychromatic erythrocytes (PCE) stain fluorescent red/orange, normochromatic erythrocytes (NCE) are unstained or stain a dull green, and micronuclei stain fluorescent bright yellow. Additional criteria for scoring micronuclei are a circular appearance and a diameter between 1/20 and 1/5 of the cell's diameter(5). 1000 polychromatic erythrocytes (PCE) from each animal were examined for micronuclei, and the ratio of PCE's to NCE's was determined for each animal by counting 1000 erythrocytes (PCE's and NCE's).

(5) Schmid, W. "The micronucleus test" Mutation Res. (1975) 31:9-15.

STATISTICAL EVALUATION

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Statistical analysis included calculation of means and standard deviations of the micronuclei data and a test of equality of group means by a standard one way analysis of variance at each time period (Snedecor and Cochran, 1971). When the ANOVA was significant, comparisons of vehicle control to dosed group means were by Duncan's Multiple Range Test (Snedecor and Cochran, 1971).

A standard regression analysis was performed to test for a dose response (Snedecor and Cochran, 1971).

Residuals from the ANOVA were analyzed for normality by Wilk's Criterion (Shapiro and Wilk, 1965). The residuals were normally distributed (values were greater than 0.01 level of significance) in more than 25% of the analyses. Therefore nonparametric analysis was not performed.

REFERENCES

Hollander, M. and Wolfe, D.A., Nonparametric Statistical Methods, John Wiley and Sons, New York, 1973.

Shapiro, S.S. and Wilk, M.B., "An analysis of variance test for normality (complete samples)", Biometrika, 52, 1965.

Snedecor and Cochran, Statistical Methods, 6th Edition, The Iowa State Press, Ames, Iowa, 1971.

**IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY:  
INHALATION DOSING METHOD: MRD-90-814, 181430**

**RESULTS**

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The results for this study are presented in a series of tables in Appendices A and B. First in the series is a summary (Table 1) found in Appendix A which condenses the data for the 24 hour sacrifice time point by listing the means and standard deviations of the micronuclei data for each dose group. Next in the series is a listing of the raw data (Tables 1-4) found in Appendix B, listed by dose group.

Target exposure concentrations for MRD-90-814 were 500 and 1,000, ppm in air, and actual mean exposure concentrations (overall mean of the individual samples) for each group were: 492 and 1,010 ppm, respectively. Methods of inhalation exposures and a presentation of exposure results are in Appendix C.

Micronucleus Assay

Two statistical analyses were performed. The initial analysis demonstrated that the positive control, 1,3 butadiene, produced a statistically significant increase ( $P < 0.01$ ) in the mean number of micronuclei in polychromatic erythrocytes. 1,3 butadiene also produced a statistically significant decrease ( $P < 0.01$ ) in the mean percent of polychromatic erythrocytes which is a measure of toxicity. The test material, MRD-90-814, also produced a statistically significant decrease in the mean percent of polychromatic erythrocytes ( $P < 0.01$ ) which was also dose related (regression coefficient at ( $P < 0.01$ )). MRD-90-814 also produced a dose related increase ( $P < 0.05$ ) in the mean number of micronuclei in polychromatic erythrocytes, but none of the test material dose groups were statistically different from the air control.

Since the positive control value may prevent the detection of a weak mutagenic response produced by the test material and since the test material did produce a statistically significant increase in micronuclei, a second statistical analysis was performed which excluded the positive control. In this second analysis, MRD-90-814 did induce a statistically significant increase ( $P = 0.05$ ) in the mean number of micronucleated polychromatic erythrocytes at 500 and 1,000 ppm in air when compared to the air control. This increase was also dose related (regression coefficient at ( $P < 0.05$ )). The test material also produced a statistically significant decrease ( $P < 0.01$ ) in the mean percent of polychromatic erythrocytes at 1,000 ppm in air which was also dose related (regression coefficient at  $P < 0.01$ ).

CONCLUSIONS

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This study is a modification of the experimental design employed by Cunningham et al (6). The method of dosing was modified from using nose-only to whole body. Other modifications include increasing the number of animals in each group from 5 to 10 and increasing the number of polychromatic erythrocytes evaluated from 500 to 1,000. Using this modified procedure, MRD-90-814 was tested for the induction of micronuclei at 24 hours following in vivo exposure using B6C3F1 mice.

