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**AAMRL-TR-90-061**



# **THE ACUTE INHALATION TOXICITY OF TRIETHYLBORANE SPONTANEOUS OXIDATION PRODUCTS: IMMEDIATE AND DELAYED EXPOSURE**

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### TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-90-061

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



JAMES N. McDOUGALL, Maj, USAF, BSC  
Deputy Director, Toxic Hazards Division  
Harry G. Armstrong Aerospace Medical Research Laboratory

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## PREFACE

This is one of a series of technical reports describing results of the experimental programs conducted at the Toxic Hazards Research Unit, NSI Technology Services. This document serves as a final report on the acute inhalation toxicity of triethylborane fuel additive. The research described herein began in April 1988 and was completed in April 1989 under U.S. Air Force Contract No. F33615-85-C-0532. Melvin E. Andersen, Ph.D., and Harvey J. Clewell III, Lt Col, USAF, served consecutively as Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory/Toxic Hazards Division during the course of this investigation.

The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #85-23, 1985, and the Animal Welfare Act of 1966, as amended.

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## ABBREVIATIONS

aTEB	Aged triethylborane
BW	Body Weight
CNS	Central nervous system
CO <sub>2</sub>	Carbon dioxide
CV	Coefficients of variation
°C	Degrees Celsius
cm	Centimeter
DEEOB	Diethylethoxyborane
EDEOB	Ethyldiethoxyborane
fTEB	Fresh triethylborane
GC	Gas chromatographic
g	Grams
H <sub>2</sub> O	Water
h	Hour(s)
IR	Infrared
i.d.	Inside diameter
ig	Intragastric
ip	Intraperitoneal
kg	Kilogram
L	Liter
LC <sub>50</sub>	Lethal concentration - 50%
LC <sub>67</sub>	Lethal concentration - 67%
LC <sub>100</sub>	Lethal concentration - 100%
LD <sub>50</sub>	Lethal dose - 50%
MS	Mass spectral

mg	Milligram
min	Minute
mL	Milliliter
msec	Millisecond
N <sub>2</sub>	Nitrogen (molecular)
NRC-COT	National Research Council – Committee on Toxicology
O <sub>2</sub>	Oxygen (molecular)
p	Probability
ppm	Parts per million
r <sup>2</sup>	Coefficient of correlation squared
TBB	Tributylborane
TEB	Triethylborane
TEOB	Triethoxyborane
USAF	United States Air Force
V <sub>E</sub>	Minute ventilation
vs	versus

## SECTION 1

### INTRODUCTION

Triethylborane (TEB) is employed in U.S. Air Force (USAF) high performance aircraft engines as a fuel additive to increase ignition speed and retard engine flameout at the reduced pressures of high altitude flight. At present, little is known about the potential toxicity of exposure to TEB fumes released during ground-based operational procedures. However, clinical reports on personnel accidentally exposed to TEB fumes suggest that TEB induces both respiratory and central nervous system (CNS) dysfunction. Similar responses have been reported for workers accidentally exposed to related organoborane compounds such as pentaborane and decaborane. (Rozendaal, 1951; Krackow, 1953; Lowe and Freeman, 1957; Jacobson, 1958; Hill et al., 1958; Sim, 1958; Cordasco et al., 1962; Hart et al., 1984). Several studies have been conducted examining the toxicity of TEB-related organoboranes in animals, demonstrating predominantly CNS and respiratory effects (Svirbely, 1954 and 1955; Weeks et al., 1964; Weir et al., 1964). However, investigation of TEB toxicity, per se, in animals is limited to the work of Rinehart (1960). In this investigation rats were exposed to TEB via either ip, ig, or inhalation routes. The 4-h inhalation LC<sub>50</sub> of what was reported to be pure TEB vapor was nominally 700 ppm. However these data have been considered suspect because the TEB in the exposure environment was neither characterized quantitatively or qualitatively nor was temporal stability of the exposure concentrations monitored. Likewise, clinical observations of respiratory distress in the exposed animals were not substantiated by histopathologic examinations. In light of clinical observations of apparent TEB CNS and pulmonary toxicity and the paucity of TEB-specific toxicity studies, several USAF agencies and the National Research Council - Committee on Toxicology (NRC-COT) (Marzulli, 1985) strongly urged that additional studies be conducted.

Although TEB is relatively stable in solution with nonhalogenated hydrocarbons such as hexane, it is extremely labile in air and this instability complicates the determination of the inhalation toxicity of this material. Liquid TEB autoignites (often explosively) when exposed to air, and TEB vapor, at concentrations exceeding 1150 to 1300 ppm, undergoes complete spontaneous combustion to form boron oxide, H<sub>2</sub>O, and CO<sub>2</sub>. At concentrations below 1150 to 1300 ppm, TEB rapidly oxidizes to diethylethoxyborane (DEEOB), then ethyldiethoxyborane (EDEOB), and ultimately to triethoxyborane (TEOB) (Callery Chemical, 1978 - see Figure 1). Given the appropriate stoichiometry (1.5 mole O<sub>2</sub> per mole TEB), these oxidations proceed very rapidly. Preliminary evidence suggests that the initial oxidation occurs within milliseconds after initial contact of TEB with air, and proceeds to completion in a matter of minutes. The precise reaction kinetics of TEB oxidation in air have not been determined. However, similar rapid oxidation of tributylborane (TBB) has been observed by Brown

et al. (1986, and personal communication). These investigators demonstrated a complete oxidation of TBB, within 60 min, by a pathway analogous to that postulated, under conditions designed to minimize the reaction rate (minus 70 °C in the presence of the oxygen scavenger tetrahydrofuran).

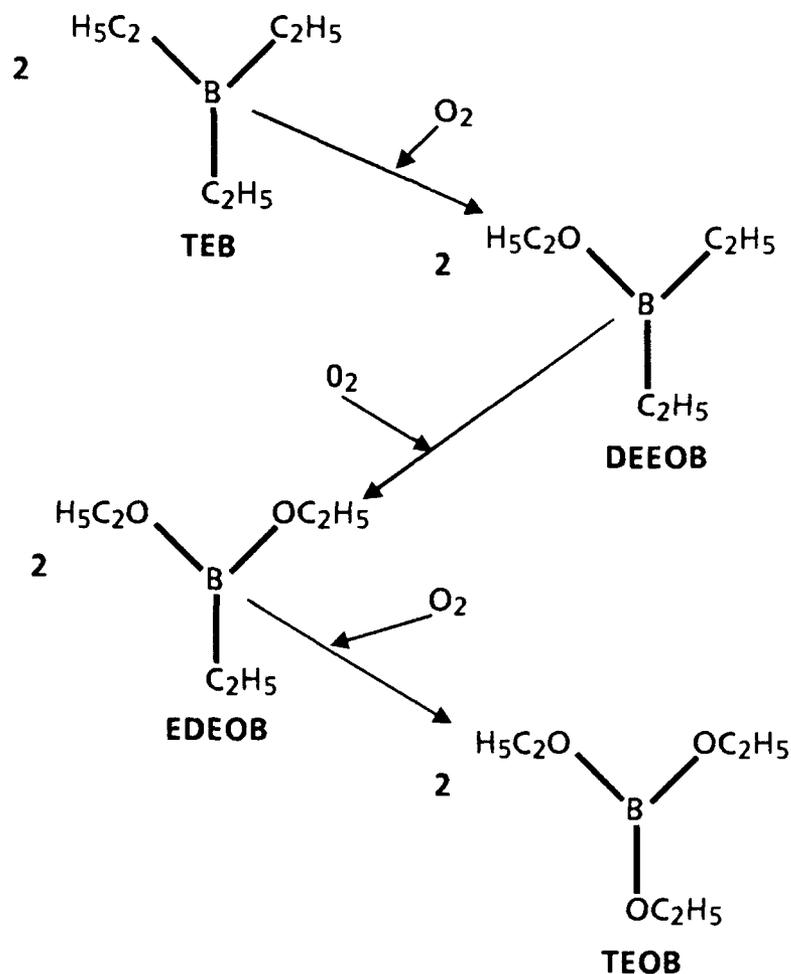


Figure 1. Spontaneous, Noncombustive Oxidation of TEB.

Because of the rapid oxidation of TEB in air and the possibility that TEB toxicity might be a function of the extent to which this oxidation has progressed, the acute inhalation toxicity of TEB was determined not only as a function of concentration but also as a function of the time after initiation of oxidation as well. At various exposure concentrations animals were exposed for 1 h to TEB oxidation product vapors in which either 36 msec or 4.6 min had elapsed between the initiation of the oxidation process and delivery to the exposure apparatus. For the purpose of clarity, fresh TEB (fTEB) refers, in actuality, to the mixture of TEB and its DEEOB, EDEOB, and TEOB oxidation derivatives delivered to the exposure chamber 36 msec after initial mixing of pure TEB vapor with oxygen. Aged TEB (aTEB) vapor refers to the atmosphere resulting from a 4.6-min delay between the initiation of TEB oxidation and delivery to the exposure chamber.

## SECTION 2

### METHODS AND MATERIALS

#### 2.1 TEST MATERIAL

Two commercial sources of liquid TEB were used in the course of this investigation. One hundred grams of TEB supplied by Aldrich Chemical Company, Inc. (Milwaukee, WI - Lot No. 00525CP) was used for initial attempts at analytical methods development. Triethylborane (1.2 kg) for subsequent methods development, calibration standards, and exposures was supplied by Callery Chemical Company, Inc. (Callery, PA - Lot No. 853-S). Both supplies of TEB were received in sealed vessels containing a pure N<sub>2</sub> headspace and precautions were instituted to ensure that all procedures for transfer, use, and subsequent storage were conducted to maintain the integrity of this headspace. The physicochemical properties of TEB are shown in Table 1.

