

American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005
202-682-8308



000534193P

FYI-AX-1287-0589 SUPP

Sequence B

84-850000052

02/29/88

Robert T. Drew, Ph.D.
Director
Health and Environmental Sciences Department

January 29, 1988

Mr. Lee Thomas
Administrator, U.S. EPA
401 M Street, SW
Room 1200, West Tower
Washington, DC 20460

Reporting No. 131

Dear Mr. Thomas:

Preliminary draft reports have been previously submitted, enclosed are final reports for:

1. (Identification number: FYI-AX-0887-0563) Mutagenicity Test on ASTM D-3734 Type I C-9 in an in vitro Cytogenetics Assay Measuring Sister Chromatid Exchange Frequencies in Chinese Hamster Ovary. Final Report from Hazleton Laboratories America Inc.
2. (Identification number: FYI-AX-0887-0563) Mutagenicity Test on ASTM D-3734 Type I C-9 in an in vitro Cytogenetics Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary. Final Report from Hazleton Laboratories America Inc.
3. (Identification number: FYI-AX-0887-0563) Mutagenicity Test on ASTM D-3734 Type I C-9 in the Ames Salmonella/Microsome Reverse Mutation Assay. Final Report from Hazleton Laboratories America Inc.
4. (Identification number: FYI-AX-0887-0563) Mutagenicity Test on ASTM D-3734 Type I C-9 in the CHO/HGPRT Forward Mutation Suspension Assay. Final Report from Hazleton Laboratories America Inc.
5. (Identification number: FYI-AX-1287-0589) Evaluation of the C-9 Aromatic Hydrocarbons for Mutagenic Potential - Bone Marrow Cytogenetics Tests in Rats. Final report from International Research and Development Corporation.

Please note that this information is provided in accordance with the full disclosure policy of the API and does not constitute a formal submission as required by the C9 test rule.

We will continue to keep you apprised of the progress of this research. If you have any questions about it, please communicate with me.

cc w attach - Nancy Merrifield, Test Rules Dev. Br.

Sincerely,

Robert T. Drew, Ph.D.

DATE RECEIVED _____

RECEIVED BY _____

RETURN TO Meryl L. Kane
Information Specialist
Health and Environmental Sciences
American Petroleum Institute
1220 L Street, NW
Washington, DC 20005

040029

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Health and Environmental
Sciences Department

*F4I-AX-1288-0539 SUPPL
Sequence 15*

HESD Publ. No.:

EVALUATION OF C9 AROMATIC HYDROCARBONS FOR MUTAGENIC POTENTIAL-BONE MARROW CYTOGENETICS TEST IN RATS

Final Report

Study Conducted Under Contract PS-6A By:
International Research and Development Corporation
Mattawan, Michigan

January 1988

FOREWORD

API PUBLICATIONS NECESSARILY ADDRESS PROBLEMS OF A GENERAL NATURE WITH RESPECT TO PARTICULAR CIRCUMSTANCES. LOCAL, STATE, AND FEDERAL LAWS AND REGULATIONS SHOULD BE REVIEWED

API IS NOT UNDERTAKING TO MEET DUTIES OF EMPLOYERS, MANUFACTURERS, OR SUPPLIERS TO WARN AND PROPERLY TRAIN AND EQUIP THEIR EMPLOYEES, AND OTHERS EXPOSED, CONCERNING HEALTH AND SAFETY RISKS AND PRECAUTIONS, NOR UNDERTAKING THEIR OBLIGATIONS UNDER LOCAL, STATE, OR FEDERAL LAWS.

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American Petroleum Institute
Health and Environmental Sciences Department

QUALITY ASSURANCE STATEMENT

Study Title: Evaluation of C9 Aromatic Hydrocarbons For
Mutagenic Potential-Bruce Marrow Cytogenetics Test
In Rats

Testing Facility Number: 418-030

API Product Safety Number: PS-64

This study was reviewed by API Quality Assurance personnel on the
dated indicated below for compliance with applicable Good
Laboratory Practice regulations.

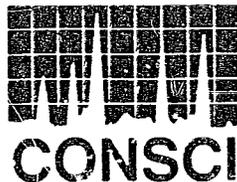
Copies of report by API Quality Assurance personnel are available
upon written request to the Director of the Health and Environ-
mental Sciences Department of the American Petroleum Institute of
his designee.

<u>Date(s) of Inspection/Review</u>	<u>Type of Inspection</u>
September 14-16, 1987	Site visit-preliminary data audit
October 19-21, 1987	Site visit-draft final report and raw data audit
January 4, 1988	Final Report review with provisional acceptance
January 21, 1988	Final Report acceptance

Rodney C. Anderson
Rodney C. Anderson, M.S.
Quality Assurance Associate

January 21, 1988
Date

MATERIAL SPECIFICATION
ASTM D3734 TYPE I
C-9 AROMATIC HYDROCARBONS



American Petroleum Institute
1220 L Street N.W.
Washington, D.C. 20005

February 14, 1986

Attn: Dr. C. E. Holdsworth

Certificate # : 51219001
Sample Description : C9 Aromatic Naphtha
Date Received : December 19, 1985

CAPILLARY GAS CHROMATOGRAPHY ANALYSIS *

<u>Component Name</u>	<u>Wt. %</u>	<u>LV. %</u>
Total non-aromatics	< 0.10	< 0.12
C6 + C7 aromatics	0.01	0.01
m-Xylene	0.05	0.05
p-Xylene	0.02	0.02
o-Xylene	3.17	3.14
Isopropylbenzene	2.76	2.79
n-Propylbenzene	3.95	3.99
1-Methyl-3-ethylbenzene	15.85	15.98
1-Methyl-4-ethylbenzene	6.13	6.19
1,3,5-Trimethylbenzene	8.09	8.14
1-Methyl-2-ethylbenzene	5.78	5.72
1,2,4-Trimethylbenzene	39.18	39.13
tert-Butylbenzene	<0.20	<0.20 #
Isobutylbenzene	0.12	0.12
sec-Butylbenzene	0.11	0.11
1-Methyl-2-isopropylbenzene	0.01	0.01
1,2,3-Trimethylbenzene	5.49	5.35
1-Methyl-4-isopropylbenzene	0.07	0.07
Indane (2,3-Dihydroindene)	0.96	0.87
1,3-Diethylbenzene	1.16	1.18
1-Methyl-3-n-propylbenzene	0.60	0.60
1-Methyl-4-n-propylbenzene/n-Butylbenzene	0.82	0.83
1,2-Diethylbenzene	0.89	0.89
1,4-Diethylbenzene/1,3-Dimethyl-5-ethylbenzene	0.12	0.12
1-Methyl-2-n-propylbenzene	0.17	0.17
1,4-Dimethyl-2-ethylbenzene	0.48	0.48
1,3-Dimethyl-4-ethylbenzene	0.59	0.59
1,2-Dimethyl-4-ethylbenzene	2.28	2.28
1,3-Dimethyl-2-ethylbenzene	0.04	0.04
1,2-Dimethyl-3-ethylbenzene	0.23	0.22

<u>Component Name</u>	<u>Wt. %</u>	<u>LV. %</u>
1,2,4,5-Tetramethylbenzene	0.18	0.18
Unidentified C11 compounds	0.14	0.17
1,2,3,5-Tetramethylbenzene	0.19	0.19
5-Methylindan	0.04	0.03
Naphthalene	0.02	0.02
	<u>100.00</u>	<u>100.00</u>

* All peak ID's confirmed by GC/MS analysis.

Quantified by GC/MS due to coelution with 1,2,4 TMB peak.

ASTM D-86 Distillation

<u>Volume % Distilled</u>	<u>deg F @760mm *</u>
IBP	320
5	322
10	323
20	324
30	325
40	326
50	327
60	328
70	329
80	331
90	335
95	339
Dry Point	346
End Point	350
Recovered	99.0%
Residue	1.0%
Loss	0.0%

* Average of duplicate runs.

