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**PHILLIPS PETROLEUM COMPANY**  
BARTLESVILLE, OKLAHOMA 74004 918 661-6600

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HEALTH, ENVIRONMENT AND SAFETY

August 92

Compliance Audit Program  
CAP ID#: 8ECAP-0075

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Attn: Section 8(e) Coordinator  
(CAP Agreement)

Gentlemen:

Phillips Petroleum Company is submitting the enclosed sixty (60) reports (two boxes, numbered 1 and 2) of toxicological studies pursuant to category II.B.2.b of the CAP Agreement 8ECAP-0075 Reports. Reports being submitted contain no confidential business information.

We are sending an additional five boxes (box numbers 3-7) of reports of studies that have, previously, been submitted to the FYI Coordinator of the Office of Pollution Prevention and Toxics by the American Petroleum Institute (API). These are being provided solely for the Agency's convenience.

For questions concerning this correspondence, please contact Fred Marashi at 918-661-8153.

Very truly yours,

Barbara J. Price  
Vice President  
Health, Environment & Safety

Enclosure (Seven Boxes)

FFM/dh:29



Phillips Petroleum Company

"Contains NO CBI"

10

CAP Identification Number: BECAP-6J75

Pursuant to Category: II.B.2.b

~~32 SEP 2 PM 2:11~~

**Title of Study:** Acute Inhalation Study Using n-Propyl Mercaptan

**Name of Chemical:** n-Propyl Mercaptan

**CAS#:** 107-03-9

**Summary:** During exposure to the vaporized atmosphere of Phillips n-propyl mercaptan at 6920 ppm, the test rats were observed to have depressed activity, squinting, ataxia, jerking movements associated with breathing, and shaking movements. After removal from the exposure chamber, all test animals appeared depressed; this depressed appearance continued during most of the 14-day postexposure observation period.

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UBTL Sample No. 8-5045-4  
UBTL Protocol No. 8-50

FINAL REPORT

Phillips - n-Propyl Mercaptan  
Acute Inhalation  
Toxicity Screen

TR G5450-024

30 May 1981

Partial Fulfillment of Contract  
Effective October 15, 1980, with  
Phillips Petroleum Company  
Bartlesville, Oklahoma 74004

Prepared by:

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UBTL DIVISION  
UNIVERSITY OF UTAH RESEARCH INSTITUTE  
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Statement of Compliance

Acute Inhalation Toxicity Screen on Phillips n-Propyl Mercaptan  
(UBTL Sample No. 80M 05450-4)  
TR 05450-024

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Study Title

by  
William G. Yates, Ph.D.

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Final Report Dated  
30 May 1981

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1. These tests conducted in accordance with the Federal Good Laboratory Practices (21 CFR Part 58). All laboratory data which pertain to this study are recorded in UBTL Laboratory Notebook/Data File: #470,520/#44.
2. Services covered by this report produced in conformity with the Fair Labor Standards Act as amended.
3. In compliance with the Federal Good Laboratory Practices this study was inspected by the UBTL Quality Assurance Unit on:

23 March 1981, 24 March 1981, 30 April 1981, 5 May 1981

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Date(s)

and the findings of the inspection(s) were reported  
to UBTL management and to the study director on:

23 March 1981, 24 March 1981, 30 April 1981, 6 May 1981

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Date(s)

3 June 1981  
Date

  
Quality Assurance

STUDY PARTICIPANTS' SIGNATURE PAGE

William G. Yates, Ph.D. - Study Director

Signature: William G. Yates Date: 1 June 1981

Neil H. Price, M.S.

Signature: Neil H. Price Date: 1 June 1981

E. William Taggart

Signature: E. William Taggart Date: 1 June 1981

Randol N. Potter

Signature: Randolph Potter Date: 1 June 1981

Carter O. Wilsey

Signature: Carter Wilsey Date: 1 June 81

Ann C. Bagley

Signature: Ann C. Bagley Date: 1 June 81

#### SUMMARY AND EVALUATION OF TLST RESULTS

The Phillips Petroleum Company's n-propyl mercaptan was evaluated by two acute, whole-body, 4-hour exposures of 5 male and 5 female Sprague-Dawley rats to the sample in vaporized form, having mean concentrations of 6920 ppm and 8170 ppm, each derived from 4 partial period samples. The animals were observed during the exposures and for 14 days postexposure, at which time the surviving animals were sacrificed and necropsied. In accordance with the sponsor's directives, no control animals were used and no tissues were preserved for possible subsequent microscopic examination.