TABLE 1. PHYSICOCHEMICAL PROPERTIES OF TRIETHYLBORANE

Synonyms:	Triethylboron, Triethylborine, Trialkylborane
CAS No:	97-94-9
Formula:	(C <sub>2</sub> H <sub>5</sub> ) <sub>3</sub> B
Molecular Weight:	98.0
Color and Form:	Clear liquid
Melting Point:	-93 °C
Boiling Point:	95 °C
Density:	0.68 g/mL at 25 °C
Viscosity:	0.30 cP at 25 °C
Vapor Pressure:	42.6 mm Hg at 20 °C & 760 mm Hg
Solubility/H <sub>2</sub> O:	Immiscible
Stability:	Pyrophoric - liquid autoignites on exposure to air, vapor autoignites ≥ 1150 <sup>a</sup> to 1300 <sup>b</sup> ppm
Conversion Factors:	1 mg/m <sup>3</sup> = 0.25 ppm

<sup>a</sup> Autoignition atmospheric concentration observed this experiment.

<sup>b</sup> Autoignition atmospheric concentration per Callery Chemical.

#### 2.2 QUALITY CONTROL

Due to the ephemeral nature of pure TEB, in all but absolute anaerobic conditions, gas-chromatographic (GC), mass-spectral (MS) and infrared (IR)-spectral methods to determine the purity of both sources of TEB proved unsuccessful as stable spectra and chromatograms could not be generated. Hence, the manufacturer's analyses of purity (99% + for both sources) was considered accurate, and further attempts to assess TEB purity were not made.

### 2.3 ANIMALS

The investigation was conducted in two phases: The first was a pilot study conducted at maximum attainable concentrations of either fTEB or aTEB, and the second phase was an acute toxicity study in which animals were exposed to various concentrations of either fTEB or aTEB. Fischer 344 (F-344) rats of both genders, 12 to 13 weeks of age at exposure, were obtained from Harlan Industries (Indianapolis, IN) for the pilot investigation; and F-344 rats, 11 to 14 weeks of age at exposure, were purchased from Charles River Breeding Laboratories (Kingston, NY) for the second phase of the investigation. Upon receipt, all animals were quarantined for a two-week period and were committed to study following observation and random selection of representative animals for quality control analysis. Prior to the exposures and during a 14-day postexposure observational period, animals were group-housed (two to three per unit) in clear plastic cages with wood chip absorbent bedding. Housing units were maintained in single-pass laminar flow facilities. Food and water were provided ad libitum with the exception of withholding food for a 12-h period prior to sacrifice. All animals were maintained on a 12-h diurnal cycle.

Animals were assigned to experimental groups using a body weight normalized random selection procedure. For the pilot investigation, 12 animals of each sex were assigned to each of three experimental groups. One group each was exposed to comparable concentrations (approximately 960 ppm) of either fTEB or aTEB and one group was exposed for approximately one hour to air to serve as controls. Half of the animals in each group were designated for evaluation of acute toxicity after a 14-day postexposure observation period and half of the animals were scheduled for serial sacrifice to evaluate the development and recovery from pulmonary insult at 4 h, 24 h, and 7 days postexposure. Six animals of each sex were assigned to each of seven experimental groups for the second phase (LC<sub>50</sub> determination) of the investigation. No provisions were made to include subgroups for serial, postexposure pathologic evaluation of potential pulmonary or CNS lesions. In addition to a control group, animals were exposed to either fTEB or aTEB at one of three concentrations (approximately 370, 600, or 780 ppm).

### 2.4 INHALATION EXPOSURE APPARATUS

The animals were exposed to either fTEB or aTEB using a 48-port, nose-only exposure apparatus described by Raabe et al. (1973). The chamber flow was the reverse of the direction used during conventional operation of this exposure chamber. Operating the exposure chamber in this manner allowed for delivery of the TEB reaction products through ports in the exhaust (normal operation) manifold which were located directly in front of each animal's nose. Chamber discharge occurred through what is normally considered the chamber inlet. In addition, only exhaust manifolds on one side of the chamber were used, thereby accessing only 24 exposure ports for the study. At

normal chamber flow rates of 10 to 11 L/min, reverse flow operation allowed a toxicant delivery system configuration in which the minimum transit time between initiation of TEB oxidation, in an isokinetic gas mixing chamber, and delivery to the midpoints in the exposure (exhaust) manifolds was, on average, 36 msec (Figure 2). In this configuration, 0.4 cm i.d. stainless steel tubing 50.8 cm in length was employed to transport (at mean flow = 10.67 L/min) fTEB to the midpoints of the exposure chamber; alternately for aTEB exposures, the chamber toxicant delivery system was configured with ducting (7.21 cm i.d. by 1,249.7 cm length - mean flow = 11.12 L/min) in which transit time from the point of initiation of TEB oxidation to delivery at midpoints in the exposure manifolds was, on average, 4.6 min (Figure 3). Additional experiments, not described herein, were performed to determine linearly consistent correction coefficients to account for delivery system losses of TEB vapor and hence equate exposure chamber concentrations of fTEB and aTEB to original TEB in N<sub>2</sub> concentrations (average 8%, or 1.08 multiplication factor) given the different delivery configurations and ducting for concentration sampling.

To control and coordinate the delivery of time-point-specific TEB oxidation products to the exposure apparatus, it was necessary to artificially reproduce a normal breathing atmosphere. Normoxic exposure atmospheres were accomplished by metering, at appropriate ratios, N<sub>2</sub> and O<sub>2</sub> from compressed gas sources. Exposure chamber O<sub>2</sub> concentrations were monitored continuously during the exposures. (EC Oxygen Monitor, Model 5590, Hudson Ventronics Corp., Temecola, CA.)

## 2.5 ATMOSPHERE GENERATION

A specialized generation apparatus for these experiments was developed which would maximize control over TEB vapor production and delivery to the exposure apparatus as well as provide for the safety of the operator and the surrounding environs. As noted previously, TEB was supplied from the manufacturer in a pressurized dewar containing a head of pure N<sub>2</sub> from which liquid TEB was delivered through a siphon by increasing the N<sub>2</sub> pressure head. An integrated system of remotely controlled, pneumatically actuated (pressurized N<sub>2</sub>) control (either normally open/closed as required) and metering valves were used for precise delivery of liquid TEB to an N<sub>2</sub> driven evaporator (Figure 4). The generation apparatus was sealed in a primary containment unit of aluminum and Plexiglas construction under a pure N<sub>2</sub> atmosphere at  $\approx$  15 cm H<sub>2</sub>O subambient pressure.

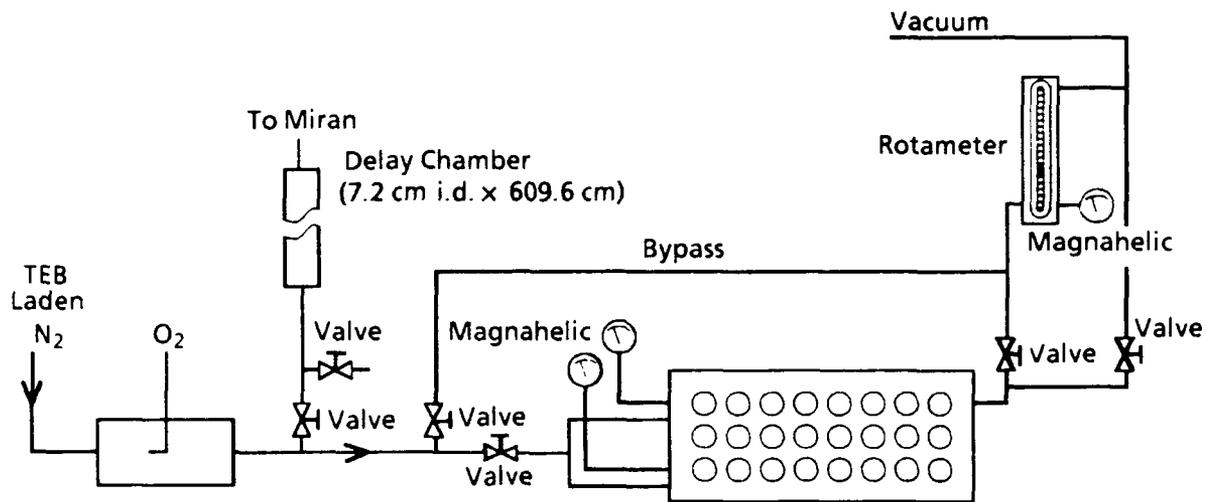


Figure 2. Exposure System Configuration for fTEB Exposures.