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I. SYNOPSIS

Four groups of 15 male and 15 female Sprague-Dawley rats were exposed, six hours per day for five consecutive days, to either clean air or 153, 471 or 1,540 ppm of C₉ aromatic hydrocarbon vapors. A fifth group of five males and five females served as positive controls and received an injection of 40 mg/kg cyclophosphamide. Five males and five females from each exposure group were injected with 4 mg/kg colchicine, six hours later (which was 6, 24 and 48 hours after the last exposure), the animals were sacrificed, bone marrow collected, and chromosome spreads prepared. The positive control animals were evaluated at the 24-hour interval. Under the exposure conditions used for this study, C₉ aromatic hydrocarbon vapor did not induce chromosome or chromatid aberrations at any exposure level, and therefore, was considered to be non-mutagenic.

C. E. Ulrich
Charles E. Ulrich, B.S.
Study Director

1-8-88
Date

International Research and Development Corporation

V. EXPERIMENTAL DESIGN

This study consisted of one negative control group, one positive control group, and three test groups. The negative control group and each test group consisted of fifteen male and fifteen female rats, while the positive control group consisted of five males and five females. The negative control group was exposed to clean air, while the test groups were exposed to desired concentrations of approximately 150, 500 or 1,500 ppm C₉ aromatic hydrocarbons (C₉AHC). Exposures were conducted 6 hours per day for 5 consecutive days. The positive control group was treated with cyclophosphamide at 40 mg/kg via intraperitoneal injection.

At 6, 24 and 48 hours after the last exposure, 5 males and 5 females from the negative control, and each test group were sacrificed (6 hours after an injection of 4 mg/kg colchicine) for collection of bone marrow for slide preparation. The positive control animals were sacrificed at 24 hours after the clastogenic agent injection. Table 1 presents a summary of the experimental design.

C. E. Ulrich
Charles E. Ulrich, B.S.
Study Director

1-8-88
Date

418-030

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D. GENERAL OBSERVATIONS

1. Mortality

All animals were observed for mortality and signs of overt toxicity twice each day - prior to and again after each day's exposure. On non-exposure days the animals were observed once in the morning and once in the afternoon.

2. Body Weights

Body weights were recorded prior to exposure initiation and again prior to colchicine dosing

E. NECROPSY AND BONE MARROW SLIDE PREPARATION

Approximately 6 hours prior to the time of sacrifice, 6, 24 or 48 hours after the last exposure, each animal was injected with 4mg/kg of colchicine (1ml/kg, 4mg/ml of Hanks Balanced Salt Solution (HBSS)).

Animals were sacrificed by intraperitoneal sodium pentobarbital administration. Both femurs were removed and the marrow collected in HBSS warmed to 37°C. The cells were swollen in 0.56% hypoconic KCl, and were fixed in 3 parts methanol, one part glacial acetic acid. The cells from each rat were spread on four microscopic slides and stained with Giemsa.

The slides were scanned under 100X magnification for well resolved metaphase spreads. Such spreads were examined and scored under oil at 1000X magnification. If available, fifty spreads were evaluated for each animal.

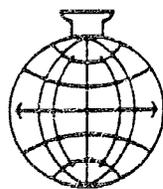
F. DATA HANDLING AND STATISTICAL METHODS

The following chromosome/chromatid aberration values were calculated for individual animal, and summarized by sex and sexes combined for each group:

- Total number of aberrations and frequency of aberrations per metaphase.
- Percent of metaphases with one or more aberrations
- Percent of metaphases with two or more aberrations

C. E. Ulrich
Charles E. Ulrich
Study Director

1-8-88
Date



International Research
and Development Corporation

MATTAWAN MICHIGAN U.S.A. 49071 TELEPHONE (616) 668-3336

SPONSOR: American Petroleum Institute

TEST ARTICLE: C₉ Aromatic Hydrocarbons

SUBJECT: Evaluation of C₉ Aromatic Hydrocarbons for Mutagenic
Potential - Bone Marrow Cytogenetics Test in Rats

DATE OF SUBMISSION: December 22, 1987

418-030

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I. SYNOPSIS

Four groups of 15 male and 15 female Sprague-Dawley rats were exposed, six hours per day for five consecutive days, to either clean air or 153, 471 or 1,540 ppm of C₉ aromatic hydrocarbon vapors. A fifth group of five males and five females served as positive controls and received an injection of 40 mg/kg cyclophosphamide. Five males and five females from each exposure group were injected with 4 mg/kg colchicine, six hours later. At 6, 24 and 48 hours after the last exposure, the animals were sacrificed, bone marrow collected, and chromosome spreads prepared. The positive control animals were evaluated at the 24-hour interval. Under the exposure conditions used for this study, C₉ aromatic hydrocarbon vapor did not induce chromosome or chromatid aberrations at any exposure level, and therefore, was considered to be non-mutagenic.

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II. QUALITY ASSURANCE STATEMENT

Study Title: Evaluation of C₉ Aromatic Hydrocarbons for Mutagenic Potential - Bone Marrow Cytogenetics Test in Rats

Test Article: C₉ Aromatic Hydrocarbons

The conduct of this study has been subjected to periodic inspections. The dates of inspection and the dates that findings were reported to management and the Study Director are listed on the following page.

This report has been reviewed by the International Research and Development Corporation Quality Assurance Department in accordance with the United States Environmental Protection Agency Good Laboratory Practice Regulations of May 2, 1984.

Approved By: Margery J. Wirtz 102/22/87
Margery J. Wirtz, B.S. Date
Director of Quality Assurance

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Quality Assurance Inspections

<u>Dates of Inspections</u>	<u>Dates of Reports to Management and to the Study Director</u>
4/25/87	4/25/87
5/04/87	5/07/87
5/07/87	5/13/87
5/12/87	5/15/87
8/12/87	10/14/87
10/14/87	

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III. OBJECTIVE

The objective of this study was to evaluate C₉ aromatic hydrocarbons for the potential to produce structural chromosomal aberrations in rats.

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IV. TEST MATERIAL

The test material was received from the Sponsor's repository (Experimental Pathology Laboratories, Herndon, VA) as follows:

<u>Date</u>	<u>Description</u>	<u>IRDC No.</u>
7-1-86	Six 55-gallon drums of "C-9 Aromatic Naphtha"	8953-1 to 8953-6
2-17-87	Six 55-gallon drums of "C-9 Aromatic Naphtha ASTM D-3734 Type 1"	8953B-1 to 8953B-6

Portions of drums 8953-6 and 8953B-1 were used for this study.

The composition of the test material in each drum used during the animal exposures was analyzed using methods described below. As each drum was opened, not more than 2 weeks before use, three 1 ml aliquots were accurately weighed into separate 50 ml volumetric flasks, diluted to the mark with internal standard solution, and then diluted again 5:10. Each solution was injected into the GC and the weight percent of each compound, including the $\geq C_{10}$'s fraction was calculated. Data from the three aliquots were averaged and the results reported to the Study Director before the drum was used on study. The results for all drums used on study were in agreement with the test material composition defined by the protocol, and shown in the following table:

<u>Compound</u>	<u>Weight Percent</u>
o-xylene	3.20
cumene	2.74
n-propylbenzene	3.97
4-ethyltoluene	7.05
3-ethyltoluene	15.1
2-ethyltoluene	5.44
1,3,5-trimethylbenzene	8.37
1,2,4-trimethylbenzene	40.5
1,2,3-trimethylbenzene	6.18
$\geq C_{10}$'s	6.19
Total	98.74

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V. EXPERIMENTAL DESIGN

This study consisted of one negative control group, one positive control group, and three test groups. The negative control group and each test group consisted of fifteen male and fifteen female rats, while the positive control group consisted of five males and five females. The negative control group was exposed to clean air, while the test groups were exposed to desired concentrations of approximately 150, 500 or 1,500 ppm C₉ aromatic hydrocarbons (C₉AHC). Exposures were conducted 6 hours per day for 5 consecutive days. The positive control group was treated with cyclophosphamide at 40 mg/kg via intraperitoneal injection.