During exposure to the vaporized atmosphere of Phillips n-propyl mercaptan at 6920 ppm, the test rats were observed to have depressed activity, squinting, ataxia, jerking movements associated with breathing, and shaking movements. After removal from the exposure chamber, all test animals appeared depressed; this depressed appearance continued during most of the 14-day postexposure observation period. No animals died during the 4-hour exposure nor during the 14-day postexposure period. Abnormalities observed during necropsy were hemorrhage in the thymus, spots and areas of hemorrhage in the lungs, and a white spot on one kidney. The organs of one male and two female animals appeared normal.

During exposure to the vaporized atmosphere of Phillips n-propyl mercaptan at 8170 ppm, the test rats were observed to have depressed activity, squinting, and ataxia. After removal from the exposure chamber, these symptoms were observed 10 minutes postexposure as was labored breathing. The depressed appearance continued during most of the 14-day postexposure observation period for the surviving animals. No animals died during the 4-hour exposure, but two female animals died during the first postexposure day; at necropsy, these animals showed extensive areas of hemorrhage in the lungs and a gaseous small intestine filled with green/yellow liquid. Abnormalities observed during necropsy after elective termination at day 14 included spots in the lungs, red lungs, red and enlarged adrenals, and gaseous intestines filled with yellow liquid. The organs of two male animals appeared normal.

By mutual agreement with the sponsor, animal exposures at chamber concentrations higher than 8170 ppm were not attempted for the reasons described in the Discussion section.

## INTRODUCTION

UBTL was contracted by the Phillips Petroleum Company of Bartlesville, Oklahoma, to perform acute, whole-body, inhalation exposures on Phillips n-propyl mercaptan (UBTL Sample No. 80M 05400-4). The two experiments described here were performed according to UBTL Acute Inhalation Toxicity Screen Protocol No. 08-50, included as Appendix A. The specific procedures used and the results obtained from the experimentation are presented in this final report.

The test animals for this study were received on 23 April 1981 and the last were necropsied on 19 May 1981. Thus, the duration of the study was approximately 4 weeks.

The original data are stored in UBTL Laboratory Notebooks 470 and 520 and UBTL Laboratory Data File 044.

## TEST SUBSTANCE IDENTIFICATION

According to information received from the Phillips Petroleum Company, the Phillips n-propyl mercaptan test sample contained n-propyl mercaptan as the major component. The test sample was received in a one gallon metal container and the neat substance was used as received in this study.

## SPECIFIC METHODS

The methods described in the Acute Inhalation Toxicity Screen Protocol (UBTL No. 08-50), included as appendix A, were followed except as described in the section on Deviations from Protocol.

Specific methods, which are not detailed in the Acute Inhalation Toxicity Screen Protocol (UBTL No. 08-50), are detailed here. These

specific methods were the same for each of the two experiments and include specific descriptions of the exposure chamber, the atmosphere generation and chamber operation, and the atmosphere sampling and analysis.

#### Exposure Chamber

Test animals were exposed in a rectangular dynamic exposure chamber with conical top and bottom sections having a total volume of approximately 184 liters (approximate inside dimensions 50 cm x 50 cm x 50 cm, not including top and bottom cones). References 1-6 describe chamber design.

#### Atmosphere Generation and Chamber Operation

The test atmosphere was produced by dilution of thermally vaporized test material with humidified compressed air and nitrogen as described below.