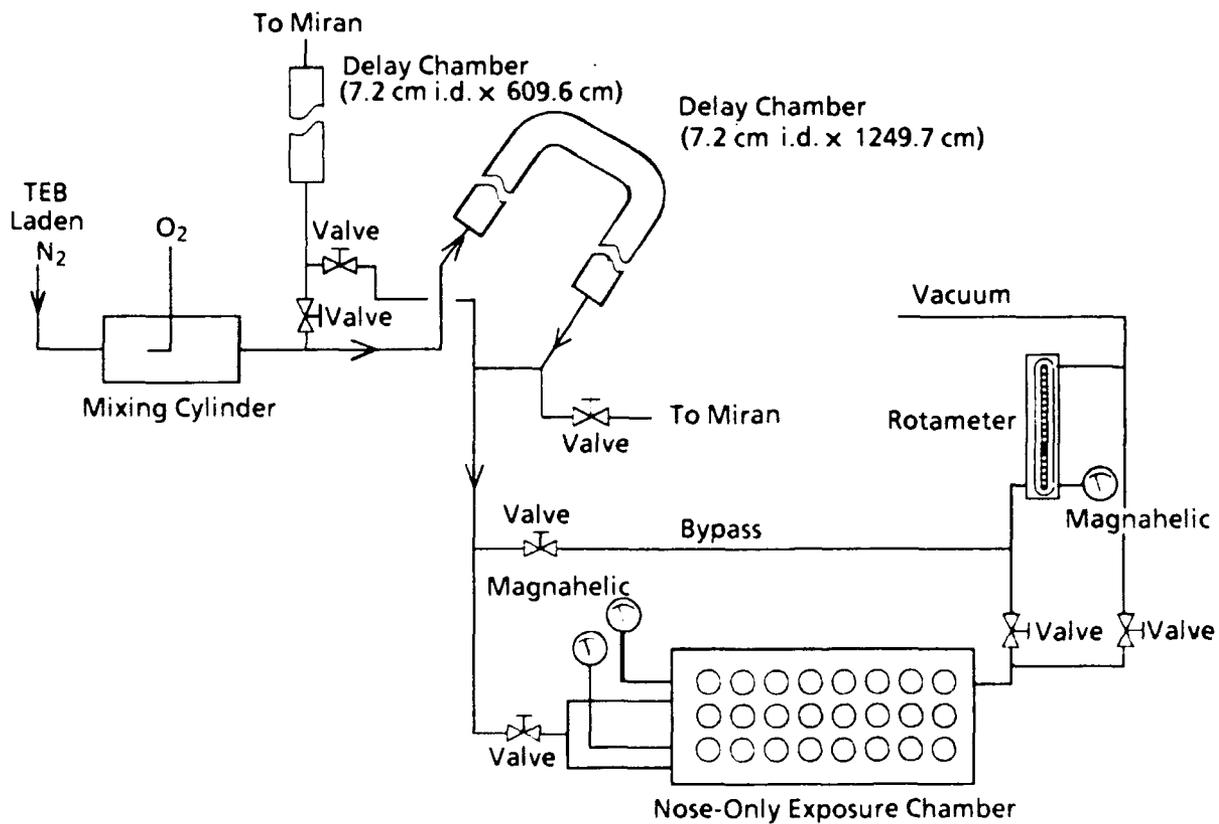


Figure 3. Exposure System Configuration for aTEB Exposures.

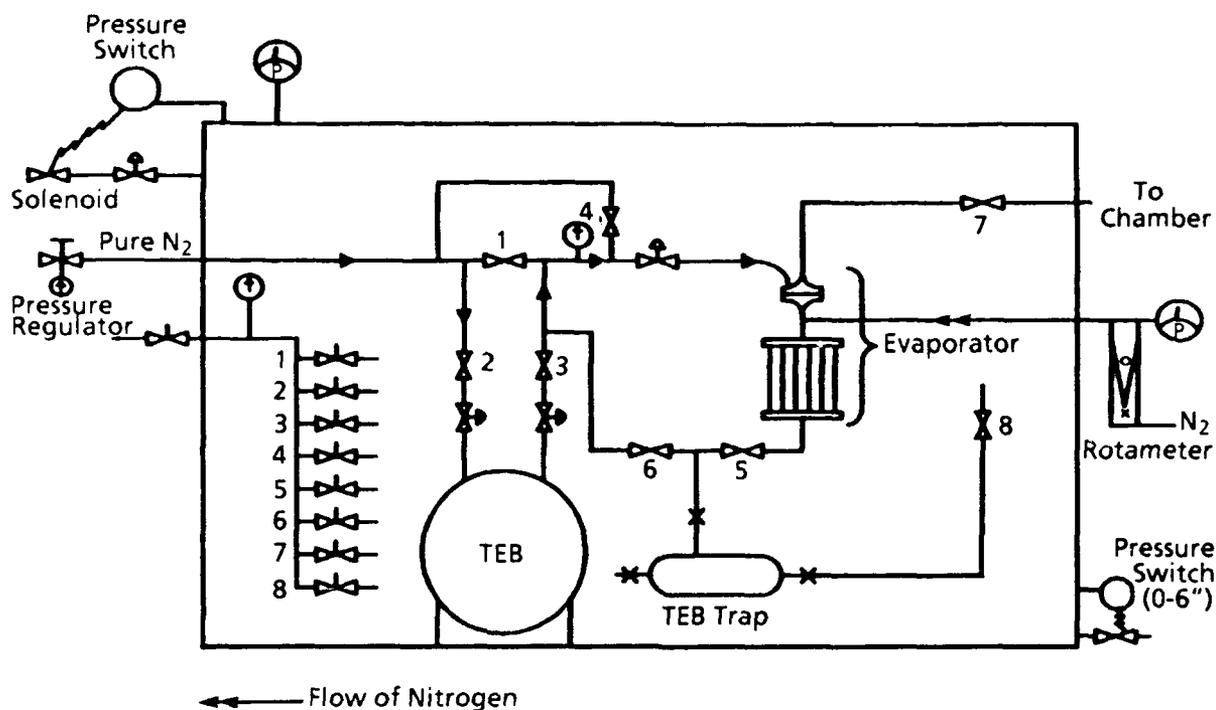
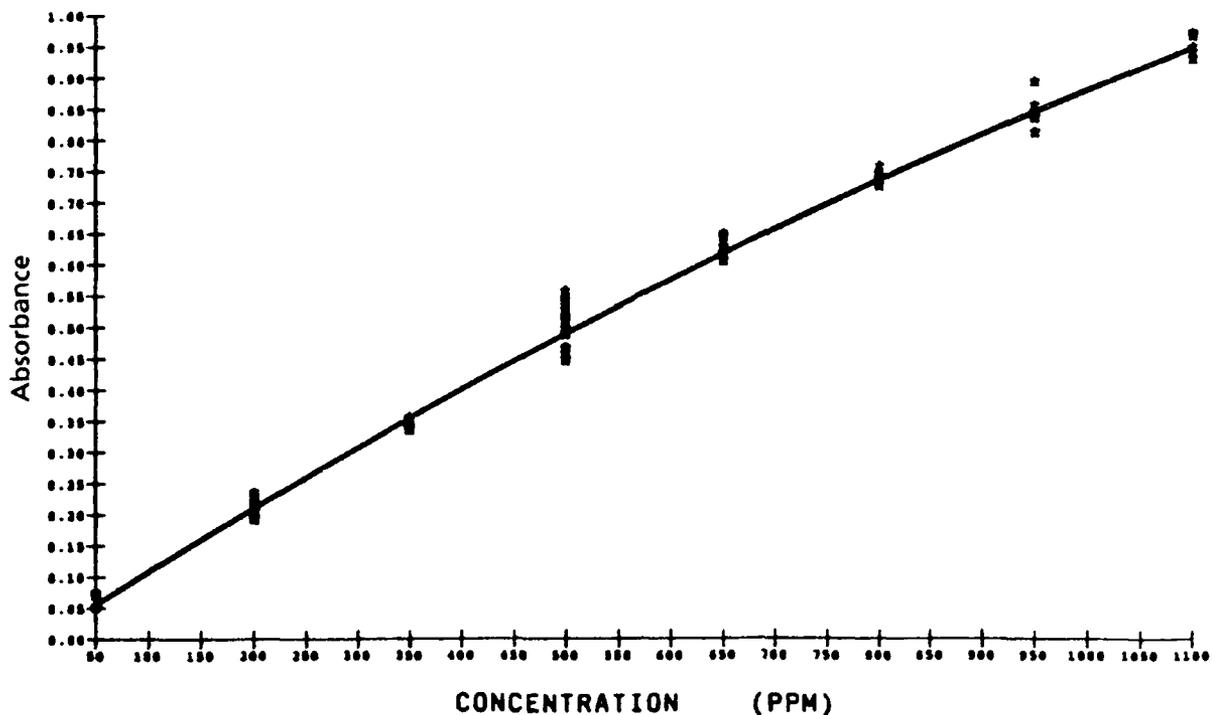


Figure 4. TEB Generation System. Numbered valves are those that are remotely controlled by corresponding, exterior, manual control valves.

## 2.6 ANALYTICAL CHEMISTRY

Infrared spectroscopy (Miran 980, Foxboro, S. Norwalk, CT) was used to determine TEB concentration in the exposure atmospheres. During methods development experiments, TEB was found to produce highly fluctuating, unstable absorbance spectra up to 3 min after mixing TEB vapor laden  $N_2$  with  $O_2$ ; however, absorbance at  $925\text{ cm}^{-1}$  wave number between 3 and 7 min after mixing with  $O_2$  was stable and proportional to the quantity of TEB originally vaporized in the pure  $N_2$ . There was less than a 3% difference in absorbance for a given sample between the 3- and 7-min delay period. Absorbance at 5 min delay time (after mixing with  $O_2$ ) proved to be reproducible and nearly linear to initial TEB in  $N_2$  concentrations over a range of 50 to 1100 ppm. Regression analysis of the calibration data was performed and second order polynomial fit (based on standard goodness-of-fit tests,  $r^2 = 0.992718$ ) was used for data interpretation (Figure 5). Samples for IR analysis were drawn from a point just prior to entry into the exposure chamber. For fTEB exposures the IR sample was transported through an aluminum delay tube (7.21 cm i.d. x 609.6 cm length - mean flow = 5.0 L/min) to allow the samples to reach steady state conditions (5.0 min delay) before analysis. As noted above, correction factors to account for sample transport system loss were determined at various concentrations of the calibration curve. For aTEB exposures a 0.4 cm i.d. x 61 cm tube was used to transport samples directly from the sampling port to the IR, a delay tube was not necessary because

approximately 4.6 min of aging occurred in the inlet system delay loop configuration prior to sampling.



$$-1.935988e-07 * X^{**2} + 1.06915e-03 * X + 3.723244e-03$$

Figure 5. TEB/IR Absorbance Calibration Curve.

The exposure atmospheres also were analyzed by GC (Varian 1200, Varian Associates, Sugar Land, TX) to observe for presence of TEB remaining in the atmospheres and to compare "fingerprints" of the mixtures of fTEB and aTEB oxidation products. Although positive identification of the various peaks in the exposure atmospheres was not possible, a comparison of the fTEB and aTEB chromatograms at various concentrations affirmed qualitative similarity of the exposure environments as a function of concentration. Fingerprints of fTEB and aTEB were unique regardless of exposure concentration. Typical chromatograms for TEB in N<sub>2</sub>, fTEB, and aTEB are shown in Figure 6.