Based on the overall results obtained from two statistical analyses, this test material appears to induce a statistically significant increase ( $P=0.05$ ) in micronucleated polychromatic erythrocytes at both 500 and 1,000 ppm in air which is also dose related ( $P<0.05$ ). A statistically significant decrease ( $P<0.01$ ) in the mean percent of polychromatic erythrocytes was also observed. This decrease which was dose related (regression coefficient at  $P<0.01$ ), is an indication of bone marrow cytotoxicity. The positive control, 1,3 butadiene, and the negative control, air, responded in an appropriate manner consistent with results from previous studies. Therefore, under the conditions of this assay, MRD-90-814 may be marginally clastogenic in bone marrow of B6C3F1 male mice at 500 and 1,000 ppm in air when evaluated 24 hours after last dose administration.

GLP DEVIATIONS

A single protocol was used to define two test systems, one using mice and one using rats. Two separate reports were prepared, one for the mice and one for the rats.

(6)Cunningham, M. J., Choy, W.M., Arce, G.T., M.J., Rickard, L.B., Vlachos, D. A., Kinney, L.A. and Sarrif, A.M. "In vivo sister chromatid exchange and micronucleus induction studies with 1,3 butadiene in B6C3F1 mice and Sprague-Dawley rats." Mutagenesis, 1: 449-452 (1986).

IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY; 181430; MRD-90-814

APPENDIX A  
DATA REPORT SUMMARY  
TABLE 1

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Males

SACRIFICE TIME	DOSE	N	MEAN PCE	STD PCE	MEAN MNE	STD MNE
24 hours	Air Control	10	55.82 R	3.15693	3.1 R+	1.52388
	500 ppm MRD-90-814	10	53.36	2.72527	5.0 *	2.35702
	1000 ppm MRD-90-814	10	49.08 **	2.72103	5.3 *	2.21359
	1000 ppm Butadiene	10	42.03 **	4.47935	33.1 **	3.90014

- N = Number of animals in dose group
- MEAN PCE = Mean % PCE for dose group
- STD PCE = Standard deviation of mean % PCE
- MEAN MNE = Mean micronuclei per 1,000 polychromatic erythrocytes
- STD MNE = Standard deviation of mean micronuclei
- \* Significantly different from control at P=0.05
- \*\* Significantly different from control at P<0.01
- R Significant regression coefficient at P<0.01
- R+ Significant regression coefficient at P<0.05

APPENDIX B - BONE MARROW MICRONUCLEUS ASSAY  
 DATA REPORT  
 TABLE 1

2

STUDY NUMBER: 181430  
 SEX: Males

TEST MATERIAL: MRD-90-814

DOSE GROUP	PIN NUMBER	BODY WT. (GRAMS)	PCE	NCE	% PCE	MNE/1000 PCE	% MNE/PCE
Control Air	KAD 617	25.25	571	429	57.1	2	.2
	KAD 650	26.02	510	490	51.0	4	.4
24	KAD 602	26.32	583	417	58.3	0	0
	KAD 626	26.36	557	443	55.7	3	.3
Hour	KAD 630	27.73	602	398	60.2	3	.3
Control Air	KAD 613	26.81	531	469	53.1	2	.2
	KAD 612	27.67	588	412	58.8	5	.5
24	KAD 635	27.96	513	487	51.3	3	.3
	KAD 610	28.42	553	447	55.3	5	.5
Hour	KAD 632	28.86	574	426	57.4	4	.4

PCE=Polychromatic Erythrocyte, MNE=Micronucleated PCE, NCE=Normochromatic Erythrocyte,  
 BODY WT.=Body Weight, % MNE/PCE=[(MNE/1000 PCE)/1000] x 100, %PCE=PCE/(PCE+NCE) x 100

IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY; 181430; MRC-90-814

APPENDIX B - BONE MARROW MICRONUCLEUS ASSAY  
DATA REPORT  
TABLE 2

22

STUDY NUMBER: 181430  
SEX: Males

TEST MATERIAL: MRC-90-814

DOSE GROUP	PIN NUMBER	BODY WT. (grams)	PCE	NCE	% PCE	MNE/1000 PCE	% MNE/PCE
1,000 ppm MRD-90-814	KAD 638	24.97	531	469	53.1	3	.2
	KAD 645	26.13	496	504	49.6	8	.8
24	KAD 622	26.23	515	485	51.5	5	.5
	KAD 639	26.52	454	546	45.4	4	.4
Hour	KAD 646	27.09	520	480	52.0	4	.4
1,000 ppm MRD-90-814	KAD 618	27.46	481	519	48.1	2	.2
	KAD 620	27.58	485	515	48.5	9	.9
24	KAD 647	28.01	455	545	45.5	7	.7
	KAD 628	28.03	466	534	46.6	6	.6
Hour	KAD 624	28.14	505	495	50.5	5	.5

