Report

Study Title:

1,3-Butadiene: Whole-Body Inhalation Exposure of Rats and Mice

LRRI Protocol Number: FY04-051 Exposure Only

Sponsor: American Chemistry Council
Project Number OLF-121.0-BD-Exp-LRRI

Test Facility: [redacted]

Study Director: [redacted]

Sponsor Representative for ACC Study: Elizabeth Moran, PhD

Study Monitor for ACC Study: Robert Barter, Ph.D., DABT Exxon Mobil

[redacted] Study Director [redacted] Date

Sponsor Representative: Elizabeth Moran, PhD Date

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1.0 STUDY SCHEDULE

Study Initiation Date:	July 2004
Experimental Start Date:	October 4, 2004
Experimental Termination Date:	November 5, 2004
Report Date:	July 18, 2005

2.0 OBJECTIVE OF STUDY

The American Chemistry Council (ACC) Olefins Panel requested a study of inhalation exposure of male and female Sprague-Dawley rats and male and female B6C3F1 mice to 1,3-butadiene. The study objective was to collect blood and tissues for transfer to the University of North Carolina (UNC), where the samples are being analyzed for butadiene-derived hemoglobin adducts and tissue DNA adducts. The exposures were completed according to the final approved protocol, and samples were successfully shipped to UNC for analysis.

2.1 Good Laboratory Practices Standards

This study was conducted in conformance with specific sections of the U.S. Environmental Protection Agency's Good Laboratory Practice regulations. These included 40 CFR, Part 792, as amended at 54 FR 34034: Sections 792.1(c), 792.1330 (e), 792.33 (a), (b), and (c) and 792.83. There was no official LRRI Quality Assurance Oversight of this work.

2.2 Animal Welfare Standards

The [redacted] animal facilities are directed by an American College of Laboratory Animal Medicine (ACLAM)-certified Attending Veterinarian who supervises animal health. The LRRI is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (File #000200). The [redacted] has a current approved Animal Welfare Assurance filed with NIH, Office of Protection from Research Risks (Assurance #[redacted]) and is a USDA-registered facility (Registration #[redacted]) in good standing that is regularly inspected by USDA veterinarians. The Study Protocol was approved by the [redacted] and Use Committee (IACUC).

3.0 TEST FACILITY

3.1 Facility

[redacted]

Courier Address and Location of Laboratory:

[redacted]

3.2 Key Study Personnel

Study Director and Chemist: [redacted]

Veterinary Pathologist: [redacted]

Attending Veterinarian: [redacted]

Aerosol Scientist: [redacted]

4.0 TEST AND CONTROL ARTICLES

4.1 Components of Test and Control Articles

Table 1. Components of Test and Control Articles.

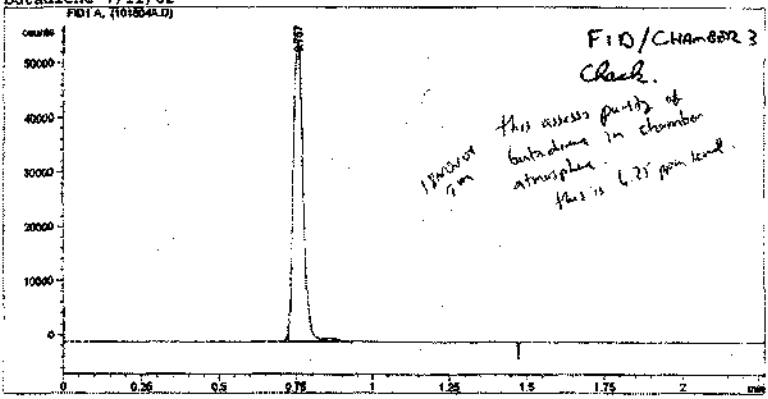
Component	CAS	Source	Storage Conditions	Exp. Date
Test Article: 1,3-Butadiene	106-99-0	Matheson	20–25°C	NA
Control Article	106-99-0	SCOTT 424402L	20–25°C	8/2006