## 2.7 STATISTICAL ANALYSES

Comparisons of mean body weights were performed using a multivariate analysis of covariance for repeated measures test (Barcikowski, 1983). LC<sub>50</sub> determinations were made using a logistic regression analysis (Bates and Watts, 1988). Histopathology data from the pilot investigation were analyzed using a log-linear model, three-way contingency analysis (Dixon, 1990). Histopathology data from the LC<sub>50</sub> study were analyzed using a two-factor analysis of variance and Scheffe's multiple

comparison test (Barcikowski, 1983). Unless otherwise specified, data reported are mean  $\pm$  standard deviation.

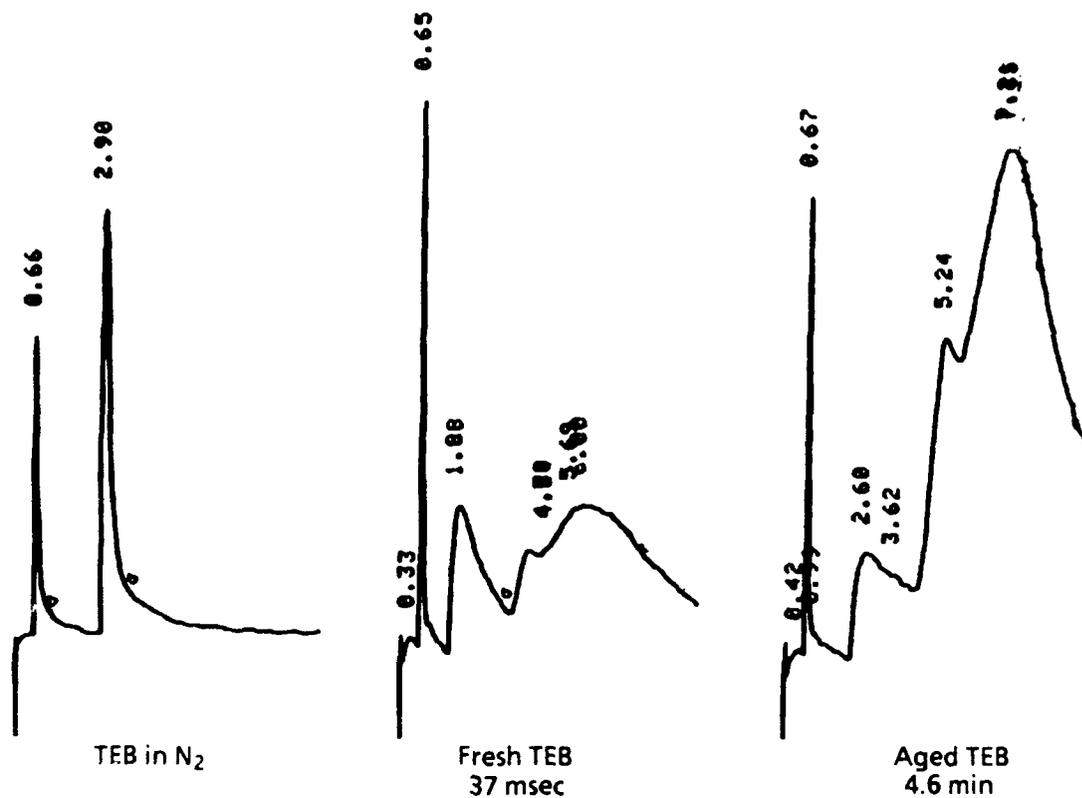


Figure 6. Typical Chromatograms for Unreacted TEB (in N<sub>2</sub>), fTEB and aTEB.

## SECTION 3

### RESULTS

#### 3.1 EXPOSURES

Mean concentrations of the fTEB and aTEB exposures in the pilot investigation were  $956 \pm 57.8$  ( $n = 26$ ) and  $897 \pm 14.6$  ( $n = 29$ ) ppm, respectively, and remained stable throughout the exposure with coefficients of variation (CV) of 6.0 and 1.7%, respectively (Figures 7 and 8). Pilot study exposure durations were only 52 min because in the first (fTEB) exposure mortality at the time was 100%.

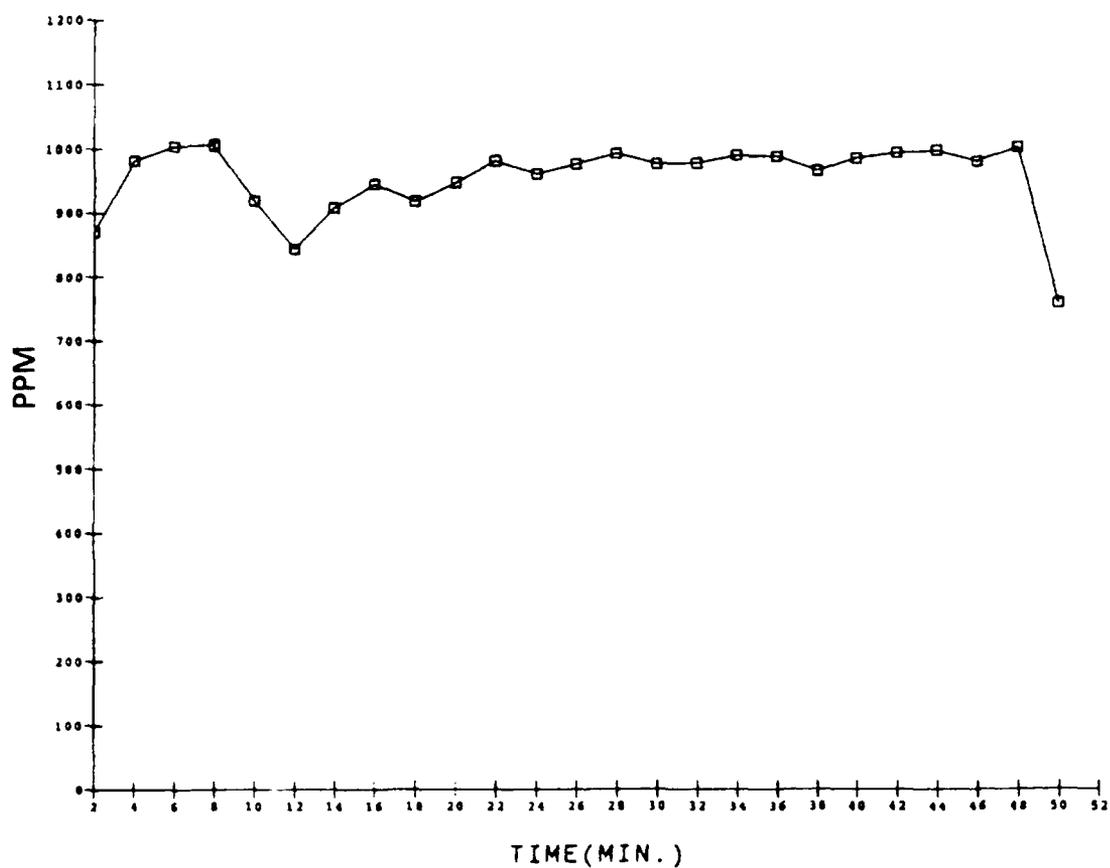


Figure 7. Concentration Profile for 956 ppm fTEB Exposure.

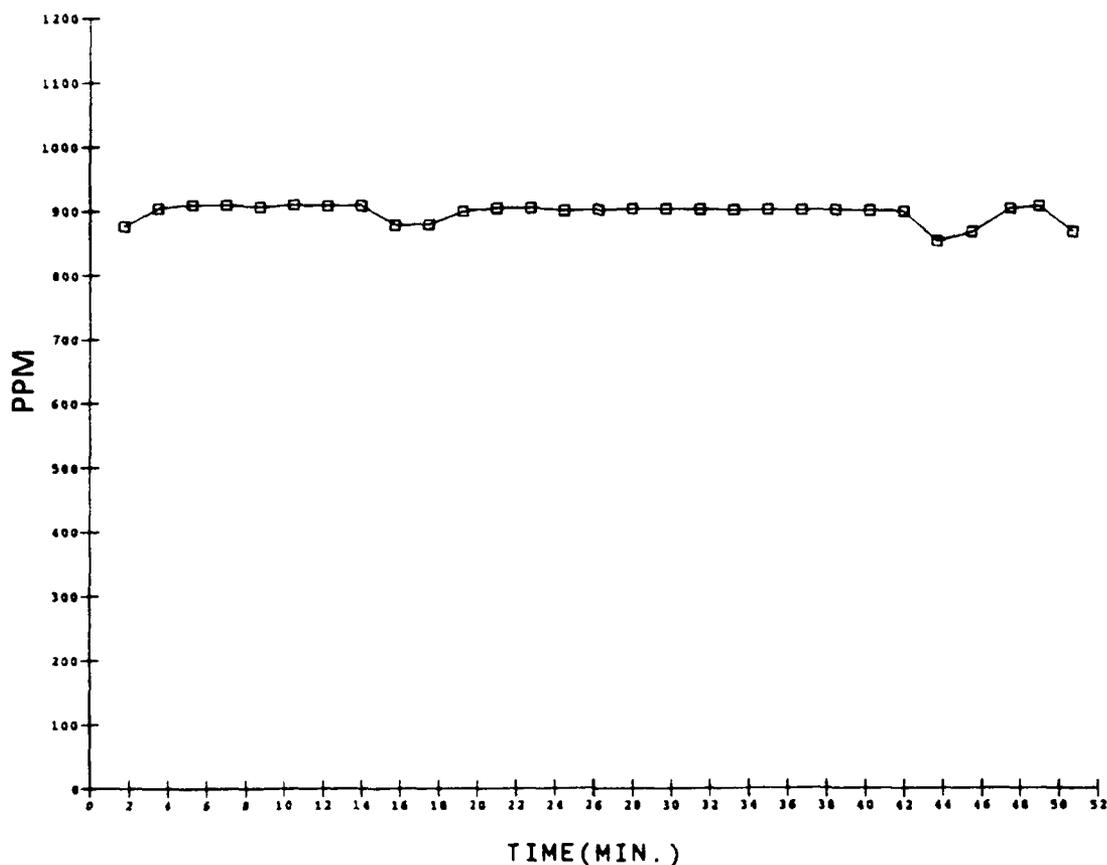


Figure 8. Concentration Profile for 897 ppm aTEB Exposure.