PCE=Polychromatic Erythrocyte, MNE=Micronucleated PCE, NCE=Normochromatic Erythrocyte,  
BODY WT.=Body Weight, % MNE/PCE=[(MNE/1000 PCE)/1000] x 100, %PCE=PCE/(PCE+NCE) x 100

IN VIVO MURINE BONE MARROW MICRONUCLEUS ASSAY; 181430; MRD-90-814

APPENDIX B - BONE MARROW MICRONUCLEUS ASSAY  
DATA REPORT  
TABLE 3



STUDY NUMBER: 181430  
SEX: Males

TEST MATERIAL: MRD-90-814

DOSE GROUP	PIN NUMBER	BODY WT. (grams)	PCE	NCE	% PCE	MNE/1000 PCE	% MNE/PCE
500 ppm MRD-90-814	KAD 641	24.95	530	470	52.0	10	1.0
	KAD 606	25.33	513	487	51.3	2	.2
24	KAD 604	26.20	561	439	56.1	3	.3
	KAD 615	26.29	495	505	49.5	4	.4
Hour	KAD 643	27.32	517	483	51.7	7	.7
500 ppm MRD-90-814	KAD 611	27.34	569	431	56.9	4	.4
	KAD 629	27.97	576	424	57.6	3	.3
24	KAD 607	28.10	508	492	50.8	6	.6
	KAD 648	28.14	537	463	53.7	5	.5
Hour	KAD 642	28.15	530	470	53.0	6	.6

PCE=Polychromatic Erythrocyte, MNE=Micronucleated PCE, NCE=Normochromatic Erythrocyte,  
BODY WT.=Body Weight, % MNE/PCE=[(MNE/1000 PCE)/1000] x 100, %PCE=PCE/(PCE+NCE) x 100

IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY; 181430; MRD-90-814

APPENDIX B - BONE MARROW MICRONUCLEUS ASSAY  
DATA REPORT  
TABLE 4



STUDY NUMBER: 181430  
SEX: Males

TEST MATERIAL: MRD-90-814

DOSE GROUP	PIN NUMBER	BODY WT. (grams)	PCE	NCE	% PCE	MNE/1000 PCE	% MNE/PCE
1,000 ppm	KAD 631	25.27	448	552	44.8	36	3.5
1,3 Butadiene	KAD 621	25.36	423	577	42.3	30	3.0
24	KAD 609	25.88	421	579	42.1	39	3.9
	KAD 616	26.06	403	597	40.3	31	3.1
Hour	KAD 623	26.79	392	608	39.2	34	3.4
1,000 ppm	KAD 636	27.15	354	646	35.4	35	3.5
1,3 Butadiene	KAD 627	27.55	410	590	41.0	36	3.6
24	KAD 608	27.91	496	504	49.6	25	2.5
	KAD 633	28.51	374	626	37.4	33	3.3
Hour	KAD 649	29.39	482	518	48.2	32	3.2

PCE=Polychromatic Erythrocyte, MNE=Micronucleated PCE, NCE=Normochromatic Erythrocyte,  
BODY WT.=Body Weight, % MNE/PCE=[(MNE/1000 PCE)/1000] x 100, %PCE=PCE/(PCE+NCE) x 100

APPENDIX C  
INHALATION EXPOSURE PROCEDURES AND RESULTS

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## EXPERIMENTAL METHODS

### Animal Procedures

The experimental and control groups were each contained in one cubic meter stainless steel and glass chambers. Each group was exposed for six hours per day (plus time for chamber equilibration) for two consecutive days either to room air (Group I) or to vapors of the test materials (Groups II, III, and V).

During each exposure, in-chamber observations of each group were recorded at hourly intervals.