4.2 Identity and Purity of Test and Control Articles

Test article identity was confirmed by mass spectrometric (gas chromatography/mass spectrometry, GCMS) analysis. GCMS analysis was conducted both on a certified reference standard (from Scott Specialty Gases) and the test article atmosphere. Both the spectra and chromatographic retention time of the test article was matched to the certified standard. Purity was confirmed by analysis of chamber atmospheres on a flame ionization detector (FID). Test atmosphere was injected directly on to a GC-FID and the instrument response was integrated. The GC was configured with a standard 30 meter DB-1 capillary column with a 10.5 minute elution time that spanned from 50-250°C oven temperature at 10°C per minute. The 1,3-butadiene chromatographic peak is reported as a percentage of any other observed responses. Figure 1 shows the chromatogram and area % of butadiene (100 %) on the chromatogram. The same chromatographic conditions were also utilized on a 5972 Hewlett Packard Mass Spectrometer to give the spectra and library match to 1,3-butadiene given in Figure 2.

Bd purity check chamber 3

Injection Date : 10/18/04 2:24:08 PM
Sample Name : Bd purity check Vial : 1
Acq. Operator : dak Inj Volume : Manually
Method : C:\HPCHEM\2\METHODS\BD2.M
Last changed : 10/18/04 2:15:39 PM by dak
butadiene 7/11/02



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

3L Bag (tedlar) pulled from chamber and injected done on GC FID.

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area counts*s	Height [counts]	Area %
1	0.757	BB	0.0343	1.23648e5	5.48528e4	1.000e2
Totals :				1.23648e5	5.48528e4	

Results obtained with enhanced integrator!

*** End of Report ***

Figure 1.
Gas chromatography — flame ionization analysis of 1,3-butadiene test atmosphere to determine purity. Only one major peak corresponding with 1,3-butadiene was found.

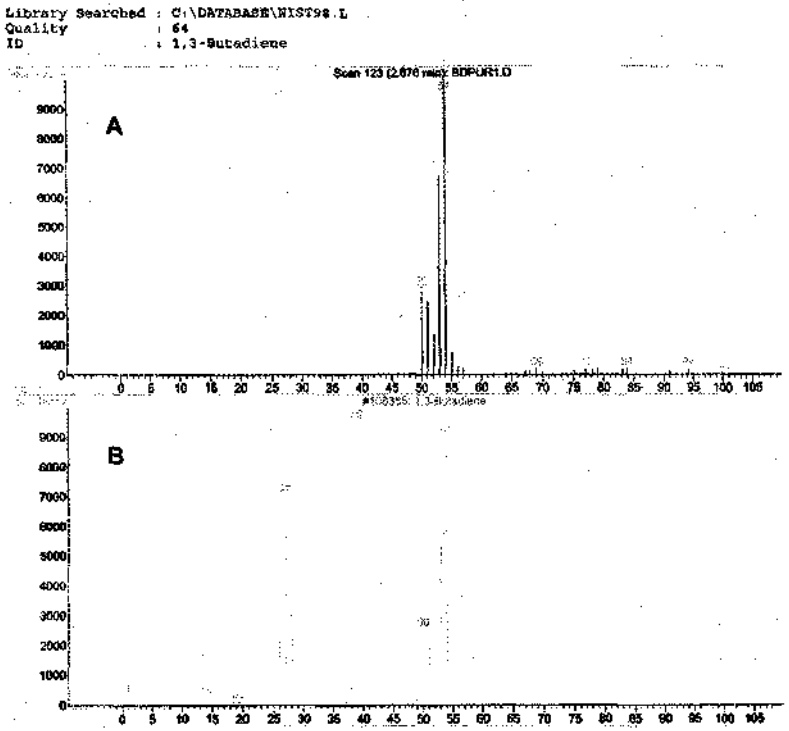


Figure 2.

Mass spectra of 1,3-butadiene test atmosphere (A) and a reference spectra (B) for 1,3-butadiene. Note that in A, mass was only monitored above 50 mass units.

4.3 Disposition

All unused Test and Control Articles remaining at study completion have been retained until the expiration date.