Mean fTEB and aTEB concentrations in the low-level exposure group were  $335 \pm 32.7$  ( $n = 111$ ) and  $399 \pm 27.8$  ( $n = 111$ ) ppm, respectively, and also were stable with CVs of 9.6 and 7.0 % (Figures 9 and 10). Mean concentrations of fTEB and aTEB in the midlevel exposures were  $617 \pm 133.1$  ( $n = 111$ ) and  $575 \pm 96.7$  ( $n = 111$ ) ppm, respectively, and were relatively variable with CVs of 16.9 and 21.6% (Figures 11 and 12). High-level exposure concentrations for fTEB and aTEB were  $741 \pm 40.8$  and  $821 \pm 56.9$  ppm, and were stable with CVs of 5.5 and 6.9%, respectively (Figures 13 and 14). All of these exposures were 1 h in duration.

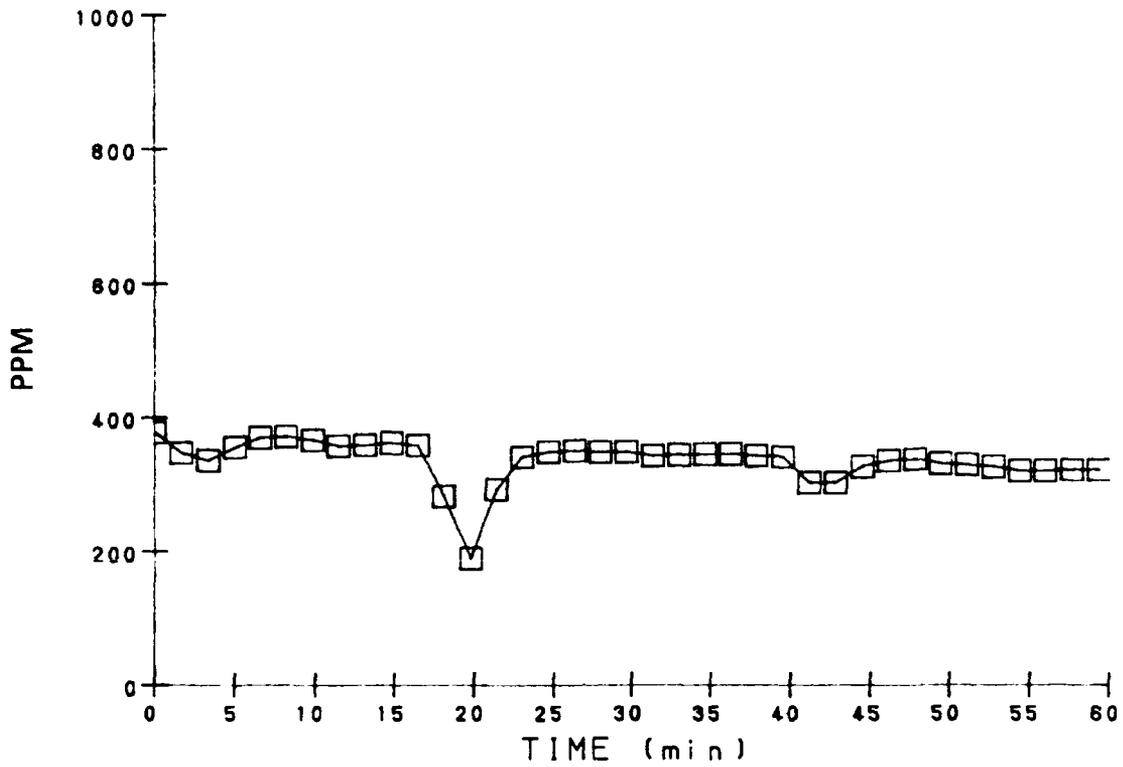


Figure 9. Concentration Profile for 335 ppm fTEB Exposure.

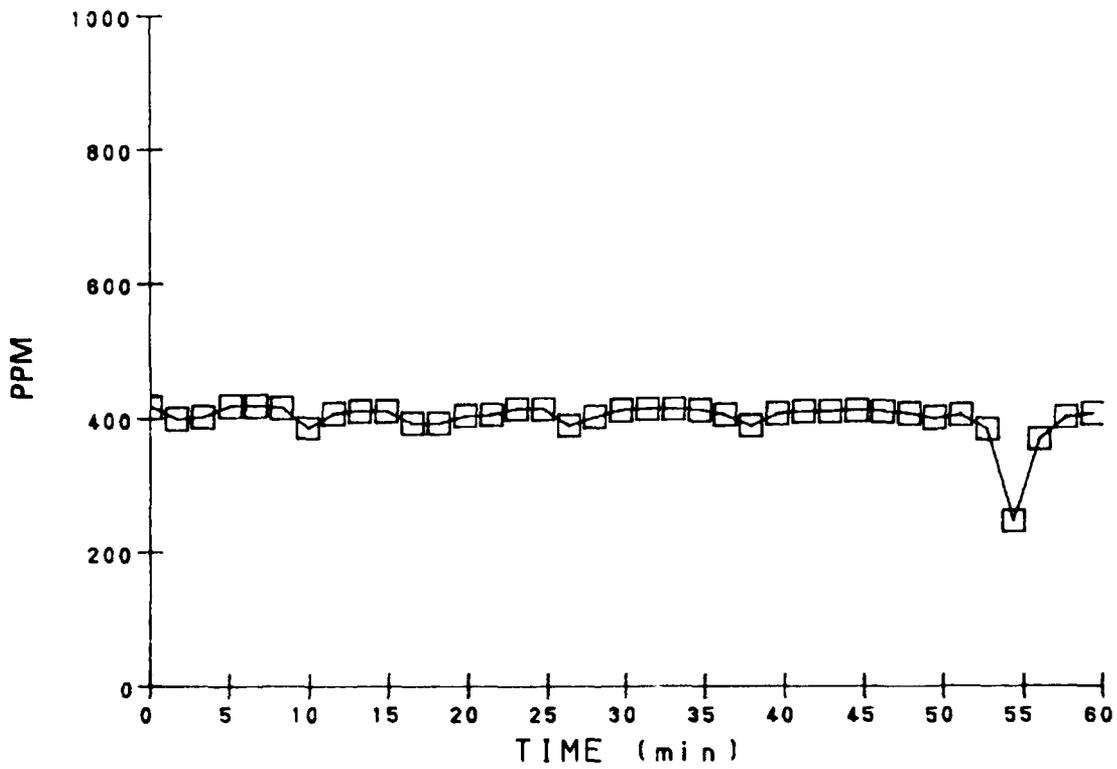


Figure 10. Concentration Profile for 399 ppm aTEB Exposure.

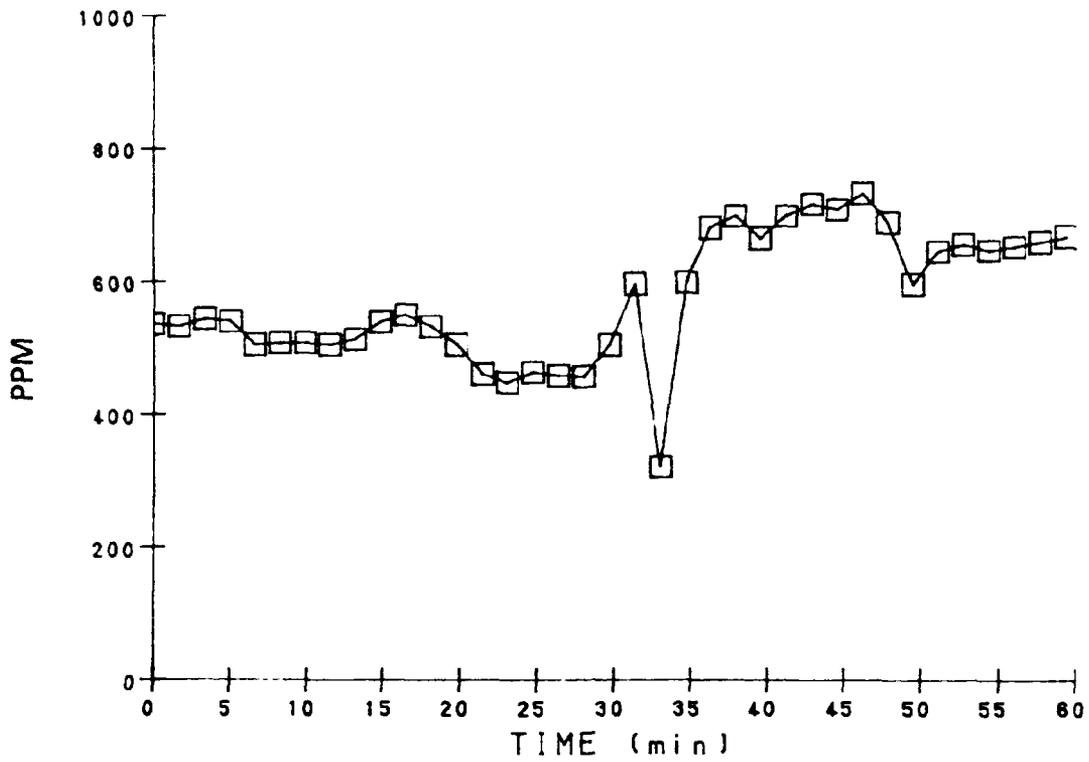


Figure 11. Concentration Profile for 617 ppm fTEB Exposure.