At 6, 24 and 48 hours after the last exposure, 5 males and 5 females from the negative control, and each test group were sacrificed for collection of bone marrow for slide preparation. The positive control animals were sacrificed at 24 hours after the clastogenic agent injection. Table 1 presents a summary of the experimental design.

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VI. MATERIALS AND METHODS

A. ANIMALS

The Sprague-Dawley derived albino rats (Charles River CD®) for this study were received from the Charles River Breeding Laboratories, Inc., Portage, Michigan on April 21, 1987 under IRDC Purchase Order No. 3515. From the time of receipt to study termination, the animals were individually housed in suspended wire-mesh cages. During the 15-day quarantine period the animals were randomly allotted to the five groups and the various sacrifice intervals. The rats were individually identified with Monel® metal ear tags. During the quarantine period the animals were housed in rooms which were controlled for temperature and relative humidity with a 12-hour photo period, in accordance with the standards outlined in the "Guide for the Care and Use of Laboratory Animals" (DHEW No. NIH 85-23, 1985). During the non-exposure hours, the animals were maintained under similar conditions within the exposure chambers.

At the initiation of the exposure period, both male and female rats were seven weeks of age and were considered acceptable for use on the study based on general appearance.

B. ANIMAL DIET

Purina® Certified Rodent Chow® #5002 was available ad libitum, except during times of exposure. Each lot number of diet used was recorded. Each lot of diet was analyzed by Raltech Scientific Services for the presence of pesticides, heavy metals and aflatoxins. Tap water was available ad libitum. The drinking water at IRDC was analyzed quarterly for the presence of pesticides, heavy metals and coliforms. Results of these analyses are stored in the Archives of IRDC and are available upon Sponsor request.

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C. TEST MATERIAL ADMINISTRATION

1. Animal Exposures

All groups were exposed simultaneously in four 16m³ glass and stainless-steel exposure chambers. The chambers used for this study were also being used for animal exposures with the same material and at the same desired exposure levels, for IRDC study numbers 418-031 and 418-035. The Group V animals were exposed to clean air along with the Group I negative controls.

Due to logistical constraints imposed by the 6-hour sacrifice interval, the fifth day's exposure was conducted from approximately midnight until 6 A.M. In addition, the start of exposure on that day was staggered for each group to allow adequate time for slide preparation.

Prior to each day's exposure, the animal cages were removed from the chambers, excreta pans were removed, feed was removed, and the animals returned to the chamber for the appropriate exposure duration. After each day's exposure, the animals were again removed from the chambers, the chambers were cleaned, feed and excreta pans returned, and the animals replaced in the chamber.

Chamber ventilation air was provided by an HVAC system separate from the general laboratory air handling system. This air was particulate-filtered, and controlled for temperature and humidity. Chamber-air flow rate, temperature and relative humidity were recorded at approximately half-hour intervals during each day's exposure. Individual daily mean data can be found in Appendix A, while means and standard deviations of the daily means are presented in the following table:

Group No.	Chamber No.	Temp. (°C)		Relative Humidity (%)		Chamber Air Flow (L/min)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
I	C-4	24	1.7	58	5.5	3430	121
II	C-1	24	1.5	44	4.4	3600	101
III	C-10	24	1.2	47	2.3	3210	28
IV	C-3	24	1.2	45	6.2	3630	164
V		Same as C-4					

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2. Generation of Exposure Atmospheres

The exposure atmospheres were generated with the system shown schematically in Figure 1. Nitrogen, heated to 200°C by passage through a one-liter stainless-steel cylinder fitted with a 1500-watt band heater, was introduced at the bottom of a glass column 7.6cm in diameter and 30cm long, which was packed with glass beads. The liquid test material was delivered by a fluid metering pump (FMI) from a stainless steel safety can, through Teflon® tubing, to the bottom quarter of the column. The test material was vaporized as it flowed up the column co-current with the nitrogen flow. The vapors were passed to the chamber-inlet where dilution with chamber ventilation air reduced the concentration to the desired exposure level. The operating parameters for the generation systems are shown in the following table:

Parameter	Group No. and (Chamber No.)		
	II (C-1)	III (C-10)	IV (C-3)
FMI pump type	RPG-20-1/4	RPG-50-3/8	SYX-72-3/8
FMI pump setting	4.5	2.5	5.5
Approximate liquid flow rate (ml/min)	2.9	9.0	27.4
Ammeter setting for heater (A)	10	10	10
Nitrogen flow rate (L/min)	150	150	150
Chamber-air flow rate (L/min)	3200-4000	3200-4000	3200-4000
Ammeter setting for heat tapes (A)	NA	NA	2

3. Analysis of Exposure Atmospheres

a. Nominal

A nominal exposure concentration was determined for each exposure by weighing the test material reservoir before and after each day's exposure and dividing the difference in weight by the total volume of air that passed through the chamber during that exposure. The total

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air volume was calculated by multiplying the arithmetic mean of the half-hourly measurements of chamber-air flow rate by the exposure duration. The nominal concentration was converted from g/L to ppm by use of the following equation:

$$\text{ppm} = \frac{g}{L} \times \frac{R}{M} \times \frac{T}{P} \times 10^6 = \text{g/L} \times 2.06 \times 10^5$$

- Where: g = weight of test material used during the exposure, in grams
 L = volume of air that passed through the chamber during the exposure, in liters
 R = universal gas constant, $\frac{62.36\text{L} \cdot \text{mm Hg}}{\text{mole} \cdot \text{K}}$
 M = gram molecular weight of the test material, 120g/mole
 T = nominal laboratory temperature, 294°K
 P = nominal laboratory barometric pressure, 740mm Hg
 10⁶ = ppm conversion factor