### Exposure Equipment and Procedures

MRD-90-814 (Groups II and III):

Figure 1 presents a schematic of the Groups II and III exposure/generation system

The test material, MRD-90-814, was delivered from a reservoir via a laboratory pump to the inside of a cylindrical glass vapor generator. The glass vapor generator was impressed with a spiral indentation as a channel for the test material. The spiral indentation was heated with heating tape. The temperature of the vapor generator was regulated by a variable autotransformer and monitored by an Omega telethermometer with an iron-constantin probe. As it flowed down the heated generator, the test material evaporated and the resulting vapors mixed with room air as both were drawn up through the generator and into a one cubic meter glass and stainless steel exposure chamber. The exposure chambers operated at an exhaust flowrate of 200 liters per minute (measured by a calibrated orifice meter), providing one air change every 5.0 minutes and a theoretical equilibration time (T<sub>99</sub>) of 23.0 minutes.

MRD-89-362 (Group V):

Figure 2 presents a schematic of the Group I and V exposure/generation system.

The test material, MRD-89-362 (1,3 butadiene) was received from Matheson Gas Products, East Rutherford, New Jersey as a liquid under pressure in a gas cylinder. The material was delivered from its cylinder via a pressure regulator to a 1/8" coil of copper tubing submerged in a slightly heated water bath. The test material volatilized within the heated copper coil and the resulting vapors were metered by a calibrated rotameter into the exposure chamber. The exposure chamber operated at an exhaust flowrate of 200 liters per minute (measured by a calibrated orifice meter), providing one air change every 5.0 minutes and a theoretical equilibration time (T99) of 23.0 minutes.

The control group chamber received room air only, and was also operated at 200 liters per minute.

#### Chamber Temperature and Relative Humidity

Dry bulb/wet bulb hygrometers were used to continuously monitor chamber temperature and relative humidity and were recorded at approximately thirty minute intervals.

#### Chamber Static Pressure

Chamber static pressure was maintained slightly negative to the room and monitored continuously by Magnehelic gauge and recorded at approximately thirty minute intervals.

## Concentration Determinations

Exposure concentrations were determined on both a nominal and actual basis. The nominal concentrations for Groups II and III (MRD-90-814) were calculated by weighing the reservoir containing the test material before and after exposure and dividing the net loss in weight by the total volume of air passing through the chamber during the exposure.

The nominal concentration for Group V (MRD-89-362) was estimated by dividing the mean test material delivery rate (LPM) by the total flow of air through the chamber and multiplying by the concentration of the pure test material (one million ppm).

Actual exposure concentrations for all groups were determined by on-line gas chromatography (GC). A Hewlett-Packard 5880A gas chromatograph, equipped to sequentially sample up to 16 separate sample streams, was programmed to analyze and report on samples hourly. Six sample streams were actually used in this study, one to a 1000 ppm reference standard of the positive control (MRD-89-362) vapor and one to each of the five exposure chambers. The remaining unused streams were capped. All active streams were updated continuously to provide fresh samples for analysis.

During the trials for this study, samples were drawn from four points around the horizontal center plane of each of the exposure chambers to determine the homogeneity of test material distribution. On-line gas chromatography, as described above, was used for this procedure.

Operating Conditions

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Gas Chromatograph:	Hewlett Packard 5880A
Detector:	Flame Ionization
Column:	6' x 1/8", 20% SP-2100, 80/100 Supelcoport
Gas Flows (cc/min):	H2 - 30 Air - 400 carrier gas - N2 - 25
Oven Temperature Profile:	Isothermic - 75°C
Detector Temperature:	225°C
Injector Temperature:	75°C
Sample Loop Size:	2 cc
Chart Speed:	1.0 cm/min
Attenuation:	2 ↑ 12

Calibration Procedure

Figure 3 presents a schematic of the test material calibration systems used for this study.

MRD-90-814 (Groups II and III):

The GC was calibrated with an AID Model 360 Standards Generator. The standards generator provided a dynamically prepared reference standard of the test material vapors in air. Reduced to its basics, the device consists of a temperature controlled oven containing four glass diffusion tubes filled with the test material.

The diffusion tubes (glass bulbs with long narrow necks) evolve vapors at a stable rate controlled by the temperature of the oven. A regulated source of compressed air dilutes the vapors to the desired concentration and transports them to the GC for analysis.

The diffusion tubes are weighed before and after installation in the oven and the weight loss calculated. This value, divided by the time spent in the oven, yields weight loss per unit time (ug/min) - the basic measure of the vapor generation. By varying the amount of dilution air introduced with the vapors, a range of concentrations can be produced.

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To calibrate the GC, a series of four concentrations were created which spanned the target exposure levels for the study. Five replicate samples were taken at each level. The mean area counts of the five samples taken at each level and the corresponding concentration were manually entered into the GC's External Standard calibration function. A multilevel regression analysis was calculated by the GC and used to determine actual chamber concentrations.