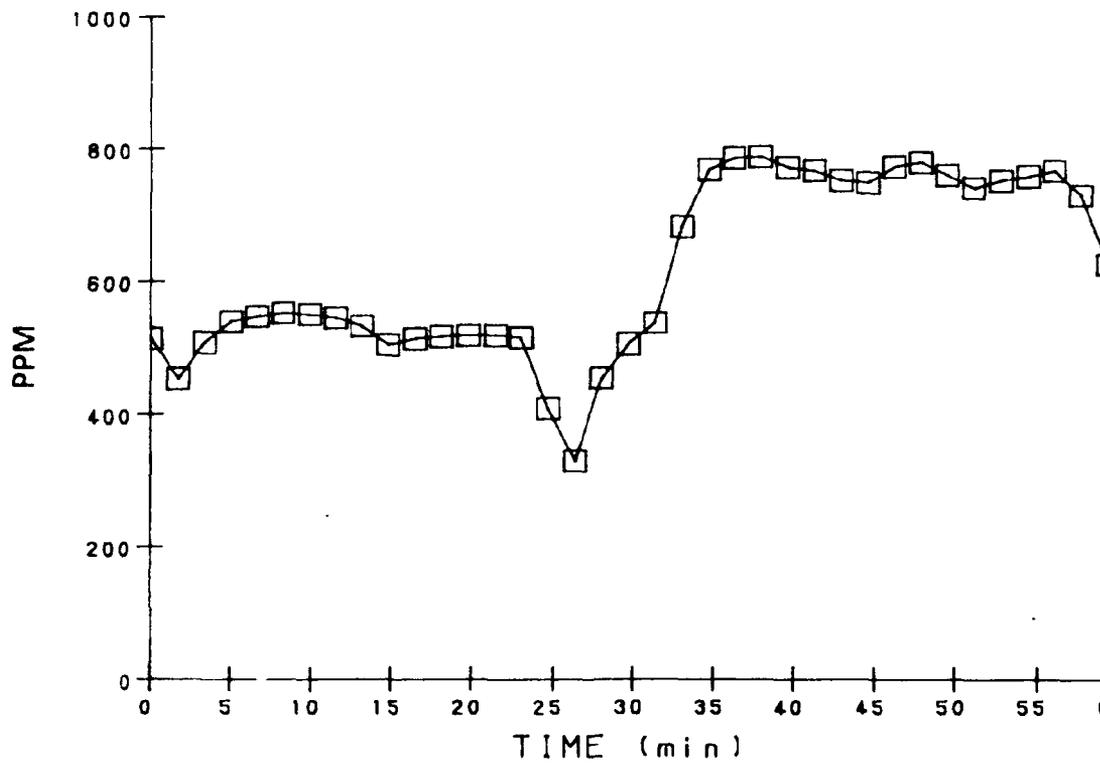


Figure 12. Concentration Profile for 575 ppm aTEB Exposure.

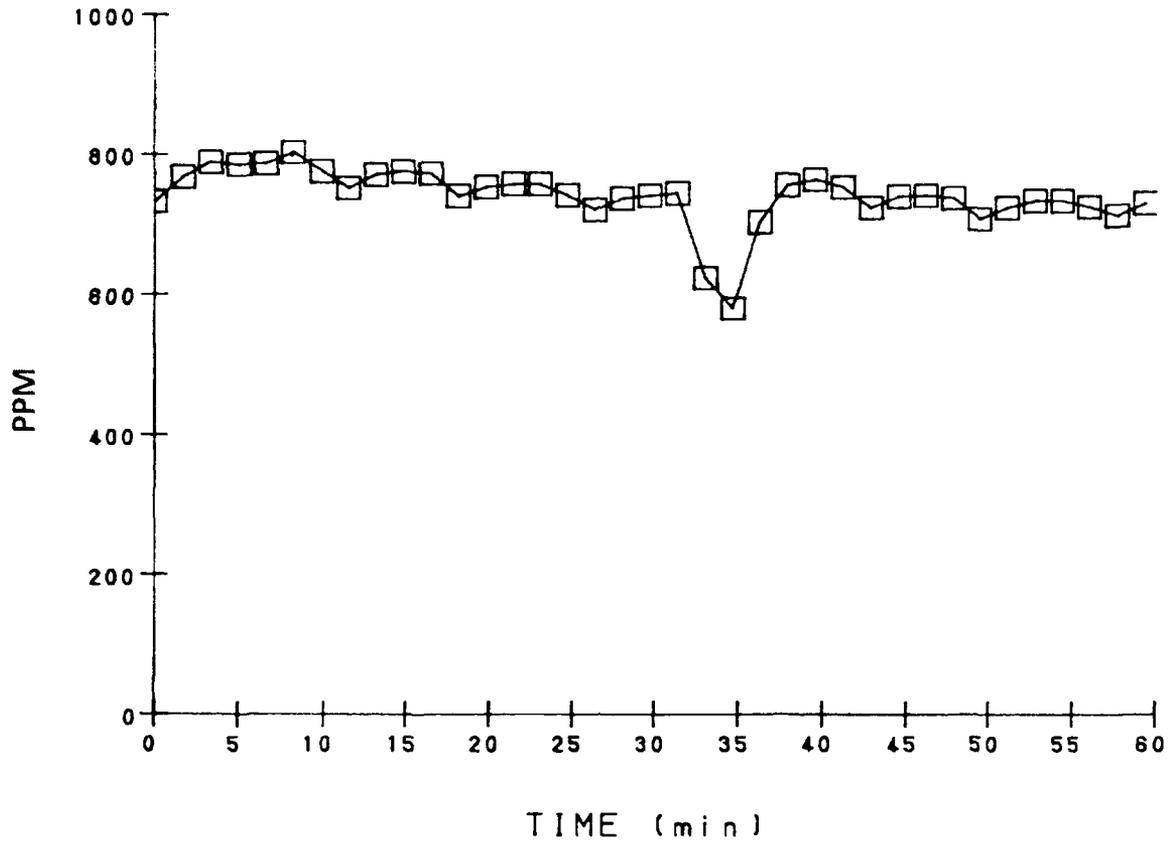


Figure 13. Concentration Profile for 741 ppm fTEB Exposure.

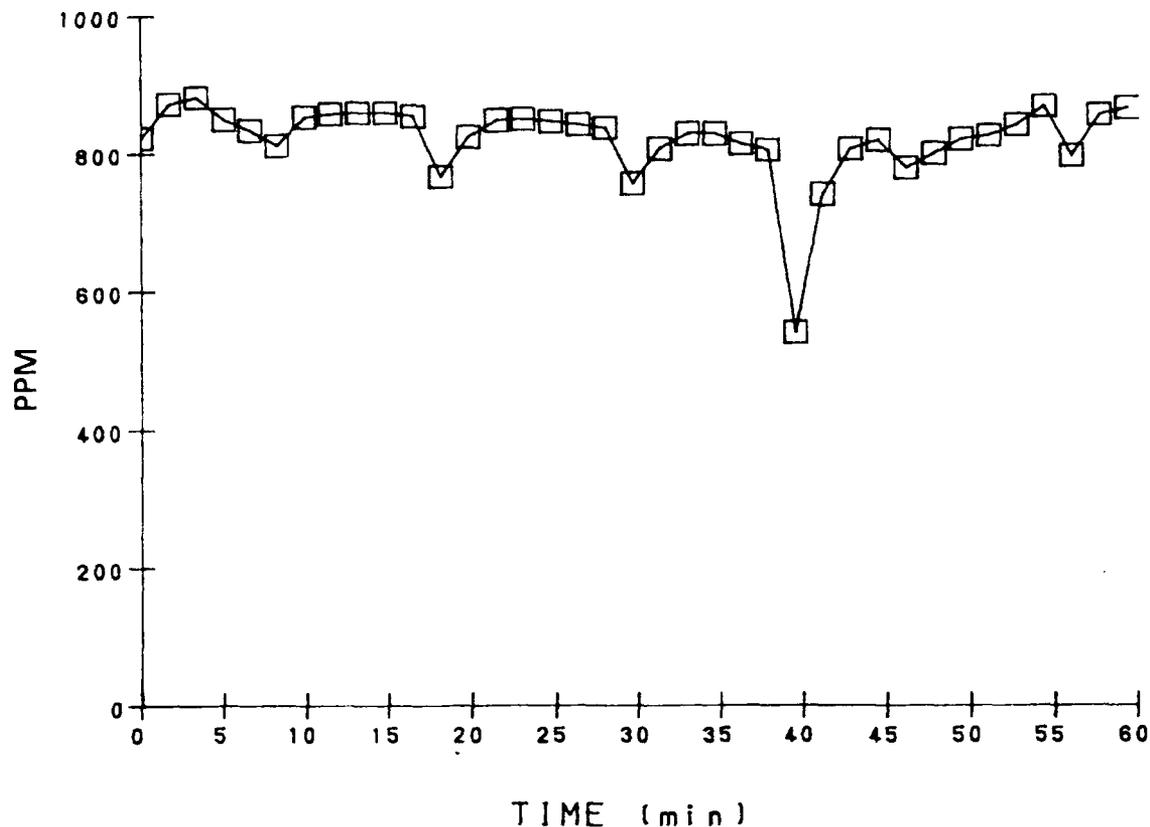


Figure 14. Concentration Profile for 821 ppm aTEB Exposure.

### 3.2 ACUTE TOXICITY

#### Pilot Investigation

During the pilot investigation of fTEB exposure 21 of 23 animals expired, including all 12 of the animals assigned to the 14-day observation subgroup and 9 of the animals assigned to the serial sacrifice subgroup. Due to a malfunctioning exposure tube assembly one animal was excluded from the study. The 2 animals surviving exposure were moribund at 24 h postexposure and were sacrificed. Hence, 956 ppm fTEB was considered an LC<sub>100</sub> concentration. Of the 24 animals exposed to aTEB, a total of 10 died during the exposure and 2 died within 24 h. No other deaths were recorded during the 14-day observation period. Of these 12 deaths, 8 were among animals assigned to the observation subgroup, technically an LC<sub>67</sub> exposure concentration (Table 2). Five of the 8 animals that died from aTEB exposure were male. The remaining 4 animals in the observation subgroup survived the 14-day observation period. Of the 8 animals assigned to the aTEB serial sacrifice subgroup, 2 were sacrificed 4 h postexposure, 2 were sacrificed at 24 h postexposure, and 2 each were sacrificed 7 and 14 days postexposure. None of the animals exposed to the artificial atmosphere,

without TEB, expired during the study. Evaluation of the general health of exposed animals as indicated by body weight maintenance over time was not possible; however, observed differences in the incidence of mortality between exposure groups could not be attributed to initial differences in exposure group body weight (Table 3).