b. Actual

The actual exposure concentrations were monitored with a gas-phase infrared spectrophotometer (IR). Exposure concentrations were reported to the nearest 1 ppm for concentrations less than 1,000 ppm, and to the nearest 10 ppm for concentrations above 1,000 ppm. Each chamber, including the control, was sampled by drawing chamber atmosphere through a Teflon® sample line, through an automatic sampling system, into the IR. The automatic sampling system allowed, by means of solenoid valves, each chamber to be sampled sequentially for approximately seven minutes of every hour. Both the automatic sampling system and the recording of actual concentrations were controlled by a Hewlett-Packard Model 3388A laboratory computer. The operating conditions for the IR were as follows:

```

-----
Instrument:                Miran 1A
Wavelength:                3.46micm
Range:                     1 Absorbance Unit Full Scale
Slit Width:                1 mm
Closed-Loop Volume:        5.64L
Pathlength Setting:        1.54
Gain Setting:              X10
Cell Pressure:             -1 psig
Meter Response Time:       10 seconds
Zero Gas:                  Chamber Supply Air
-----
  
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The IR was calibrated by use of the closed loop technique as recommended by the manufacturer. The closed-loop calibration consisted of injections of various volumes of the test material into the closed loop. The concentrations of test material in the closed loop was calculated as follows:

$$\text{conc. (ppm)} = \frac{V}{L} \times \frac{R \times T}{M} \times \frac{D}{P} \times 10^{-3} \times 10^6 = V \times 34.176$$

V = liquid volume of test material, in mL

L = volume of closed loop, 5.64L

R = universal gas constant, $\frac{62.36L \cdot \text{mmHg}}{\text{mole} \cdot ^\circ\text{K}}$

M = gram molecular weight of the test material, 120g/mole

T = nominal laboratory temperature, 294°K

D = density of test material, 0.86g/mL

P = IR cell pressure 1 psig below laboratory barometric pressure or 688mmHg

10^{-3} = mL to mcl conversion factor

10^6 = ppm conversion factor

The volumes of test material injected into the closed loop and the corresponding vapor concentrations are shown in the following table:

<u>Volume of Test Material Injected (mcl)</u>	<u>Cumulative Injection Volume (mcl)</u>	<u>Vapor Concentration in closed loop (ppm)</u>
1.5	1.5	51
1.5	3	103
5	8	273
5	13	444
10	23	786
10	33	1130
10	43	1470
10	53	1810

Each calibration point was replicated three times, and a standard curve of mean instrument response versus closed-loop vapor concentration was generated. Before each day's exposure the standard curve was verified by the injection of liquid test material into the closed loop at the concentrations shown in the table on the following page.

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<u>Volume of Test Material Injected (mcl)</u>	<u>Cumulative Injection Volume (mcl)</u>	<u>Vapor Concentration in closed loop (ppm)</u>
3	3	103
12	15	513
30	45	1540

If the measured concentrations for all three points were within $\pm 10\%$ of the actual closed-loop concentrations, the IR was considered to be in calibration.

In addition the accuracy of the analytical system was confirmed by the use of vapor standards prepared by vaporizing liquid test material into Tedlar® gas bags and then analyzing the bag atmosphere. The concentration of the test material vapors in the gas bags were calculated as follows:

$$\text{ppm} = \frac{V}{L} \times \frac{R \times T}{M} \times \frac{D}{P} \times 10^{-3} \times 10^{-6} = \frac{V}{L} \times 179.2$$

- Where: V = liquid volume of test material in mcl
 L = volume of air added to gas bag in liters
 R = universal gas constant, $\frac{62.36 \text{ L-mmHg}}{\text{mole} \cdot ^\circ\text{K}}$
 M = gram molecular weight, 120g/mole
 T = nominal laboratory temperature, 294°K
 P = nominal laboratory barometric pressure, 740mmHg
 10^{-3} = conversion factor, ml to mcl
 10^{-6} = ppm conversion factor

The gas bags were prepared as shown below:

<u>Desired Exposure Concentration (ppm)</u>	<u>Bag Volume (L)</u>	<u>Volume of Liquid Test Material (mcl)</u>	<u>Calculated Bag Conc. (ppm)</u>	<u>Acceptable Analyzed Range (ppm)</u>
150	100	84	151	135-166
500	100	280	502	450-550
1500	100	840	1510	1350-1660

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If the analyzed concentration of the gas bag was not within $\pm 10\%$ of the calculated concentration, a second bag was prepared and analyzed. If the second bag was also not within $\pm 10\%$ of the calculated concentrations, the Study Director was notified. The results of the gas bag checks are presented in Table 1.

c. Gas Chromatographic Analysis of Chamber Atmospheres

Once each day for the first five exposure days the composition (on a weight percentage basis) of the test material within each exposure chamber was determined by use of gas chromatography (GC). The compositional analysis was performed to demonstrate complete vaporization of the test material, not to define exposure concentrations.

The chamber samples for GC analysis were collected on two-stage (400 mg stage 1, 200 mg stage 2) charcoal tubes (SKC, Inc.) under the following sampling regimens:

<u>Desired Exposure Conc. (ppm)</u>	<u>Sample Flow Rate (cc/min)</u>	<u>Sample Duration (min)</u>	<u>Total Volume of sample (L)</u>
0	200	300	60
150	200	250	50
500	200	75	15
1,500	200	25	5

After the samples were collected the charcoal from each stage was placed in separate vials. Internal Standard Solution, prepared by adding 300 μ l of styrene to a 200ml volumetric flask and diluting to the mark with carbon disulfide, was added to each vial (4 ml for stage 1, 1 ml for stage 2). Each vial was then capped and the charcoal was allowed to desorb on a shaker for at least 150min. Approximately 1 μ l aliquots were drawn from the vials and injected into a calibrated GC (see below for

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details of calibration). The ratio of the peak area for the component to peak area of interval standard was determined for the following compounds/components:

o-xylene, cumene, n-propylbenzene (PB), 4-ethyltoluene (4-ET), 3-ethyltoluene (3-ET), 2-ethyltoluene (2-ET), 1,3,5-trimethylbenzene (1,3,5-TMB), 1,2,4-trimethylbenzene (1,2,4-TMB), and 1,2,3-trimethylbenzene (1,2,3-TMB). In addition, unidentified components with retention times greater than 1,2,3-TMB were defined as >C10's.