The neat test material was fed into a vaporization chamber consisting of a glass condensation column filled with glass beads. The tee on the input end of the glass condensation column was connected through a rotameter with a metering valve to a source of compressed nitrogen and compressed air for flushing the vaporized material and to a metering pump (Fluid Metering, Inc., #RFTSY/250) with Teflon tubing. The Teflon tubing extended through the tee to a distance of about 5 cm into the glass condensation column, insuring delivery of the liquid material into the heated portion of the device for vaporization. One side of the tee on the output end of the glass condensation column was connected through a rotameter to a source of clean humidified compressed air for dilution of the vaporized test material and the other side of the tee was connected to another glass condensation column with temperature controlled water passing through the outer water jacket to maintain a constant atmosphere temperature. The output from the generation system was connected to the exposure chamber with stainless steel tubing.

The temperature of the glass vaporization chamber was controlled by a proportional controller (Drake Wilcox). That device provided the

electrical power for an immersion heater mounted in steel pipe upstream from the glass vaporization chamber. The vaporization chamber was mounted vertically with laboratory hardware within a ventilated safety cabinet. The vaporization chamber temperature was maintained at 160°C for vaporization of the test material used in this study. References 7-8 describe atmosphere generation.

The compressed air used for dilution of the vaporized material was cleaned by passing it through a bed of activated charcoal and a HEPA filter. The compressed air flow rate was monitored with a rotameter and was calibrated with a spirometer and an electric timer. The compressed air was humidified by injection of water vapor into the dilution air. The relative humidity was adjusted to approximately 50% by use of an electronic hygrometer (Weather Measurement Company) with its sensor mounted in the exposure chamber.

The chamber temperature was monitored by an electric thermometer (Fenwall) and was calibrated by a mercury-in-glass thermometer (ERTCO B 44264, -1 to 51°C) mounted inside of the chamber. References 9-10 describe chamber monitoring and related topics.

The mass flow rate of the liquid test material was determined by placing the test material container on a top loading balance (Mettler #P1210N) and measuring the weight loss over a known interval of time measured with an electric timer (Precision Scientific).

The nominal mass concentration (mg/L) of the generated atmosphere was determined by dividing the test liquid mass flow rate (mg/min) by the total gas flow rate (L/min).

The concentration of test compound was monitored continuously by use of an organic vapor monitor (HNU #201). However, these data were used for monitoring purposes only and were not used to calculate the actual concentration of the test atmosphere.

Preliminary experiments were performed prior to the formal exposures in order to establish chamber parameters and confirm chamber concentrations.

#### Atmosphere Sampling and Analysis

The test atmosphere was sampled through ports in the exposure chamber with glass midget impingers containing 15 ml of acetone sorbent.

The air flow through each of the impinger sets was controlled by needle orifices connected to a vacuum manifold system. The sampling rate for each impinger set was calibrated using a soap film flow meter (Alltech) and an electric timer (Precision Scientific), prior to sampling the exposure atmosphere. Using the calibrated sampling rate and the sampling interval, the collection volume for each impinger set was determined.

In order to take samples of the exposure atmosphere, the impingers were connected together in series in sets of two and placed in a special holder and clamped onto the exterior of the exposure chamber. Two impingers in series were used to insure the adsorption capacity of the acetone sorbent. The impingers were then connected to a fixed glass sampling line which extended into the exposure chamber and to hoses from the vacuum sampling manifold which was controlled by a sample timer. Sampling intervals were set on the timer which was then actuated and would shut off automatically. At the end of the sampling interval, the impingers were removed from the chamber and the sample time was recorded.

After sampling, both ends of the impinger were sealed with plastic tape and stored at room temperature until analyzed.

Calibration standards were prepared by placing 15 ml acetone sorbent and 5 ml of an acetone/benzene internal standard solution into six separate glass crimp top septum vials with Teflon lined caps. The vials which contained the acetone/benzene internal standard solution were weighed on an analytical balance (Mettler H20T) and aliquots of the neat test material over an appropriate range were added to each vial. The vials were weighed and the net test material added to each vial was determined.

Test atmosphere samples and calibration standards were analyzed by gas chromatography in the following manner. The test atmosphere impingers were disassembled and 5 ml of internal standard solution were added to

... The samples were mixed and transferred to sealable glass vials with Teflon lined caps. All samples and calibration standards were analyzed using a Hewlett-Packard 5710A gas chromatograph equipped with a flame ionization detector. A 3 ft x 0.25 in glass column packed with 80/100 mesh Chromosorb 102 and operated at 190°C was used for sample analysis.