**TABLE 2. LETHALITY IN RATS FOLLOWING EXPOSURE TO TEB (PILOT STUDY)**

Exposure Concentration (ppm)	Mortality Males	Mortality Females	Total
956 (fTEB) <sup>a</sup>	6/6	6/6	12/12
897 (aTEB) <sup>b</sup>	5/6	3/6	8/12
Control	0/6	0/6	0/12

<sup>a</sup> Fresh TEB.

<sup>b</sup> Aged TEB.

**TABLE 3. PREEXPOSURE BODY WEIGHTS<sup>a</sup> OF CONTROL AND EXPOSURE RATS (PILOT STUDY)**

	Control	fTEB <sup>b</sup>	aTEB <sup>c</sup>
Males	270.3 ± 9.10	251.8 ± 11.60	257.5 ± 9.50
Females	179.8 ± 5.94	175.7 ± 6.77	175.4 ± 7.66

<sup>a</sup> All data are mean ± standard deviation of body weight in grams.

<sup>b</sup> Fresh TEB.

<sup>c</sup> Aged TEB.

Clinical observations during the fTEB exposure indicated that approximately 70% of the animals exhibited either hyperpnea, gasping, or Cheyne-Stokes pattern breathing. Bronchospastic hiccough was evident in animals that expired during exposure. Likewise, all animals exhibiting abnormal breathing responses appeared hyperactive as evidenced by escape/avoidance responses in the exposure apparatus. Animals surviving exposure exhibited apneustic breathing with minimal inspiratory effort and subsequently a probable reduction in tidal volume. Hyperactivity in these animals was not evident and all were cyanotic on removal from the exposure apparatus. During the aTEB exposure, approximately 50% of the animals demonstrated abnormal breathing patterns and hyperactivity. All 10 of the animals that expired during exposure were hyperpneic and appeared to experience broncospasm prior to death. The surviving animals either did not evince abnormal breathing or were moderately apneustic. No breathing pattern anomalies or hyperactivity were observed in control exposure animals.

#### LC<sub>50</sub> Determination

Mortality rates of the animals exposed to either fTEB or aTEB in this study are shown in Table 4. No animals exposed to the lowest concentrations of fTEB and aTEB (335 and 399 ppm, respectively) expired during the course of the investigation. One animal each expired after midlevel exposure to fTEB or aTEB (617 and 575 ppm, respectively); interestingly, both were males. The animal exposed to

fTEB died during the exposure and the animal exposed to aTEB died within 24 h of exposure. At higher levels of fTEB and aTEB (741 and 821 ppm, respectively), 5 of 12 animals expired from fTEB exposure and 10 of 12 animals expired from aTEB exposure. All 5 animals that died from fTEB exposure were male, 3 died during the exposure and 2 died within 24 h. Six of the 10 animals that died from aTEB exposure were male. One male and 2 females died during exposure, 2 males and 2 females died on the day of exposure, and the 3 additional deaths occurred within 2 days of exposure and all were males.

TABLE 4. LETHALITY IN RATS FOLLOWING EXPOSURE TO TEB (LC<sub>50</sub> STUDY)

Exposure Concentration (ppm)	Mortality Males	Mortality Females	Total
335 (fTEB) <sup>a</sup>	0/6	0/6	0/12
399 (aTEB) <sup>b</sup>	0/6	0/6	0/12
617 (fTEB)	1/6	0/6	1/12
575 (aTEB)	1/6	0/6	1/12
741 (fTEB)	5/6	0/6	5/12
821 (aTEB)	6/6	4/6	10/12
Control	0/6	0/6	0/6

<sup>a</sup> Fresh TEB.

<sup>b</sup> Aged TEB.

The overall LC<sub>50</sub> for TEB (both fTEB and aTEB, both genders; calculations did not include pilot study data) was 738 ppm (692 to 806). The LC<sub>50</sub> for fTEB only (both genders) was greater at 766 ppm (699 to 779) and the LC<sub>50</sub> for aTEB only (both genders) was less at 709 (629 to 798). The combined (both fTEB and aTEB) LC<sub>50</sub> for males was 676 ppm (591 to 719), whereas the combined LC<sub>50</sub> for females was >821 ppm. Numbers in parentheses are lower and upper 95% fiducial limits. The general health of animals surviving exposures, as indicated by body weight maintenance, did not demonstrate exposure concentration, type of TEB, or gender-specific effects (Table 5).

During exposures (all concentrations, either fTEB or aTEB), the majority of animals exhibited labored, shallow breathing which persisted for up to 24 h in animals surviving exposure. Animals exposed at the mid- and high-level concentrations of fTEB or aTEB also exhibited tremors during exposure, cyanosis, and a persistent general lassitude lasting up to 3 days postexposure.

TABLE 5. BODY WEIGHT<sup>a</sup> VS. TIME POSTEXPOSURE TO TEB (LC<sub>50</sub> STUDY)

Concentration (ppm)	Sex	Preexposure Weight	Postexposure Weight			
			2 Days	4 Days	7 Days	14 Days
335 (fTEB) <sup>b</sup>	Male	227.7 ± 20.3	225.9 ± 6.6	228.7 ± 7.5	239.4 ± 8.7	258.2 ± 10.0
	Female	146.1 ± 7.8	147.2 ± 7.7	147.3 ± 5.9	154.6 ± 7.2	162.3 ± 8.4
339 (aTEB) <sup>c</sup>	Male	239.4 ± 10.6	223.5 ± 9.0	232.9 ± 7.3	241.6 ± 6.7	256.0 ± 5.7
	Female	146.8 ± 6.5	146.2 ± 5.9	150.6 ± 7.2	154.3 ± 5.1	158.0 ± 6.6
617 (fTEB) <sup>b</sup>	Male	250.3 ± 12.7	229.5 ± 9.4 <sup>d</sup>	234.4 ± 9.3 <sup>d</sup>	249.7 ± 10.4 <sup>d</sup>	271.1 ± 12.4 <sup>d</sup>
	Female	151.4 ± 6.3	148.7 ± 1.0	151.9 ± 2.6	159.0 ± 5.0	166.0 ± 5.9
575 (aTEB) <sup>c</sup>	Male	239.3 ± 18.9	217.2 ± 18.6 <sup>d</sup>	223.3 ± 17.6 <sup>d</sup>	233.7 ± 18.3 <sup>d</sup>	255.6 ± 17.9 <sup>d</sup>
	Female	145.9 ± 6.4	146.9 ± 4.8	153.0 ± 6.1	157.9 ± 4.9	162.6 ± 5.6
741 (fTEB) <sup>b</sup>	Male	248.9 ± 13.1	235.9 ± 0.0 <sup>e</sup>	232.7 ± 0.0 <sup>e</sup>	242.9 ± 0.0 <sup>e</sup>	270.3 ± 0.0 <sup>e</sup>
	Female	154.9 ± 10.9	151.9 ± 6.7	158.4 ± 6.4	160.9 ± 6.8	169.8 ± 6.6
821 (aTEB) <sup>c</sup>	Male	256.5 ± 12.8	f	f	f	f
	Female	154.7 ± 7.6	148.5 ± 11.8 <sup>g</sup>	152.0 ± 14.1 <sup>g</sup>	158.6 ± 12.5 <sup>g</sup>	174.2 ± 9.6 <sup>g</sup>
Control	Male	258.0 ± 14.6	255.7 ± 12.9	263.1 ± 14.1	265.3 ± 18.3	279.1 ± 14.0
	Female	160.7 ± 5.3	160.8 ± 6.5	164.5 ± 7.5	165.0 ± 7.5	167.0 ± 7.5

<sup>a</sup> All data are mean ± standard deviation of body weight in grams.

<sup>b</sup> Fresh TEB.

<sup>c</sup> Aged TEB.

<sup>d</sup> n = 5 (one animal died).

<sup>e</sup> n = 1 (five animals died).

<sup>f</sup> All animals died.

<sup>g</sup> n = 2 (four animals died).

### 3.3 PATHOLOGY

#### Pilot Investigation

The primary finding attributable to TEB exposure was multifocal reddened lung parenchyma in rats exposed to fTEB. Epistaxis also occurred in rats exposed to fTEB; however, the nasal turbinates of these animals were not collected for histopathologic examination. Chocolate-brown blood covered the perinasal and perioral facial regions and was found in the nares of many of the rats exposed to fTEB. The irritant effect of TEB was primarily documented by histopathologic evaluation of the tracheas of rats in all groups and the turbinates of rats exposed to aTEB.

Pulmonary edema in rats exposed to fTEB was the most important histopathologic finding. The edema was found most frequently around pulmonary vasculature and less frequently in airway lumen and alveoli. Overall, there was no difference in the severity of pulmonary edema in the 2 rats euthanized at 24 h postexposure to fTEB than that encountered in animals that died during exposure. However, these 2 animals tended to have greater accumulation of interstitial edematous fluid. Animals that died within 24 h of aTEB exposure developed pulmonary edema at least as early as 4 h postexposure. No respiratory tract lesions were present in rats exposed to aTEB at 7 and 14 days postexposure. Nasal and tracheal epithelial necrosis occurred in a few of the rats that were exposed to fTEB and was primarily limited to the ventral half of the tracheal circumference.

Other significant findings were centrolobar hepatocytic, cytoplasmic, vacuolar degeneration, and congestion in animals exposed to both fTEB and aTEB. The vacuoles frequently contained a homogeneous eosinophilic material suggestive of a proteinaceous cell product. Acute to subacute multifocal myocarditis was observed frequently in all exposure groups.

An incidence summary of significant lesions observed in animals exposed to fTEB and aTEB in the pilot investigation shown in Table 6. Significant incidence of pulmonary and hepatic lesions were found predominantly in animals (of either genders) exposed to fTEB. Lesions found in aTEB-exposed animals were predominant in males.