The weight percent for each compound was calculated by dividing the amount of each compound by the sum of the amounts of the individually quantitated compound and multiplying by 100 minus the weight percent of the >C10's. The calculations are shown below:

$$RFy = \frac{[CM]}{[ISS]} \times \frac{AIS}{Ay} \times \frac{wt. \%y_{CM}}{1}$$

$$Amt. y = \frac{Ay \times RFy \times [ISS] \times EV}{AIS}$$

$$wt. \% \geq C10 = \frac{AC10}{\sum Ay + AC10} \times 100$$

$$wt. \%y = \frac{Amt. y}{\sum Amt. y} \times (100 - wt. \% \geq C10)$$

Where: RFy = response factor for compound y
 [CM] = concentration of calibration mixture in working standard in mg/ml
 [ISS] = concentration of internal standard, 1.36mg/ml
 AIS = area of internal standard from chromatogram
 Ay = area from chromatogram for and individual compound (o-xylene, cumene, PB, 1,3,5-TMB, 1,2,3-TMB, 1,2,4-TMB, 2-ET, 3-ET, 4-ET)
 wt. %y_{CM} = weight percent of individual compound y in calibration mixture
 Amt. y = amount of individual compound y, in mg
 EV = elution volume, 4ml for stage 1 samples and 1ml for stage 2 samples
 wt. % > C10 = weight percent of compounds with retention times > 1,2,3-TMB
 AC10 = area from chromatogram for compounds with retention times > 1,2,3-TMB
 wt. % y = weight percent of individual compound y

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Both stages of the charcoal tubes were evaluated. At no time was there any evidence of breakthrough into the second stage. The GC was calibrated with a mixture of known composition prepared to be similar to the test material. The GC operating parameters for the charcoal tube analysis and for analysis of the neat liquid (as described above under Test Material) are shown in the following table:

Instrument:	Varian 2400 Gas Chromatograph
Column:	6' X 2mm ID glass
Packing:	5% SP-1200/1.75% Bentone 34 on 100/120 mesh supelcoport
Detector:	Flame ionization
Temperatures	
Injector:	160°C
Column:	75°C
Detector:	200°C
Gas Flow Rates	
N ₂ :	30cc/min at 60psig
H ₂ :	40cc/min at 40psig
Air:	300cc/min at 60psig
Electrometer Attenuation	
Setting:	16 X 10 ⁻¹¹ A
Injection Volume:	1μcl
Integrator:	HP3388A
Chart Speed:	0.5cm/min
Chart Display	
0-15 min:	26
15-40 min:	22
Threshold Setting	
0-15 min:	6
15-40 min:	1

Results of the GC analysis of the vapor composition of the exposure atmospheres are presented in Appendix B.

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D. GENERAL OBSERVATIONS

1. Mortality

All animals were observed for mortality and signs of overt toxicity twice each day - prior to and again after each day's exposure. On non-exposure days the animals were observed once in the morning and once in the afternoon.

2. Body Weights

Body weights were recorded prior to exposure initiation and again prior to colchicine dosing

E. NECROPSY AND BONE MARROW SLIDE PREPARATION

Approximately 6 hours prior to the time of sacrifice, 6, 24 or 48 hours after the last exposure, each animal was injected with 4mg/kg of colchicine (1ml/kg, 4ug/ml of Hanks Balanced Salt Solution (HBSS)).

Animals were sacrificed by intraperitoneal sodium pentobarbital administration. Both femurs were removed and the marrow collected in HBSS warmed to 37°C. The cells were swollen in 0.56% hypotonic KCl, and were fixed in 3 parts methanol, one part glacial acetic acid. The cells from each rat were spread on four microscopic slides and stained with Giemsa.

The slides were scanned under 100X magnification for well resolved metaphase spreads. Such spreads were examined and scored under oil at 1000X magnification. If available, fifty spreads were evaluated for each animal.

F. DATA HANDLING AND STATISTICAL METHODS

The following chromosome/chromatid aberration values were calculated for individual animal, and summarized by sex and sexes combined for each group:

- Total number of aberrations and frequency of aberrations per metaphase.
- Percent of metaphases with one or more aberrations
- Percent of metaphases with two or more aberrations

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Where possible each of the above data sets were to be evaluated by the Kruskal-Wallis multiple-group comparison test at the 0.05 significance level. Significant differences were then further evaluated by the Mann-Whitney U test at the 0.05 significance level.

G. DATA STORAGE

All data, slides and reports from the study, will be retained for at least 10 years after completion of the study and stored in the IRDC Archives and will be made available for inspection upon request by personnel authorized by the Sponsor. Appropriate samples of the test article will be retained for 10 years following completion of the study.

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VII. RESULTS

A. EXPOSURE CONCENTRATIONS

Exposures for this study were conducted from May 7 1987 through May 11, 1987. The daily mean nominal and analytically (IR) determined actual exposure concentrations are shown in Table 3, and are summarized below:

Group No.	Chamber No.	Exposure Concentration (ppm)			
		Nominal		Actual	
		Mean	S.D.	Mean	S.D.
II	C-1	150	6.0	153	9.6
III	C-10	426	38.0	471	13.1
IV	C-3	1360	59	1540	48

The fact that the nominal exposure concentrations were lower than the actual measured levels, particularly at the highest level, was quite unusual. After the completion of the study, it was determined that the calibration of the analytical instrument was slightly in error. Based on the gas bag data collected a few days prior to study initiation, this error was on the order of 10%, or possibly slightly less. Due to the inherent limitations of the methods used, the absolute accuracy of measurement for both nominal and actual concentrations were no better than +5%. Therefore, differences between nominal and actual concentrations on the order of 10% or less, cannot be meaningfully differentiated, and thus, the actual concentrations were considered the best estimate of animal exposure levels.

B. GENERAL OBSERVATIONS

1. Appearance, Behavior and Mortality

There were no signs of toxicity in any animals from any group during the twice daily mortality checks.

2. Body Weights

Individual animal body weights are presented in Table 4, while summarized data are presented in the following table:

Sacrifice Interval	Sex	Group No.	Body Weights (g)			
			Pre-exposure		Post-exposure	
			Mean	S.D.	Mean	S.D.
6-Hour	Males	I	228	12.3	251	14.9
		II	240	12.5	262	16.2
		III	221	10.0	228	17.1
		IV	237	14.4	237	16.6

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<u>Sacrifice Interval</u>	<u>Sex</u>	<u>Group No.</u>	<u>Body Weights (g)</u>			
			<u>Pre-exposure</u>		<u>Post-exposure</u>	