5.0 CONDUCT OF INHALATION EXPOSURES

Treatment groups were exposed to butadiene 6 hours + T_{90} (gas concentration rise and fall time, which was 6 minutes) per day, 5 days per week, for 4 consecutive weeks. Control animals were housed in similar exposure chambers and exposed to filtered air. Test atmospheres were generated from a compressed cylinder of pure (>98%) butadiene by metering gas with a

rotameter. The gas was directed to the exposure chamber through an air dilution system. Concentrations were controlled by adjusting the rotameter and dilution air rate, as necessary.

5.1 Environmental Monitoring and Exposure System

H2000 whole-body inhalation chambers (Lab Products, Inc., Maywood, NJ) were used to house the animals and to conduct the exposures. Chamber flow rates provided 12 to 15 air changes per hour. Chambers were held at approximately 1–3 inches of water negative pressure with respect to the exposure room, and animals were held in separate compartments within the chamber. Temperatures were held at 20°–26°C. Chamber environmental conditions were monitored by computer 24 hours per day except when the chambers were opened daily to inspect the animals or weekly to transfer animals to clean cages. For each chamber, the computer recorded air flow [by orifice meter], pressure [by magnehelic], temperature [by thermistor probe placed approximately 4 inches from the top cage unit in each chamber], and humidity [by Hy-Cal RH sensor]. There were no deviations from the specified environmental conditions.

5.2 Exposure System Qualification

The exposure system was qualified prior to animal exposures for: (1) uniformity of the distribution of gas concentration within each chamber (variability < 10 %); (2) within-day and between-day stability of vapor concentration (repeatability within 10 %); and (3) non-exposure hour chamber atmosphere concentrations (non-exposure concentrations below detection).

5.3 Exposure Atmosphere Monitoring

This study included three atmospheres of butadiene (1, 6.25, 62.5 ppm) plus a control (clean air) exposure atmosphere. Exposures were monitored with a hydrocarbon analyzer that was calibrated against a certified 1,3-butadiene standard from Scott Specialty gases. First, the hydrocarbon analyzer was spanned with a propane standard. To conserve the 1,3-butadiene standard, a smaller portion of the 1,3-butadiene standard was then be used to challenge the hydrocarbon analyzer and develop a correction factor for the 1,3-butadiene. The correction factor was incorporated into the calibration of the analyzer.

The hydrocarbon analyzer was used to define exposure concentrations and give a feedback to the laboratory technician to adjust dilutions if necessary. To adjust for background hydrocarbons from the animals, the target concentration was 1, 6.25, and 62.5 plus the control atmosphere hydrocarbon concentration (e.g., if background is 0.3 ppm, which is typical, the analyzer targeted 1.3 ppm at the low level). To confirm butadiene concentrations, a second measurement was made from each exposure atmosphere (at least once daily) by collection of butadiene on a 60/80 Carboxen (OI Analytical, Birmingham, AL) that was then thermally desorbed on to a gas-chromatograph (GC) with a flame ionization detector (FID). The GC-FID was calibrated with certified Scott Specialty Gas standards, and was challenged with a single standard each day before use. If the challenge was outside of 15% of the nominal value the instrument was recalibrated.

A summary of the daily average exposure atmosphere concentrations, and overall study summary, are given below in Table 2. Note that there are 5 weeks of exposure because the animals were staggered by one week to accommodate the necropsy (too many animals to

sacrifice in one day). All animals were exposed for exactly 4 weeks (5 days/week). The concentrations are a result of the hydrocarbon analyzer analysis. The hydrocarbon analyzer showed a minor response of vapor hydrocarbons in the control chamber due to the presence of trace background of organics from dilution air and rodent respiration. Each exposure day, the background concentration was confirmed to not be butadiene by the GC-FID measurement. Because of this, the background (0.15 ppm) was subtracted from each exposure level. In addition, all control atmosphere concentrations are defined as non-detectable. As Table 2 indicates, the overall average atmosphere concentrations were within 1 % of target at each exposure level. Note that at the 6.25 exposure level only two significant digits are reported for the atmospheric data because the precision of the measurement would not give greater than two significant figures.