A linear regression fit of the calibration standard weights versus detector response was performed on a programmable calculator (HP-97) and the mass of test material collected by each sampling device was determined using this linear regression equation. The sum of the two impingers of each sampling set was divided by the total collection volume of that impinger set to determine the atmosphere mass concentration (mg/L) under ambient UBTL conditions. The ppm (volume/volume) concentration under standard conditions of 25°C and 760 mmHg was subsequently calculated. References 11-15 describe the atmosphere sampling and analysis.

#### DEVIATIONS FROM PROTOCOL

No full period sample was taken during the 4-hour exposure period.

Per the request of Phillips, copies of the original data sheets are not included as an appendix.

Results are reported first for Experiment A at 6920 ppm, then for Experiment B at 8170 ppm.

Experiment A: 6920 ppm

Exposure Parameters

The exposure parameters for the test animals are listed in Table 1. The mean exposure concentration of 4 partial period consecutive samples was 6920 ppm. This concentration is 45% of the estimated lower explosive limit of 15,500 ppm.

Animal Observations

During the 4-hour exposure all of the animals showed depressed activity, squinting, ataxia, jerking movements associated with breathing, and shaking movements as shown in Table 2. The depressed activity continued during most of the 14-day postexposure observation period. No animals died during the exposure nor during the postexposure observation period. Abnormalities at necropsy consisted of hemorrhage in the thymus, spots and areas of hemorrhage in the lungs and a white spot on one kidney. The organs of one male and two female animals appeared normal.

The body weight data for the test animals are presented in Table 3.

Experiment B: 8170 ppm

Exposure Parameters

The exposure parameters for the test animals are listed in Table 4. The mean exposure concentration of 4 partial period consecutive samples was 8170 ppm. This concentration is 53% of the estimated lower explosive limit of 15,500 ppm.

#### 3.4.1. Postexposure

During the 4-hour exposure all of the animals showed depressed activity, salivating, and ataxia as shown in Table 5. In addition to these symptoms, labored breathing was noted at 10 minutes postexposure. The depressed activity continued during most of the 14-day postexposure observation period for the surviving animals. No animals died during the exposure, but two female animals died during the first postexposure day; at necropsy these animals showed extensive areas of hemorrhage in the lungs and a gaseous small intestine filled with green/yellow liquid. Abnormalities observed during necropsy after elective termination at day 14 included spots in the lungs, red lungs, red and enlarged adrenals, and gaseous intestines filled with yellow liquid. The organs of two male animals appeared normal.

The body weight data for the test animals are presented in Table 6.

#### DISCUSSION

Even though two animals died during Experiment B, additional exposures at higher concentrations were not attempted because of the UBTL safety policy not to substantially exceed 50% of the estimated lower explosive limit for a given compound. The analytical concentration of 8170 ppm achieved in Experiment B represents 53% of the estimated lower exposure limit of 15,500 ppm.

The above considerations were discussed with William C. Thomas of Phillips on 15 May 1981, and he agreed that attempting to expose animals at a higher concentration of n-propyl mercaptan was not necessary.

Table 1. Experiment A: 6920 ppm

Summary of Exposure Chamber Parameters

Phillips n-Propyl Mercaptan (UBTL 80M 100-4)

Chamber Gas Flow Rate = 51.4 L/min  
Chamber Liquid Flow Rate = 1142 mg/min  
Chamber Concentration,  
Nominal = 22.2 mg/L @ UBTL  
8290 ppm @ STP\*

Chamber Concentration, Analytical:

Sample No. 1 6410 ppm @ STP  
No. 2 7110 ppm @ STP  
No. 3 7380 ppm @ STP  
No. 4 6770 ppm @ STP  
Mean = 6920 ppm @ STP  
S.D. = 420 ppm @ STP  
C.V. = 6%

Chamber Temperature = 22°C  
Chamber Pressure = 648 mm Hg  
Chamber Relative Humidity = 50%  
Chamber Exposure Interval = 240 min

*CH<sub>3</sub>(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>SH*  
*31-2-70*  
*35*  
*28*

\*@ STP: Corrected to standard temperature of 25°C and pressure of 760 mm Hg.