			<u>Mean</u>	<u>S.D.</u>	<u>Mean</u>	<u>S.D.</u>
24-Hour	Females	I	165	4.5	172	9.5
		II	153	6.6	158	7.9
		III	163	10.5	166	11.8
		IV	158	10.8	162	10.1
	Males	I	234	8.8	280	10.6
		II	231	11.0	274	17.1
		III	239	6.0	275	12.8
		IV	228	10.5	250*	13.2
		V	252	46.4	271	18.3
	Females	I	166	8.0	191	9.2
II		159	4.3	180	6.1	
III		158	11.5	176	12.1	
IV		157	5.3	169*	8.8	
V		151	7.3	174	10.7	
48-Hour	Males	I	235	8.3	286	10.8
		II	220	10.6	269	13.8
		III	232	10.2	274	9.4
		IV	229	13.9	255*	18.7
	Females	I	154	14.3	180	17.2
		II	157	7.4	182	10.5
		III	164	5.4	182	6.2
		IV	152	4.5	167	4.6

*Statistically different from Group I (control) at $p < 0.05$.

It was apparent that post-exposure absolute body weights for Group IV males at the 24 and 48 hour intervals were about 11-12% lower than the Group I controls. Body weights of females in Group IV, as compared to Group I, were also depressed, but to a lesser extent. These differences were statistically significant.

The following table presents body weight gain data, post-exposure minus pre-exposure, for the Group IV animals:

<u>Sacrifice Interval</u>	<u>Body Weight Gain (% of Group I - Controls body weight gain)</u>	
	<u>Males</u>	<u>Females</u>
6-Hour	0	+57
24-Hour	+43	+48
48-Hour	+51	+58

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Apparently 5 days of exposure to the high level had a marked effect on body weight gain for both males and females.

C. CYTOGENETICS DATA

Individual animal bone marrow chromosome data are presented in Table 5.

Data were considered adequate for evaluation only under the following conditions: A given animal must have at least 30 readable metaphase spreads, and there must be at least 3 animals with adequate data, either of a given sex or sexes combined, for a given group. The following table presents the summarized data:

Interval	Group	Number and Sex	Number of Spreads	Number of Aberrations	% Aberr. per Metaphase	% Metaphases with	
						>1 Aberr.	>2 Aberr.
6-Hour	I	3 M	150	0	0	0	0
		3 F	250	0	0	0	0
		8 Combined	400	0	0	0	0
	II	3 M	250	0	0	0	0
		4 F	200	0	0	0	0
		9 Combined	450	0	0	0	0
	III	3 M	250	0	0	0	0
		3 F	237	0	0	0	0
		10 Combined	487	0	0	0	0
	IV	3 M	250	0	0	0	0
		4 F	200	0	0	0	0
		9 Combined	450	0	0	0	0
24-Hour	I	4 M	200	0	0	0	0
		3 F	250	1	0.4	0.4	0
		9 Combined	450	1	0.2	0.2	0
	II	3 M	250	0	0	0	0
		3 F	232	0	0	0	0
		10 Combined	482	0	0	0	0
	III	3 M	250	0	0	0	0
		3 F	250	0	0	0	0
		10 Combined	500	0	0	0	0
	IV	3 M	250	1	0.4	0.4	0
		3 F	250	0	0	0	0
		10 Combined	500	1	0.2	0.2	0
	V	4 M	203	70	34.5	16.3	10.3
		3 F	250	60	24	13.2	6.4
		9 Combined	453	130	28.7	14.6	8.2

Aberr. = Aberrations

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Interval	Group	Number and Sex	Number of Spreads	Number of Aberrations	% Aberr. per Metaphase	% Metaphases with	
						>1 Aberr.	>2 Aberr.
48-Hour	I	2 M	100	0	0	0	0
		2 F	100	0	0	0	0
		4 Combined	200	0	0	0	0
	II	2 M	100	0	0	0	0
		2 F	100	0	0	0	0
		4 Combined	200	0	0	0	0
	III	3 M	150	0	0	0	0
		1 F	50	0	0	0	0
		4 Combined	200	0	0	0	0
	IV	2 M	100	0	0	0	0
		1 F	50	0	0	0	0
		3 Combined	150	0	0	0	0

Aberr. = Aberrations

These data clearly show that the test material did not induce chromatid or chromosome aberrations in excess of negative controls at the 6 and 24-hour post-exposure intervals. Statistical analysis was not possible due to the few number of aberrations found. Much of the data for the 48-hour interval was lost due to inadequate staining of the chromosome spreads. Therefore, too few animals could be evaluated at the 48-hour interval for demonstrating possible differences between sexes. However, when data from the sexes were combined, there were 4, 4, 4 and 3 animals in Groups I, II, III and IV, respectively. No chromatid or chromosome abnormalities were observed on any of the 48-hour interval slides.

Nine of the 10 positive control animals provided adequate data for evaluation. Eight of these animals showed chromatid and chromosome aberrations and chromosome aberrations in excess of the negative controls.

D. ANIMAL DISPOSITION

All animal carcasses were discarded following the bone marrow harvest.

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VIII. SIGNATURES OF SUPERVISORY STAFF

Laboratory
Supervisor: *Ben A. Culy* 12-17-87
Ben A. Culy
Unit Supervisor
Inhalation Toxicology
Date

Study
Suparvision: *John G. Drummond* 12-17-87
John G. Drummond, Ph.D.
Section Head
Inhalation Toxicology
Date

Consulting
Cytogeneticist: *Gyula Ficsor* 12/11/87
Gyula Ficsor, Ph.D.
Date

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IX. DISCUSSION AND CONCLUSION

Five days of exposure to C9ABC at levels of approximately 1,540 ppm produced biologically significant decrements in absolute body weights and body weight gain, as compared to the air exposed group. There were, however, no other apparent signs of toxicity in any of the exposed animals.

Further, C9ABC exposure did not induce chromosome or chromatid aberrations. The data overwhelmingly support this conclusion for the 6 and 24-hour post-exposure intervals. While data were available only from 4 negative control and 3 or 4 exposed animals per treatment group at the 48-hour interval, these data also support the conclusion that C9ABC does not induce chromatid or chromosome aberrations.

To the best of my knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of the study. This study was conducted in conformance with the Good Laboratory Practice Regulations. This report accurately reflects the raw data obtained during the performance of the study.

Charles E. Ulrich
Charles E. Ulrich, B.S.
Director of Inhalation Toxicology

12-22-87
Date

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FIGURE NO. 1. SCHEMATIC DIAGRAM OF GENERATION AND EXPOSURE SYSTEM

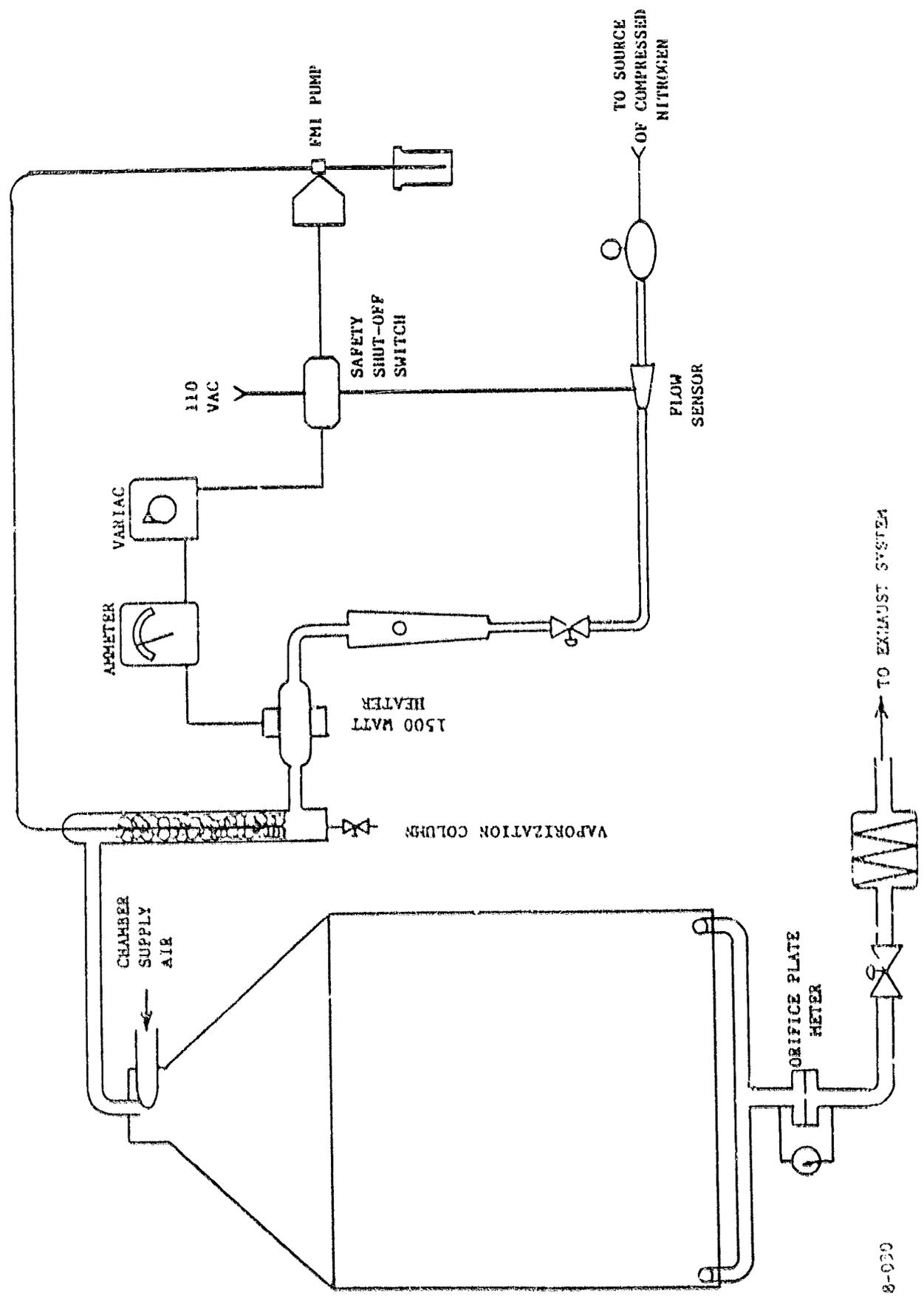


TABLE 1. Gas Bag Checks on Analytical System

Date	Calculated Conc. (ppm)	Instrument Response (mv)	Measured Conc. (ppm)	% Calculated Concentration
4-29-87	100	77.7	98	98
	251	151.7	244	97
	502	381.9	529	105
	1,000	592.6	1,080	108
	1,510	988.2	1,630	108

Conc. - Concentration

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TABLE 2. Summary of Experimental Design

Group Number	Desired Exposure Conditions	Chamber Number	Total Animals On Study		Number of Animals Evaluated At Indicated Hours Post-Dose						
			Male	Female	6		24		48		
					H	F	M	F	M	F	
I	Negative Control	C-4	15	15	5	5	5	5	5	5	5
II	150 ppm C ₉ AHC	C-1	15	15	5	5	5	5	5	5	5
III	500 ppm C ₉ AHC	C-10	15	15	5	5	5	5	5	5	5
IV	1,500 ppm C ₉ AHC	C-3	15	15	5	5	5	5	5	5	5
V	Positive Control	C-4	5	5	-	-	5	5	-	-	-