Table 2. Summary of Daily Average (ppm) and Study Statistics for the Concentration of 1,3-Butadiene in Each Respective Exposure Atmosphere.

Date	Control ppm	Target 1 ppm	Target 6.25 ppm	Target 62.5 ppm
10/4/2004	ND	1.0	6.4	65.1
10/5/2004	ND	1.1	6.3	65.5
10/6/2004	ND	1.0	6.3	64.2
10/7/2004	ND	1.0	6.3	62.9
10/8/2004	ND	1.0	6.2	62.3
10/11/2004	ND	1.0	6.2	63.0
10/12/2004	ND	1.0	6.2	63.2
10/13/2004	ND	1.0	6.2	61.9
10/14/2004	ND	1.0	6.3	63.2
10/15/2004	ND	1.0	6.3	63.5
10/18/2004	ND	1.1	6.4	63.2
10/19/2004	ND	1.0	6.4	63.4
10/20/2004	ND	1.0	6.1	62.6
10/21/2004	ND	1.0	6.3	62.5
10/22/2004	ND	1.1	6.2	63.1
10/25/2004	ND	1.0	6.4	63.8
10/26/2004	ND	1.0	6.3	63.3
10/27/2004	ND	1.0	6.3	62.4
10/28/2004	ND	1.1	6.4	63.2
10/29/2004	ND	1.0	6.3	62.7
11/1/2004	ND	1.0	6.2	62.8
11/2/2004	ND	1.0	6.0	62.0
11/3/2004	ND	1.0	6.2	62.9
11/4/2004	ND	1.0	6.3	62.1
11/5/2004	ND	1.0	6.1	62.0
Mean	0.1	1.0	6.3	63.1
Standard Deviation	N/A	0.0	0.1	0.9
%CV[a]	N/A	3.4	1.5	1.4
%Target	N/A	101.2	100.1	100.9

Note: Values listed above are Total Hydro-Carbon Readings only; ND = Not Detected
[a]CV calculated as the Standard Deviation/Mean × 100

6.0 TEST SYSTEM

6.1 Species, Breed, Vendor, and Age at Arrival

Both male and female Sprague-Dawley rats and B6C3F1 mice, 10 ± 1 weeks of age at initiation of exposures were ordered from Charles River Laboratories (Wilmington, MA).

7.0 ANIMAL CARE, HOUSING, AND ENVIRONMENTAL CONDITIONS

7.1 Animal Receipt, Housing, and Quarantine

Animals were quarantined in whole-body chambers for 14 days. [redacted] SOPs regarding AAALAC-approved animal housing, environment, and care were followed. Animals will be housed in whole-body chambers throughout the course of the quarantine and exposure.

7.2 Environmental Conditions

Environmental conditions were maintained in accordance with the recommendations in *The Guide for Care and Use of Laboratory Animals* and as outlined in [redacted] SOPs. Only study animals were housed in the animal room designated for this study. Water was available *ad libitum* from the institutional watering system. Rooms were operated on a 12-hour per day light cycle (from approximately 6 a.m. to 6 p.m.).

7.3 Diet and Drinking Water

Unlimited tap water was available at all times. Drinking water was analyzed for potential contaminants according to [redacted] SOPs. Analytes included specified heavy metals, aflatoxin, organophosphates, and chlorinated hydrocarbons. Animals were fed Teklad certified rodent food during nonexposure hours.

8.0 EXPERIMENTAL DESIGN

8.1 Group Assignment

A laboratory animal veterinarian visually examined species before they were placed on study; only animals judged to be of acceptable health were used. Animals were weighed and randomly assigned to a group by weight using a computerized data acquisition system (Path-Tox; Xybion, Cedar Knolls, NJ) operated according to [redacted] SOPs. Body weights of individual species were ± 20% of the group mean. Assignment to group and identification procedures were as specified in [redacted] SOPs.