Table 2. Experiment A: 600 ppm  
 Summary of observations Made During Exposure, Postexposure, and Necropsy  
 Phillips n-Propyl Mercaptan (BBL 808 95470-4)

Observations	Time of Observation	Number of Animals Exhibiting Specific Observation per Group of Five Animals	
		Male 5/5	Female 5/5
Depressed Activity	During Exposure, at 30 minutes and for Remainder of Exposure	5/5	5/5
Squinting		5/5	5/5
Ataxia		5/5	5/5
Jerking Movements Associated with Breathing		5/5	5/5
Shaking Movements as Though Trying to Remove Something from Coat	During Exposure, at 30 minutes	5/5	5/5
Depressed Activity	Postexposure, at 45 minutes	5/5	5/5
Squinting		5/5	5/5
Ataxia		5/5	5/5
Depressed Activity	Postexposure, During Most of 14-day Period	5/5	5/5
Squinting	Postexposure, Observed on One Day	0/5	1/5
Hemorrhage in Thymus	Necropsy, Day 14	1/5	1/5
White Spots in Lungs		1/5	0/5
Hemorrhage in Lungs		3/5	3/5
White Spot in One Kidney		1/5	0/5
Normal Appearance of All Organs		1/5	2/5

Table 3. Experiment A: 6920 ppm

Summary of Surviving Animal Body Weights, g

Phillips n-Propyl Mercaptan (UBTL 80M 05450-4)

Group	Day Prior to Exposure	Day 7	Day 14 (Necropsy)
<b>TEST MALES</b>			
T1M	228	238	260
T3M	228	247	290
T5M	237	253	283
T7M	219	246	292
T9M	235	248	292
Mean	229	246	283
S.D.	7	5	14
S.E.	3	2	6
<b>TEST FEMALES</b>			
T2F	190	196	218
T4F	195	204	222
T6F	202	196	216
T8F	228	234	257
T10F	200	207	227
Mean	203	207	228
S.D.	15	16	17
S.E.	7	7	8

Table 4. Experiment B: 8170 ppm

Summary of Exposure Chamber Parameters

Phillips n-Propyl Mercaptan (UBTL 801 15450-4)

Chamber Gas Flow Rate = 55.1 L/min  
Chamber Liquid Flow Rate = 1305 mg/min  
Chamber Concentration,  
Nominal = 23.70 mg/L @ UBTL  
8970 ppm @ STP\*

Chamber Concentration, Analytical:

Sample No. 1 8760 ppm @ STP  
No. 2 9020 ppm @ STP  
No. 3 8830 ppm @ STP  
No. 4 6050 ppm @ STP  
Mean = 8170 ppm @ STP  
S.D. = 1410 ppm @ STP  
C.V. = 17%

Chamber Temperature = 23°C  
Chamber Pressure = 640 mm Hg  
Chamber Relative Humidity = 50%  
Chamber Exposure Interval = 240 min

\*@ STP: Corrected to standard temperature of 25°C and pressure of 760 mm Hg.

Table 5. Experiment M: 8170 ppm  
 Summary of Observations Made During Exposure, Postexposure, Necropsy, and Histopathology  
 Phillips n-Propyl Mercaptan (BIBL 80M 05450-4)  
 Number of Animals Exhibiting Specific Observation per Group of Five Animals