MRD-89-362 (Group V):

MRD-89-362 was delivered as a liquid from the tank to a copper coil submerged in a slightly heated water bath. The liquid volatilized within the heated copper coil. The test material vapor, regulated by a calibrated rotameter, was metered into a 2 liter glass mixing vessel. A constant source of dilution air, regulated by a calibrated rotameter, was also metered into the mixing vessel. The test material concentration within the vessel was calculated by dividing the test material flow (cc/min) by the total flow through the vessel (cc/min) and multiplying by the concentration of the pure test material (one million ppm).

By varying the flow of the test material, a series of test material concentrations were produced which spanned the range of target concentrations for the study. Five replicate samples were taken at each concentration by the GC. The mean area counts of the five samples taken at each level and the corresponding concentrations were manually entered into the GC's External Standard calibration function. A multilevel regression analysis was calculated by the GC and used to determine actual chamber concentrations.

Once established during method development, the response of the GC was checked daily throughout the study period by sampling a 1000 ppm certified standard of MRD-89-362. The certified standard was prepared by MG Industries and served as an external check on the GC's stability during the study. The standard was sampled hourly during each set of chamber samples during the exposures.

## RESULTS

### Generation and Exposure Concentration Data

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Tables 1 and 2 summarize the relevant generation and concentration data.

The actual mean exposure concentrations (overall mean of the individual samples) for each group were: 492 ppm (Group II), 1010 ppm (Group III), and 1081 ppm (Group V). This compares to the target concentrations of 500, 1000, and 1000 ppm for Groups II, III and V respectively.

The samples of the external reference standard of the positive control (MRD-89-362) varied no more than 8.3% from the calibration value of 1000 ppm. This demonstrates the stability of the calibration used during the study.

Four distribution samples were taken at each exposure level during the trial for this study. A summary of the distribution sample locations and concentrations is presented in Table 3. The coefficients of variation (s.d./mean) for Groups II, III, and V were 0.5%, 0.5%, and 5.4% respectively, demonstrating that the test material was homogeneously distributed throughout each chamber.

### In-Chamber Animal Observations

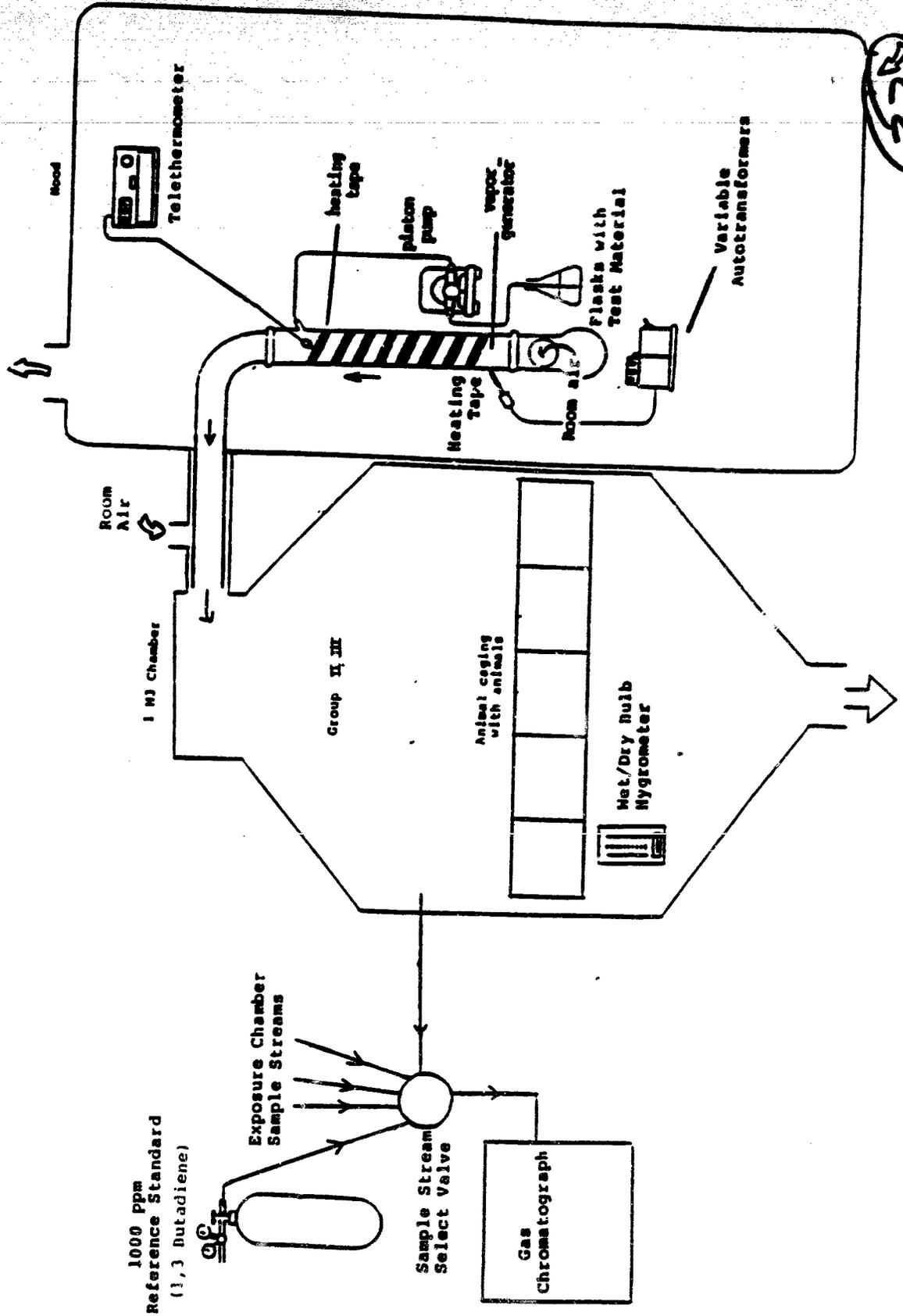
Table 4 presents the in-chamber animal observations.