TABLE 3. Daily Nominal and Mean Actual Exposure Concentrations (ppm)

Date	Group No. (Chamber No.)					
	II (C-1)		III (C-10)		IV (C-3)	
	Nominal	Actual	Nominal	Actual	Nominal	Actual
5-7-87	143	138	453	477	1430	1560
5-8-87	151	153	446	469	1360	1580
5-9-87	147	153	359	491	1270	1570
5-10-87	159	159	437	462	1380	1470
5-11-87	148	150	433	458	1380	1500

TABLE 4. Individual Animal Body Weights (g)
Group I

Sacrifice Interval	Sex	Animal No.	Pre-exposure	Post-exposure
6-Hour	M	26582	233	246
		26593	227	254
		26594	244	273
		26595	227	249
		26596	210	232
	F	26598	159	163
		26600	167	178
		26602	161	160
		26605	170	178
		26611	166	180
24-Hour	M	26583	245	278
		26585	224	267
		26586	228	275
		26590	241	295
		26592	233	285
	F	26601	171	189
		26604	157	182
		26608	158	183
		26609	171	198
		26610	174	203
48-Hour	M	26584	232	297
		26587	243	289
		26588	245	295
		26589	230	278
		26591	226	272
	F	26597	140	166
		26599	145	166
		26603	151	179
		26606	177	208
		26607	155	183

TABLE 4. Cont. Individual Animal Body Weights (g)
Group II

Sacrifice Interval	Sex	Animal No.	Pre-exposure	Post-exposure
6-Hour	M	26617	225	243
		26621	249	272
		26623	245	269
		26624	229	246
		26625	253	279
	F	26627	158	159
		26628	143	145
		26631	160	165
		26638	153	163
		26640	153	160
24-Hour	M	26612	213	247
		26614	239	283
		26618	228	266
		26619	234	284
		26626	240	288
	F	26630	161	189
		26632	155	172
		26633	153	179
		26637	162	181
		26639	162	178
48-Hour	M	26613	219	270
		26615	210	258
		26616	217	259
		26620	216	265
		26622	238	292
	F	26629	155	183
		26634	156	175
		26635	167	199
		26636	147	172
		26641	161	183

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TABLE 4. Cont. Individual Animal Body Weights (g)
Group III

Sacrifice Interval	Sex	Animal No.	Pre-exposure	Post-exposure	
6-Hour	M	26643	207	214	
		26644	221	210	
		26645	233	246	
		26649	216	226	
		26654	227	246	
	F	26659	173	179	
		26662	147	149	
		26664	164	170	
		26668	158	160	
		26669	171	173	
	24-Hour	M	26650	249	284
			26651	235	280
			26652	234	286
26653			237	267	
26656			238	256	
F		26657	139	160	
		26665	156	177	
		26666	166	189	
		26667	164	186	
		26671	166	168	
48-Hour	M	26642	234	280	
		26646	219	263	
		26647	223	273	
		26648	241	287	
		26655	241	269	
	F	26658	171	183	
		26660	161	174	
		26661	157	179	
		26663	167	190	
		26670	164	186	

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TABLE 4. Cont. Individual Animal Body Weights (g)
Group IV

Sacrifice Interval	Sex	Animal No.	Pre-exposure	Post-exposure
6-Hour	M	26673	252	261
		26675	243	245
		26679	230	230
		26680	216	217
		26686	246	234
	F	26687	154	159
		26689	143	148
		26696	172	176
		26698	163	164
		26701	157	164
24-Hour	M	26672	219	239
		26674	243	266
		26676	235	263
		26677	221	243
		26683	222	240
	F	26691	153	162
		26695	163	178
		26697	159	175
		26699	160	174
		26700	150	158
48-Hour	M	26678	207	228
		26681	235	247
		26682	229	265
		26684	245	277
		26685	228	259
	F	26688	155	173
		26690	145	161
		26692	150	167
		26693	154	170
		26694	156	165

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TABLE 4. Cont. Individual Animal Body Weights (g)
Group V

Sacrifice Interval	Sex	Animal No.	Pre-exposure	Post-exposure
24-Hour	M	26702	227	269
		26703	248	285
		26704	209	244
		26705	230	268
		26706	247	291
	F	26707	163	192
		26708	146	171
		26709	145	169
		26710	152	174
		26711	148	164

TABLE 5. Bone Marrow Chromosome Evaluations for Group I at the 6-hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with ≥ 1 Aberrations	No. of Spreads with ≥ 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with ≥ 1 Aberrations	% Spreads with ≥ 2 Aberrations
Males							
26582	50	0	0	0	0	0	0
26593	50	0	0	0	0	0	0
26594	50	0	0	0	0	0	0
26595	6	0	0	0	0	0	0
26596	3	0	0	0	0	0	0
Females							
26598	50	0	0	0	0	0	0
26600	50	0	0	0	0	0	0
26602	50	0	0	0	0	0	0
26605*	50	0	0	0	0	0	0
26611	50	0	0	0	0	0	0

*One polyploid observed

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TABLE 5. CONT. Bone Marrow Chromosome Evaluations for Group II at the 6-hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with > 1 Aberrations	No. of Spreads with > 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with > 1 Aberrations	% Spreads with > 2 Aberrations	
Males								
26617	50	0	0	0	0	0	0	
26621	50	0	0	0	0	0	0	
26623	50	0	0	0	0	0	0	
26624	50	0	0	0	0	0	0	
26625	50	0	0	0	0	0	0	
Females								
26627	50	0	0	0	0	0	0	
26628	0	0	0	0	0	0	0	
26631	50	0	0	0	0	0	0	
26638	50	0	0	0	0	0	0	
26640	50	0	0	0	0	0	0	

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TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group III at the 6-hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with ≥ 1 Aberrations	No. of Spreads with ≥ 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with ≥ 1 Aberrations	% Spreads with ≥ 2 Aberrations
Males							
26643	50	0	0	0	0	0	0
26644	50	0	0	0	0	0	0
26645	50	0	0	0	0	0	0
26649	50	0	0	0	0	0	0
26654	50	0	0	0	0	0	0
Females							
26659	50	0	0	0	0	0	0
26662	50	0	0	0	0	0	0
26664	50	0	0	0	0	0	0
26668	50	0	0	0	0	0	0
26669	50	0	0	0	0	0	0

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TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group IV at the 6-hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with ≥ 1 Aberrations	No. of Spreads with ≥ 2 Aberrations	Aberration frequency per Metaphase	% Spreads with ≥ 1 Aberrations	% Spreads with ≥ 2 Aberrations
Males							
26675	50	0	0	0	0	0	0
26675	50	0	0	0	0	0	0
26675	50	0	0	0	0	0	0
26680	50	0	0	0	0	0	0
26686	50	0	0	0	0	0	0
Females							
26687	50	0	0	0	0	0	0
26689	6	0	0	0	0	0	0
26696	50	0	0	0	0	0	0
26698	50	0	0	0	0	0	0
26701*	50	0	0	0	0	0	0

*One polyploid observed

4118-030

TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group I at the 24-Hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with ≥ 1 Aberrations	No. of Spreads with ≥ 2 Aberrations	Aberration Frequency per Metaphase	\$ Spreads with ≥ 1 Aberrations	\$ Spreads with ≥ 2 Aberrations
Males							
26583	50	0	0	0	0	0	0
26585	7	0	0	0	0	0	0
26586	50	0	0	0	0	0	0
26590	50	0	0	0	0	0	0
26592	50	0	0	0	0	0	0
Females							
26601	50	0	0	0	0	0	0
26604	50	0	0	0	0	0	0
26608	50	0	0	0	0	0	0
26609	50	1*	1	0	0.02	2	0
26610	50	0	0	0	0	0	0

*Chromatid fragment

418-030

TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group II at the 24-Hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with ≥ 1 Aberrations	No. of Spreads with ≥ 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with ≥ 1 Aberrations	% Spreads with ≥ 2 Aberrations
Males							
26612	50	0	0	0	0	0	0
26614	50	0	0	0	0	0	0
26618	50	0	0	0	0	0	0
26619	50	0	0	0	0	0	0
26626	50	0	0	0	0	0	0