Observations	Time of Observation	Male Rats		Female Rats	
Depressed Activity	During Exposure at 30 minutes	5/5	5/5	5/5	5/5
Squinting	and for Remainder of Exposure	5/5	5/5	5/5	5/5
Ataxia		5/5	5/5	5/5	5/5
Depressed Activity	Postexposure, at 10 minutes	5/5	5/5	5/5	5/5
Squinting		4/5	5/5	5/5	5/5
Ataxia		5/5	5/5	5/5	5/5
Labored Breathing		3/5	5/5	5/5	5/5
Death	Postexposure, During Day 1	0/5	0/5	2/5	2/5
Depressed Activity	Postexposure, During Most of	5/5	5/5	3/3	3/3
Squinting	14-day Period	0/5	0/5	1/3	1/3
Squinting	Postexposure, Observed on Four	2/5	2/5	2/3	2/3
Coughing	or Less Days	1/5	1/5	0/3	0/3
Extensive Areas of Hemorrhage in Lungs	Necropsy, Day 1	0/0	0/0	2/2 (died during Day 1)	2/2
Hemorrhage in Thymus		0/0	0/0	1/2	1/2
Small Intestine Gaseous and Filled with Green/Yellow Liquid		0/0	0/0	2/2	2/2
White Spots in Lungs	Necropsy, Day 14	3/5	3/5	2/3	2/3
Red Lungs		0/5	0/5	1/3	1/3
Red Adrenals		2/5	2/5	3/3	3/3
Enlarged Adrenals		0/5	0/5	3/3	3/3
Gaseous Intestine Filled with Yellow Liquid		0/5	0/5	1/3	1/3
Normal Appearance of All Organs		2/5	2/5	0/3	0/3

Table 6. Experiment #: 8170 ppm

Summary of Surviving Animal Body Weights, g

Phillips n-Propyl Mercaptan (CRTL # M 05450-4)

Group	Day Prior to Exposure	Day 7	Day 14 (Necropsy)
<b>TEST MALES</b>			
T1M	269	278	333
T3M	248	259	308
T5M	251	256	301
T7M	245	252	313
T9M	248	250	297
Mean	252	259	310
S.D.	10	11	14
S.E.	4	5	6
<b>TEST FEMALES</b>			
T2F	213	205	236
T4F	214	*	*
T6F	199	200	222
T8F	197	163	136
T10F	219	*	*
Mean	208	189	198
S.D.	10	23	54
S.E.	4	13	31

\* Died 1 day after exposure.

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APPENDIX A

UBTL Protocol No. 08-50

Acute Inhalation Toxicity Screen Protocol

## EXPOSURE FACILITY

### Exposure Chambers and Exposure Cages

Dynamic test and control chambers are used for whole-body inhalation exposures. These chambers are of rectangular shape with a total internal volume ranging from 70 to 150 liters. These chambers meet the recommended guideline for the exposure animals not to exceed approximately 5% of the chamber volume [1-6]. A large door is mounted on the front of each chamber to allow insertion and removal of the animal cages. Ports are present on the front door and on top of each exposure chamber for use in collecting atmosphere samples.

Rectangular stainless steel wire mesh cages are used to house the test animals during the exposures. These cages are rectangular with approximate outside dimensions of 38 cm x 38 cm x 23 cm. Each cage contains 10 compartments to allow for the individual caging of animals during the exposures. Each compartment is approximately 8 cm x 19 cm x 23 cm.

### Exposure Atmosphere Generation

A relatively high concentration near the vapor saturation concentration or approximately 50% of the lower explosive limit concentration of test material in air is generated, if feasible, using state-of-the-art techniques [7-10]. Depending upon the properties of the test compound and the desires of the sponsor, a given test material is generated as a gas, vapor, aerosol, or combination of these forms. The neat test material is used for generation and analysis of the exposure atmospheres unless the sponsor specifically requests otherwise in writing.

... is measured with a flowmeter and the average measured air flow into the exposure chamber is calculated using the average measured air flow into the exposure chamber.

The relative humidity of the chamber is maintained at 10% by a humidifier and measured by a psychrometer or similar device [12, 15, 16]. The intake air temperature is controlled by an exchanger system such that the temperature is  $24 \pm 2^\circ\text{C}$  within the chamber as measured by a mercurial or an electronic thermometer [11, 12, 13].

The oxygen content in the chamber is maintained at a minimum of 19% and is verified by gas chromatographic or other appropriate methods as necessary [17, 18].