On Day 1, all the mice in Groups I, II, III, and V appeared normal.

On Day 2, all the Groups I and II mice appeared normal. Some Group III and V mice exhibited decreased activity throughout the exposure.

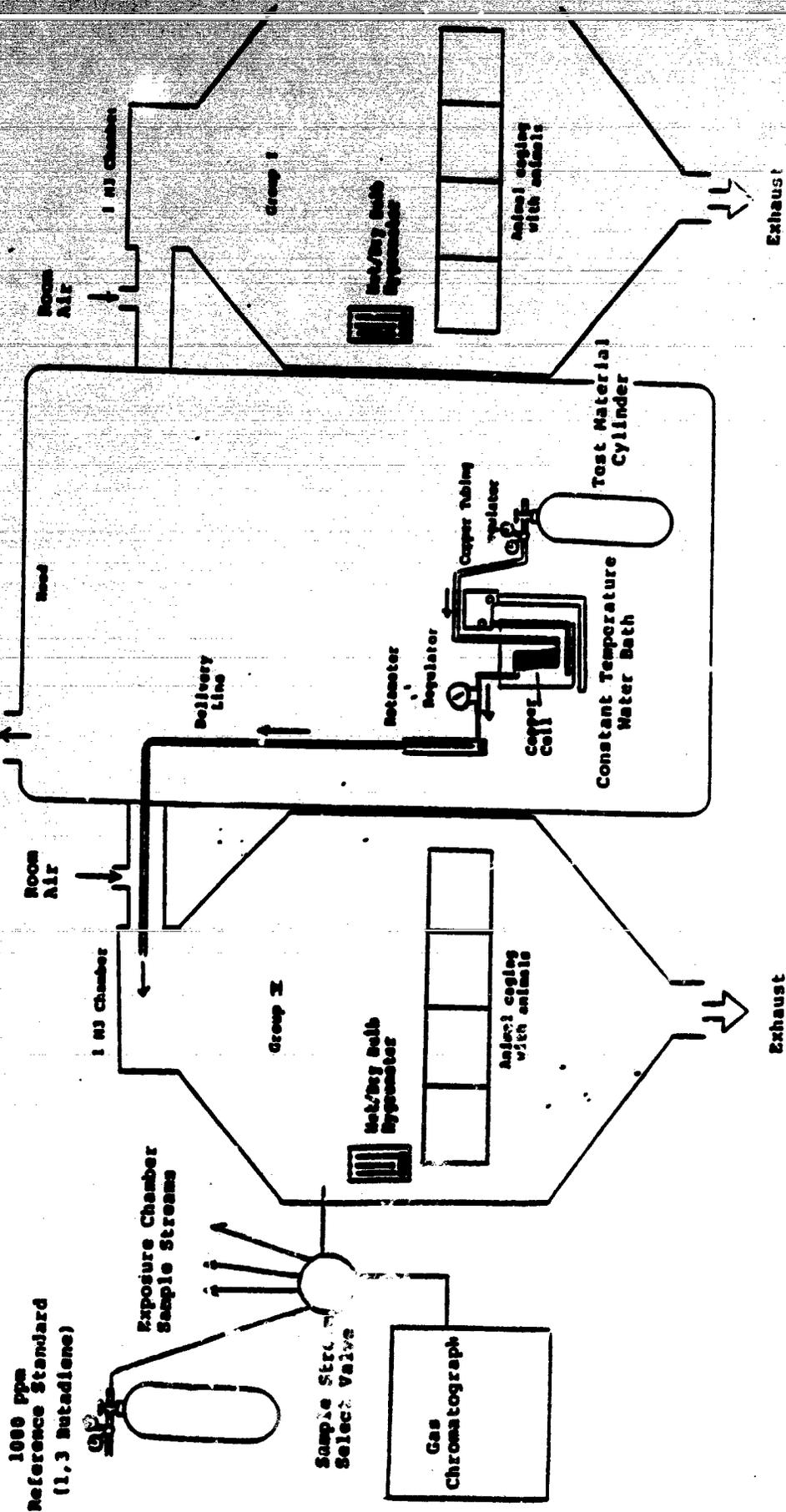
IN VIVO MAMMALIAN BONE MARROW MICRONUCLEUS ASSAY: 181430; MRD-90-814