8.2 Study Design

Table 3 summarizes the treatment groups and animal number designations for the inhalation study. Animals were sacrificed on the last day of the terminus of the four week exposures. In order to accommodate the necropsy procedures for the large amount of animals, control animals were sacrificed on a Thursday after ~4 weeks of exposure. The control animals were sacrificed during regular business hours (starting at ~8 A.M.). Animals in the exposed groups were

sacrificed in two separate groups of ~90 (separate days for males and females) on consecutive Fridays (second group was started one week later than the first).

Table 3. Animal Numbers and Study Design.

	Number of Animals per Butadiene Exposure Concentration (6 hours/day, 5 days/week, 4 weeks)				
Species	0 ppm	1 ppm	6.25 ppm	62.5 ppm	Sex
B6C3F1 Mice	A001-A022	C001-C022	E001-E022	G001-G022	Male
(10 weeks old)	B001-B022	D001-D022	F001-F022	H001-H022	Female
Sprague-Dawley Rats	I001-I011	K001-K011	M001-M011	O001-O011	Male
(10 weeks old)	J001-J011	L001-L011	N001-N011	P001-P011	Female

9.0 OBSERVATIONS AND MEASUREMENTS

9.1 Mortality and Morbidity

Animals were examined by laboratory animal technicians twice per day (morning and afternoon) on each day of the study as specified by [redacted] SOPs. Examination was oriented toward (1) identifying dead, weak, or moribund animals, and (2) documenting the onset and progression of any abnormal clinical signs. There was no morbidity or unexpected mortality in the core study animals.

9.2 Body Weights

Body weights were recorded when animals come out of quarantine. Body weights were used to ensure homogeneity of the size/weights of the test animals and to randomize the animals into exposure groups (utilizing Path-Tox software randomization function). Average (plus one-sigma standard deviations) body weights are reported in Tables 4 (rats) and 5 (mice).

Table 4. Average Body Weight — Rat/Sprague-Dawley.

Group	Control	1 ppm	6.25 ppm	62.5 ppm
		Male		
Mean	294.5	294.1	294.9	295.7
Standard Deviation	23.5	22.8	21.1	22.6
		Female		
Mean	222.9	216.45	216.06	217.49
Standard Deviation	12.3	10.04	11.07	14.84

Table 5. Average Body Weight — Mouse/B6C3F1.

Group	Control	1 ppm	6.25 ppm	62.5 ppm
		Male		
Mean	26.7	26.8	26.9	26.8
Standard Deviation	1.2	1.1	1.5	1.3
		Female		
Mean	21.2	19.54	19.50	19.44
Standard Deviation	1.3	0.78	0.79	0.82

9.3 Clinical Observations

A clinical examination was performed prior to initiation of exposures for clinical abnormalities. These observations will include, but not be limited to, the following: reactivity to general stimuli, description and severity of any convulsions, tremors, or abnormal motor movements (including posture or gait abnormalities), and description of any abnormal behaviors, emaciation, dehydration, or any other abnormal masses, lesions, or appearances. No abnormal clinical observations were observed.

9.4 Food Consumption Measurements

Food consumption was not monitored.

9.5 Necropsy and Pathology

Animals were euthanized by rapid CO_2 asphyxiation. Specific necropsy procedures were specified in a study specific procedure adapted from the UNC. The necropsy protocol was approved by Dr. James Swenberg at UNC prior to finalization. The protocol is included as an appendix to this report.

10.0 SHIPMENT OF SAMPLES

After collection, tissues and washed red blood cells were stored at ~-80°C until disposition. Samples were shipped priority overnight on dry ice to:

Pat Upton 919-966-6141
Dept of Envr Sci & Eng
348 Rosenau Hall
Chapel Hill, NC 27599

11.0 RECORDS RETENTION

All raw data and records that would be required to reconstruct the study will be maintained in the [redacted] archives for 10 years. Records retained shall include but not be limited to:

- Test Materials:
  Test material receipt storage, usage, and disposition

- Exposures:
  All exposure analytical and environmental data

- In-Life Phase:
  Animal receipt and disposition
  Quarantine observation, health assessment and release
  Sample collection

12.0 PROTOCOL CHANGES

Once finalized, there were no amendments or changes to the Study Protocol, which is included as an appendix to this report.