#### Exposure Atmosphere Sampling and Analysis

In order to confirm the exposure atmosphere concentration, appropriate state-of-the-art sampling procedures are used [19-31]. The type of sampling device employed (i.e., adsorption tube, filter, impactor, cascade impactor, etc.) depends upon the properties of the atmosphere being tested.

Analysis of the atmosphere samples taken during a given exposure is performed by the method supplied by the sponsor, with modifications as necessary. In some cases sponsor supplied analytical methods are not suitable for atmosphere analysis and therefore the sponsor may request in writing that CBTL develop a suitable analysis method for an additional charge. Gas chromatographic analytical methods are employed unless judged to be inappropriate for a given analysis.

Partial period consecutive atmosphere samples are taken at least once per hour during the four hour exposure yielding four samples. In addition, one full period sample is taken for the entire exposure interval.

At least one cascade impactor sample is taken per exposure when an aerosol is generated. The cascade impactor samples taken from aerosol



Animal Procurement and Procurement

Healthy, young adult Sprague-Dawley rats weighing 200-300 g are obtained from Simonson Labs, Gilroy, California. All animals are shipped by air carrier and checked upon arrival. Rats are placed separately in shoe box type cages with approximate dimensions of 27 cm x 27 cm x 17 cm and containing wood shaving bedding.

Upon arrival, each animal is permanently identified with a specific identification number using an ear punch procedure. UBTL accounts for all animals throughout the study. UBTL has established and adheres to standard operating procedures for housing, feeding, labeling, handling, and care of test animals as specified in 772.110-1. All incoming animals are isolated in a room separate from exposed animals for a period of at least one week to check for latent health problems and to acclimate them to the UBTL environment prior to exposure. The animal rooms are maintained at  $70 \pm 3^\circ\text{F}$  and  $45 \pm 15\%$  relative humidity and have a light cycle of 12 hours on and 12 hours off.

The animal cages are cleaned and the bedding is changed twice weekly. All animals are caged separately before, during, and after exposure. Both high quality feed (Wayne's Lab Block) and water are available to all animals continually except during the inhalation exposure.

At the time of exposure, the rat body weight ranges from approximately 200 to 300 g.

Each test animal is assigned an experimental number which corresponds to the inhalation chamber multicompartiment cage section in which it will be exposed. This number also serves as an identification number for a given animal and its organ specimens throughout the remaining data gathering processes, as shown below:

#### Animal Exposure

A test group consisting of 5 male rats and 5 female rats is exposed to the test material atmosphere.

For the exposure the test rats are placed alternately in a multicompartment cage according to sex, one rat per compartment. The caged test rats are placed in the exposure chamber and exposed to the test material atmosphere for an interval of four hours.

#### Animal Observation

When possible, observations of the test animals are made during the exposure; however, some atmospheres obscure visibility and preclude such observation. At the end of the exposure interval, the test and control animals are immediately removed from the exposure cages and examined for gross signs of toxicity. The animals are then placed in individual shoe box type cages for the post-exposure observation period. To further assure the development of valid data, observation of animals are made by a qualified technical employee or by a qualified professional scientist (e.g. veterinarian or toxicologist) hourly for the first 2 hours post-exposure if the animals appear intoxicated upon removal from the exposure chamber and at least daily throughout the remainder of the observation period. The day of the exposure is denoted as Day 0 and the routine day of surviving animal necropsy is denoted as Day 14 (fourteen days after exposure).

#### Specific Indicators of Pharratoxicity

##### Pupillary:

Sluggish  
Dilated

Excretory:

Diarrhea  
Bloody Stool

Cardiovascular:

Rapid Heart Rate  
Irregular Rate

Activity:

Normal  
Depressed  
Hyperactive

Eyes:

Squinting  
Closure  
Blinking  
Lacrimation

Respiratory:

Nasal Drainage  
Nasal Protection  
Coughing  
Sneezing  
Bloody Stains  
Salivation

CNS:

Ataxia  
Escape Behavior  
Narcosis  
Tremors  
Convulsions  
Death

The weight of each animal is determined on the day prior to exposure and 7 and 14 days after exposure.