FIGURE 1 SCHEMATIC OF CHAMBER GENERATION AND EXPOSURE SYSTEM (MRD-90-814)



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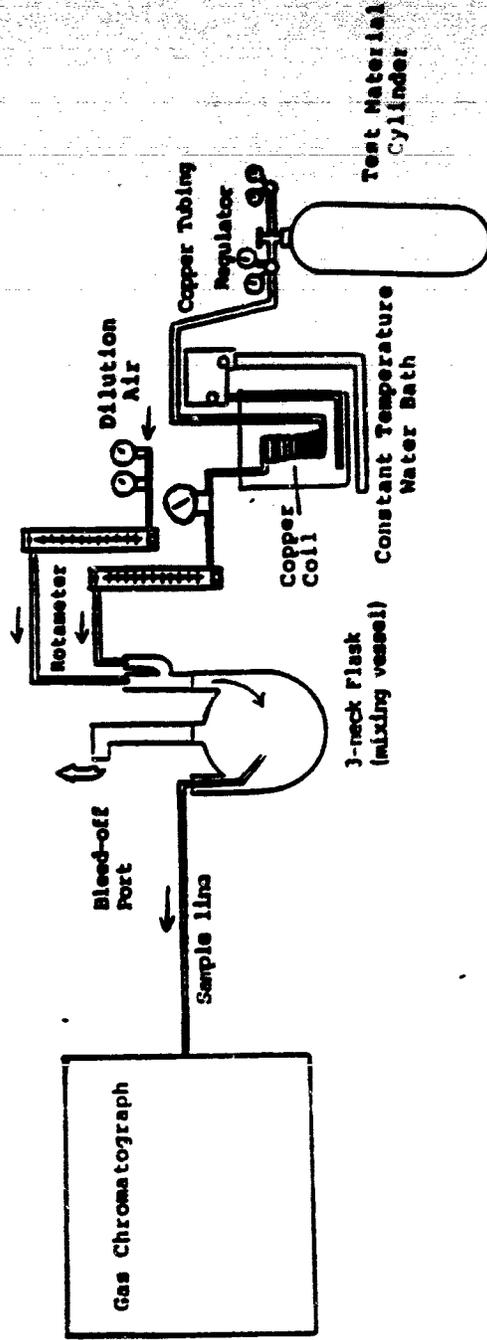
FIGURE 2 SCHEMATIC OF CHAMBER GENERATION AND EXPOSURE SYSTEM (MDO-89-362)