Females							
26630	32	0	0	0	0	0	0
26632*	50	0	0	0	0	0	0
26633	50	0	0	0	0	0	0
26637	50	0	0	0	0	0	0
26639	50	0	0	0	0	0	0

*One polyploid observed

418-050

TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group III at the 24-Hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with ≥ 1 Aberrations	No. of Spreads with ≥ 2 Aberrations	Aberration frequency per Metaphase	% Spreads with ≥ 1 Aberrations	% Spreads with ≥ 2 Aberrations
Males							
26650	50	0	0	0	0	0	0
26651	50	0	0	0	0	0	0
26652	50	0	0	0	0	0	0
26653	50	0	0	0	0	0	0
26656	50	0	0	0	0	0	0
Females							
26657	50	0	0	0	0	0	0
26665	50	0	0	0	0	0	0
26666	50	0	0	0	0	0	0
26667	50	0	0	0	0	0	0
26671	50	0	0	0	0	0	0

418-030

TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group IV at the 24-hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with ≥ 1 Aberrations	No. of Spreads with ≥ 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with ≥ 1 Aberrations	% Spreads with ≥ 2 Aberrations
Males							
26672	50	0	0	0	0	0	0
26674	50	0	0	0	0	0	0
26676	50	0	0	0	0	0	0
26677	50	1*	1	0	0.02	2	0
26683	50	0	0	0	0	0	0
Females							
26691	50	0	0	0	0	0	0
26695	50	0	0	0	0	0	0
26697	50	0	0	0	0	0	0
26699	50	0	0	0	0	0	0
26700	50	0	0	0	0	0	0

*Chromatid fragment

418-030

TABLE 5. CONT. Bone Marrow Chromosome Evaluations for Group I at the 48-hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with > 1 Aberrations	No. of Spreads with > 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with > 1 Aberrations	% Spreads with > 2 Aberrations	
Males								
26584	50	0	0	0	0	0	0	
26587	50	0	0	0	0	0	0	
26588	0	0	0	0	0	0	0	
26589	4	0	0	0	0	0	0	
26591	0	0	0	0	0	0	0	
Females								
26597	50	0	0	0	0	0	0	
26599	0	0	0	0	0	0	0	
26603	0	0	0	0	0	0	0	
26606	50	0	0	0	0	0	0	
26607	0	0	0	0	0	0	0	

418-030

TABLE 5. CONT. Some Harrow Chromosome Evaluations for Group II at the 48-Hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with > 1 Aberrations	No. of Spreads with > 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with > 1 Aberrations	% Spreads with > 2 Aberrations
Males							
26613	0	0	0	0	0	0	0
26615	0	0	0	0	0	0	0
26616	50	0	0	0	0	0	0
26620	0	0	0	0	0	0	0
26622	50	0	0	0	0	0	0
Females							
26629	0	0	0	0	0	0	0
26634	0	0	0	0	0	0	0
26635	0	0	0	0	0	0	0
26636	50	0	0	0	0	0	0
26641	50	0	0	0	0	0	0

418-030

TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group III at the 48-hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with > 1 Aberrations	No. of Spreads with > 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with > 1 Aberrations	% Spreads with > 2 Aberrations
Males							
26642	50	0	0	0	0	0	0
26646	0	0	0	0	0	0	0
26647	50	0	0	0	0	0	0
26648	50	0	0	0	0	0	0
26655	0	0	0	0	0	0	0
Females							
26673	0	0	0	0	0	0	0
26660	0	0	0	0	0	0	0
26661	0	0	0	0	0	0	0
26663	0	0	0	0	0	0	0
26670	50	0	0	0	0	0	0

416-030

TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group IV at the 48-hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with > 1 Aberrations	No. of Spreads with > 2 Aberrations	Aberration Frequency per Metaphase	% Spreads with > 1 Aberrations	% Spreads with > 2 Aberrations
Males							
26676	0	0	0	0	0	0	0
26681	0	0	0	0	0	0	0
26682	50	0	0	0	0	0	0
26684	0	0	0	0	0	0	0
26685	50	0	0	0	0	0	0
Females							
26688	0	0	0	0	0	0	0
26690	50	0	0	0	0	0	0
26692	0	0	0	0	0	0	0
26693	0	0	0	0	0	0	0
26694	0	0	0	0	0	0	0

418-030

TABLE 5. Cont. Bone Marrow Chromosome Evaluations for Group V at the 24-Hour Evaluation Interval

Animal Number/Sex	Total No. of Spreads Evaluated	Total No. of Aberrations Found	No. of Spreads with ≥ 1 Aberrations		No. of Spreads with ≥ 2 Aberrations		Aberration Frequency per Metaphase	% Spreads with ≥ 1 Aberrations		% Spreads with ≥ 2 Aberrations	
Males											
26702	50	11	7	2	0.22	14	4	21.6	15.7	0	0
26703	51	26	11	6	0.51	0	0	0	0	0	0
26704	0	0	0	0	0	0	0	0	0	0	0
26705	52	15	6	4	0.29	11.5	7.7	18.0	14	0	0
26706*	50	18	9	7	0.36	0	0	0	0	0	0
Females											
26707*	50	5	3	2	0.1	6	4	0	0	0	0
26708	50	0	0	0	0	0	0	0	0	0	0
26709*	50	19	11	4	0.38	22	8	36	18	0	0
26710**	50	34	18	9	0.68	2	2	0.04	0	0	0
26711	50	2	1	1	0.04	0	0	0	0	0	0

*One gap observed

**Six gaps observed

418-090

APPENDIX A
Daily Environment Data

418-030

Daily Mean Chamber Environment Data*

Chamber No.	Date of Exposure	Temp. (°C)		Relative Humidity (%)		Chamber Air Flow (L/min)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
C-4	May 7, 1987	25	0.5	59	3.6	3560	123
	May 8, 1987	24	0.5	53	4.1	3520	30
	May 9, 1987	25	2.3	59	8.4	3390	40
	May 10, 1987	23	1.2	67	4.2	3250	13
	May 11, 1987	21	0	54	11.8	3430	69
C-1	May 7, 1987	23	1.0	39	8.5	3430	195
	May 8, 1987	23	0.7	44	6.9	3650	132
	May 9, 1987	24	2.4	47	4.0	3590	33
	May 10, 1987	26	1.6	50	2.1	3640	39
	May 11, 1987	22	0.4	41	7.4	3690	9
C-10	May 7, 1987	24	0.5	47	0.5	3200	54
	May 8, 1987	23	0.3	46	1.7	3200	17
	May 9, 1987	24	2.2	48	3.7	3180	21
	May 10, 1987	26	1.7	49	2.1	3250	15
	May 11, 1987	23	0.5	43	4.8	3230	16
C-3	May 7, 1987	24	0.5	53	5.9	3410	41
	May 8, 1987	23	0.6	41	8.6	3590	127
	May 9, 1987	24	1.9	42	5.8	3870	165
	May 10, 1987	26	1.9	49	2.1	3630	131
	May 11, 1987	23	0.4	38	2.4	3640	73

418-030

Temp. - Temperature

S.D. - Standard Deviation

*The "n" for each daily mean was 12

APPENDIX B
Gas Chromatographic Analysis of Chamber Atmospheres

418-030

Gas Chromatographic Analysis of Chamber Atmospheres (wt %)

Chamber	Compound	Date Sample Collected					
		5-4-87	5-5-87	5-6-87	5-7-87	5-8-87	5-11-87
C-1 (150 ppm desired)	o-xylene	-	-	-	3.16	3.18	3.19
	cumene	-	-	-	2.83	2.82	2.84
	NPB	-	-	-	4.08	4.06	4.09
	4-ET	-	-	-	7.30	7.29	7.32
	3-ET	-	-	-	15.5	15.5	15.6
	2-ET	-	-	-	5.39	5.40	5.43
	1,3,5-TMB	-	-	-	8.30	8.30	8.34
	1,2,4-TMB	-	-	-	40.6	40.5	40.5
	1,2,3-TMB	-	-	-	5.94	5.99	5.93
	>C10's	-	-	-	6.90	6.94	6.77
C-10 (500 ppm desired)	o-xylene	*	3.18	3.18	3.17	3.17	3.18
	cumene	*	2.82	2.83	2.83	2.82	2.83
	NPB	*	4.08	4.07	4.08	4.08	4.08
	4-ET	*	7.31	7.29	7.31	7.29	7.28
	3-ET	*	15.5	15.5	15.5	15.5	15.5
	2-ET	*	5.40	5.40	5.41	5.41	5.40
	1,3,5-TMB	*	8.31	8.29	8.31	8.30	8.27
	1,2,4-TMB	*	40.5	40.5	40.6	40.6	40.5
	1,2,3-TMB	*	5.96	5.99	5.95	5.96	5.97
	>C10's	*	6.91	7.04	6.78	6.83	7.01
C-3 (1,500 ppm desired)	o-xylene	3.17	3.18	3.19	3.19	3.18	3.19
	cumene	2.82	2.83	2.83	2.83	2.83	2.85
	NPB	4.08	4.09	4.09	4.09	4.08	4.09
	4-ET	7.30	7.32	7.32	7.32	7.31	7.28
	3-ET	15.5	15.6	15.6	15.5	15.5	15.5
	2-ET	5.40	5.41	5.41	5.42	5.41	5.42
	1,3,5-TMB	8.32	8.32	8.33	8.33	8.32	8.30
	1,2,4-TMB	40.6	40.6	40.7	40.6	40.6	40.5
	1,2,3-TMB	5.95	5.92	5.93	5.97	5.95	5.94
	>C10's	6.83	6.73	6.65	6.79	6.81	6.84

- - Samples not collected

418-030

*Samples collected, but analytical results
were unreliable