#### Sacrifice and Necropsy

All animals living at the termination of the observation period are sacrificed. All test animals, whether dying by sacrifice or during the test or observation period, are subjected to a complete gross necropsy following their death. The method used for animal sacrifice is humane (ether euthanasia) and the same throughout the study.

Necropsies are performed by persons with training/experience equivalent to those certified by the American Society of Clinical Pathologists or American Association of Laboratory Animal Science. They are under the supervision of a qualified doctorate pathologist. All abnormalities are recorded.

Animals are necropsied as soon as possible after death but no later than 16 hours after death. If necropsy cannot be performed immediately after the animal is sacrificed or found dead, the animal is immediately refrigerated (but not frozen) at temperatures low enough to minimize tissue autolysis (4-8°C). Animals found dead upon routine clinical examination are necropsied as soon as possible to salvage usable tissues.

The gross necropsy includes an initial physical examination of the external surfaces and all orifices followed by an internal examination of tissues and organs in situ. The examination includes the following: external and internal portions of all hollow organs; cranial cavity and external surfaces of the brain; nasal cavity and paranasal sinuses; neck with its associated organs and tissues; thoracic, abdominal, and pelvic cavities with their associated organs and tissues. As instructed by the sponsor, no animal tissues are preserved.

#### SUBSEQUENT EXPERIMENTATION

Based on the results of the acute inhalation screen performed by this protocol, subsequent toxicity experimentation may be recommended to the sponsor using the Acute Inhalation Toxicity LC<sub>50</sub> Protocol (UBTL No. 09-50) in order to estimate the LC<sub>50</sub> for the test material.

#### RECORDS AND SAMPLES

All original records, raw data, an aliquot of the test material, and the test protocol are maintained in the UBTL archives.

#### PROTOCOL DEVIATIONS

Notification of deviations from this protocol are made in the final report. Sponsors wishing to make deviations in the protocol should notify UBTL in writing before the project has been initiated.

DATA PRESENTED IN THE FINAL REPORT

A copy of the test protocol is included as an appendix to the final report. Test procedures fully described in the protocol are not included in the body of the final report.

The final report includes the following items:

- A. Title Page. Including name of test protocol followed, name of test substance, name of sponsor, purchase order number or contract number of sponsor, signature of study director, and UBTL logo.
- B. Quality Assurance Statement. Including authorized signature from the quality assurance unit.
- C. Study Participants Page. Including signatures of all major participants in the study.
- D. Summary and Evaluation of Test Results. Including summary and analysis of the data and statement of the conclusions drawn from the analysis. The summary highlights any and all positive data or observations which may be indicative of toxic effects.
- E. Introduction. Including name of sponsor, name of test substance, name of test protocol followed, date of study initiation, date of study completion, and duration of study.
- F. Test Substance Identification. Including basic information about test substance supplied by sponsor, type of shipping container, container labeling, and any vehicle added to test substance by UBTL prior to testing and rationale used for selection of such vehicle.

- G. Specific Test Methods. Including specific test details not fully described in the test protocol and not considered to be deviations of the test protocol. For example, exposure chamber design and dimensions, atmosphere generation, atmosphere sampling, atmosphere analysis, desired exposure concentration, and duration of exposure.
- H. Deviations From Protocol. Including all deviations from the established protocol, with an explanation of the reasons for such deviations.
- I. Results
- 1) Exposure parameter summaries including temperature, relative humidity, chamber air flow, nominal chamber concentration, analytical chamber concentration, and for aerosol atmospheres, mass median aerodynamic diameter, geometric standard deviation, and percent of particles with aerodynamic diameter less than 10  $\mu\text{m}$ . Individual data are tabulated along with the mean, standard deviation, and standard error.
  - 2) Animal data summaries including tabulation of response data by sex; the time of death after exposure; and tabulation of body weights of test and control animals on the day prior to exposure and 7 and 14 days after exposure.
- J. References. Including statistical and any other methods employed for analyzing the raw data; a list of references to any published literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results.

APPENDIX A. COPY OF THE TEST PROTOCOL

APPENDIX B. COPIES OF THE ORIGINAL DATA SHEETS

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