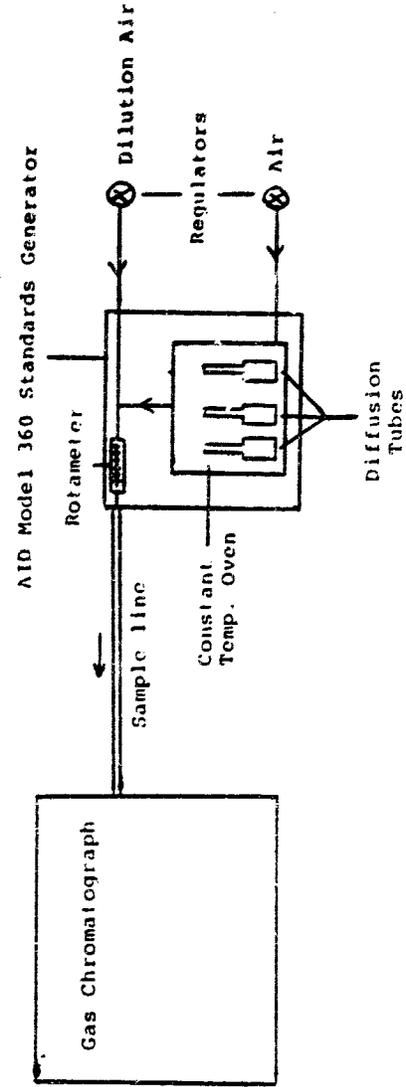
33

FIGURE 3 SCHEMATIC OF TEST MATERIAL CALIBRATION SYSTEM

G.C. CALIBRATION WITH MRD-89-362



G.C. CALIBRATION WITH MRD-90-814



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TABLE 1 SUMMARY OF EXPOSURE CONDITIONS

	Group I (Control)	Group II (MNU-90-814)	Group III (MNU-90-814)	Group V (MNU-89-362)
Chamber Airflow (Liters/minute)	200	200	200	200
Daily Exposure Duration (minutes)	360	360	360	360
Mean Chamber Concentration:				
Nominal (PPM)	0	521	1064	1010*
Actual (PPM)	0	492	1010	1001
Average Chamber Temperature (F) (Overall range: 66-76)	72	72	74	69
Average Chamber Relative Humidity (%) (Overall range: 58-81)	71	72	67	77

\* Nominal concentration estimated from delivery rate of test material.

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TABLE 2 SUMMARY OF CHAMBER CONCENTRATION SAMPLES

Exposure Number	Group I (Control) (PPM)	Group II (MRD-90-814) (PPM)	Group III (MRD-90-814) (PPM)	Group V (MRD-8(-362) (PPM)	Standard (PPM)
	0	340	900	1107	932
1	0	534	980	1092	935
31-JUL-90	0	528	979	1070	935
	0	537	1000	1417	936
	0	494	1000	1065	936
	0	486	995	1022	935
2	0	508	1186	1032	940
	0	514	952	1065	940
	0	499	1044	1019	923
	0	487	1027	1028	917
1-AUG-90	0	496	1029	1047	918
	0	480	1032	1011	918
Mean	0	492	1010	1081	930
s.d.	0	52	68	110	8.8
C.V. (s.d./mean)	0	10.6	6.7	10.2	0.9

Chamber concentration = analysis by gas chromatography.

TABLE 3 SUMMARY OF CHAMBER DISTRIBUTION SAMPLES

Location	Group II (MRD-90-814) (ppm)	Group III (MRD-90-814) (ppm)	Group V (MRD-89-362) (ppm)
Right Front	569	1010	1169
Left Front	564	1005	1114
Right Rear	569	1006	1109
Left Rear	570	998	1024
Mean	568	1005	1104
s. d.	2.7	5.0	59.5
CV% (s. d./mean)	0.5	0.5	5.4

NOTE: Distribution samples were taken during 27-Jul-90 method development trial.

TABLE 4 SUMMARY OF IN-CHAMBER ANIMAL OBSERVATIONS  
31-JUL-90

	TIME (minutes into the exposure)					
	60	120	180	240	300	360
Group I (Control)						
NO OBSERVED ABNORMALITIES	ALL	ALL	ALL	ALL	ALL	ALL
Group II (MRD-90-814)						
NO OBSERVED ABNORMALITIES	ALL	ALL	ALL	ALL	ALL	ALL
Group III (MRD-90-814)						
NO OBSERVED ABNORMALITIES	ALL	ALL	ALL	ALL	ALL	ALL
Group V (MRD-89-362)						
NO OBSERVED ABNORMALITIES	ALL	ALL	ALL	ALL	ALL	ALL

Key:  
 FEW = 10%-30% of group with observation.  
 SOME = 40%-60% of group with observation.  
 MOST = 70%-90% of group with observation.  
 ALL = 100% of group with observation.

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