#### LC<sub>50</sub> Determination

In this investigation gross observations of vascular engorgement and organ discolorations suggestive of vascular congestion or hemorrhage were found in all TEB exposure groups, as well as in some control animals. Hemorrhage was occasionally confirmed by histopathologic examination. Congestion was histopathologically confirmed for the lung, liver, and kidneys of some animals. Except for those tissues with concurrent diagnoses of edema (primarily lung), the vascular alterations were considered agonal rather than exposure-related.

TABLE 6. SUMMARY OF SELECTED MICROSCOPIC LESIONS (PILOT STUDY)

Organ-Lesion	Incidence %					
	Males			Females		
	Control	fTEB <sup>a</sup>	aTEB <sup>b</sup>	Control	fTEB <sup>a</sup>	cTEB <sup>b</sup>
Nasal turbinates	(7) <sup>c</sup>	(0) <sup>d</sup>	(12)	(8)	(0) <sup>d</sup>	(12)
Epithelial necrosis	0	-	50 <sup>e</sup>	0	-	8
Congestion	0	-	33 <sup>e</sup>	0	-	8
Hemorrhage	0	-	8	0	-	0
Trachea	(8)	(12)	(12)	(8)	(11)	(12)
Autolysis	0	25 <sup>e</sup>	8	0	36 <sup>e</sup>	0
Epithelial necrosis	0	42 <sup>e</sup>	42 <sup>e</sup>	0	27 <sup>e</sup>	8
Hemorrhage	0	8	8	0	18	0
Fibrin deposition	0	0	0	0	18	0
Lung	(8)	(12)	(12)	(8)	(11)	(12)
Alveolar edema	0	17	0	25	0	0
Perivascular edema	0	33 <sup>e</sup>	17	25	55 <sup>e</sup>	8
Interstitial pneumonitis	13	0	0	25	0	0
Epithelial necrosis	0	0	8	0	0	0
Congestion	0	92 <sup>e</sup>	17	0	91 <sup>e</sup>	8
Hemorrhage	0	0	17	0	0	0
Arterial mineralization	0	17	0	0	9	0
Liver	(8)	(12)	(12)	(8)	(11)	(12)
Hepatocytomegaly	0	0	17	0	0	0
Degeneration <sup>f</sup>	0	92 <sup>e</sup>	67 <sup>e</sup>	0	73 <sup>e</sup>	25 <sup>e</sup>
Hepatocytic necrosis	0	8	8	0	0	8
Congestion	0	58 <sup>e</sup>	17	0	82 <sup>e</sup>	8
Kidney	(8)	(12)	(12)	(8)	(11)	(12)
Renal tubular necrosis	0	0	8	0	0	0
Hyaline droplet degeneration	13	17	17	0	0	0
Congestion	0	17	0	0	18	0
Heart	(8)	(12)	(12)	(8)	(11)	(12)
Myocarditis, acute	0	8	8	0	18	0
Myocarditis, subchronic	63	17 <sup>g</sup>	8 <sup>g</sup>	38	9 <sup>g</sup>	8 <sup>g</sup>

<sup>a</sup> Fresh TEB.

<sup>b</sup> Aged TEB.

<sup>c</sup> Values in parentheses are number of animals from which tissues were harvested.

<sup>d</sup> Tissues not harvested.

<sup>e</sup> Significantly greater than controls,  $p \leq 0.05$ , Bates-Watts log-linear, three-way contingency analysis.

<sup>f</sup> Hepatocytic, cytoplasmic, vacuolar.

<sup>g</sup> Significantly less than controls,  $p \leq 0.05$ , Bates-Watts log-linear, three-way contingency analysis.

The histopathologic diagnoses and their corresponding incidences for females and males are listed in Tables 7 and 8, respectively. Pulmonary congestion and perivascular edema were considered to be dose-related and gender-related effects attributable to fTEB and aTEB exposure. Significant pulmonary congestion and perivascular edema were found in females exposed to aTEB; whereas, these sequelae were found at significant levels in males exposed to both fTEB and aTEB. Subjectively, the severity of the pulmonary congestion and perivascular edema was judged to range from minimal to slight. Significant hepatocytic degeneration and congestion was found in females exposed to the highest concentration of aTEB; whereas, the significant incidence of the same lesions was found in males exposed to the highest concentrations of both fTEB and aTEB.

A gender-related effect was disclosed for the occurrence of necrotizing myocarditis; however, a dose-related effect was not observed and could not be substantially related to TEB exposure. Neither dose nor TEB-type effects could be related to various renal lesions found among all exposure groups, including controls; however, a variety of background renal lesions was more prevalent in males. Renal lesions were not considered to be influenced by either type of TEB exposure, nor were they considered to affect the impact of TEB (either fTEB or aTEB) exposure on other tissues.

TABLE 7. SUMMARY OF SELECTED MICROSCOPIC LESIONS LC<sub>50</sub> STUDY – FEMALE RATS

Organ-Lesion	Incidence %						
	Control	f335 <sup>a</sup>	a339 <sup>b</sup>	f617 <sup>a</sup>	a575 <sup>b</sup>	f741 <sup>a</sup>	a721 <sup>b</sup>
Liver	(6) <sup>c</sup>	(6)	(6)	(6)	(6)	(6)	(6)
Hepatocytic degeneration	0	0	0	0	0	0	50 <sup>e</sup> [0.5] <sup>d</sup>
Congestion	0	0	0	0	0	0	67 <sup>c</sup> [2.2]
Heart	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Necrotizing myocarditis	0	17 [0.2]	0	0	0	0	0
Mineralization	0	0	0	33 [0.31]	0	0	0
Kidney	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Hyaline droplet accumulation	0	0	0	0	0	0	17 [0.2]
Tubular mineralization	100 [1.0]	100 [1.0]	100 [1.0]	100 [1.0]	100 [1.0]	100 [1.0]	100 [1.0]
Congestion	100 [2.2]	0	0	0	0	100 [2.0]	83 [2.5]
Lung	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Interstitial pneumonitis	0	0	17 [0.2]	0	0	0	0
Congestion	0	0	0	0	0	0	50 <sup>e</sup> [0.8]
Perivascular edema	0	0	0	0	0	0	50 <sup>e</sup> [1.2]
Alveolitis	0	0	0	0	0	0	17 [0.3]

<sup>a</sup> Fresh TEB followed by concentration in ppm

<sup>b</sup> Aged TEB followed by concentration in ppm

<sup>c</sup> Number of animals from which tissues were harvested.

<sup>d</sup> Severity scoring system defined as 0 = no lesion; 1 = minor or very slight; 2 = slight; 3 = moderate; 4 = marked; 5 = severe. Group scores are calculated by dividing the sum of individual scores by number of affected animals.

<sup>e</sup> Statistically different from controls,  $p \leq 0.05$ ; two-factor analysis of variance and Scheffe's multiple comparison test.

TABLE 8. SUMMARY OF SELECTED MICROSCOPIC LESIONS LC<sub>50</sub> STUDY – MALE RATS

Organ-Lesion	Incidence %						
	Control	f335 <sup>a</sup>	a339 <sup>b</sup>	f617 <sup>a</sup>	a575 <sup>b</sup>	f741 <sup>a</sup>	a721 <sup>b</sup>
Liver	(6) <sup>c</sup>	(6)	(6)	(6)	(6)	(6)	(6)
Hepatocytic degeneration	50 [1.0] <sup>d</sup>	0	0	0	17 [0.3]	50 [0.8]	50 [0.5]
Congestion	33 [0.7]	0	0	0	0	83 <sup>e</sup> [3.0]	83 <sup>e</sup> [2.2]
Heart	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Necrotizing myocarditis	33 [0.3]	33 [0.8]	33 [0.8]	83 <sup>e</sup> [1.8]	50 [1.2]	50 [0.8]	50 [1.2]
Mineralization	33	0	0	17	33	0	0
Kidney	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Hyaline droplet accumulation	100 [3.2]	100 [1.2]	100 [2.3]	100 [2.3]	100 [2.6]	67 [1.2]	100 [1.8]
Hyaline casts	100 [1.0]	100 [1.0]	100 [1.0]	100 [1.0]	100 [1.0]	83 [1.0]	100 [1.0]
Tubular mineralization	83 [0.8]	33 [0.3]	83 [0.8]	100 [1.0]	100 [1.0]	100 [1.0]	67 [0.7]
Cystic distal conv. tubule	0	0	0	0	20 [0.2]	0	0
Congestion	83 [2.0]	0	0	0	0	83 [3.3]	100 [3.2]
Distal conv. tubule necrosis	0	0	0	0	0	0	50 <sup>e</sup> [1.2]
Lung	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Congestion	0	0	0	0	17	83 <sup>e</sup> [1.7]	67 <sup>e</sup> [1.7]
Perivascular edema	0	0	0	0	0	83 <sup>e</sup> [1.5]	83 <sup>e</sup> [2.0]
Hemorrhage	0	0	0	0	0	17 [0.3]	17 [0.3]
Alveolitis	0	0	0	0	0	17 [0.3]	0
Alveolar edema	0	0	0	0	0	0	17 [0.5]

<sup>a</sup> Fresh TEB followed by concentration in ppm.

<sup>b</sup> Aged TEB followed by concentration in ppm.

<sup>c</sup> Number of animals from which tissues were harvested.

<sup>d</sup> Severity scoring system defined as 0 = no lesion; 1 = minor or very slight; 2 = slight; 3 = moderate; 4 = marked; 5 = severe. Group scores are calculated by dividing the sum of individual scores by number of affected animals.

<sup>e</sup> Statistically different from controls,  $p \leq 0.05$ ; two-factor analysis of variance and Scheffe's multiple comparison test.

## SECTION 4

### DISCUSSION

The establishment of 1-, 12-, and 24-h emergency exposure limits (EELs) and 7-day chronic exposure limits (CELs) for the inhalation of TEB fumes is essential for the protection of personnel handling this material. Although not preferred by the NRC-COT, the use of LC<sub>50</sub> data as a basis for formulating EELs and CELs for TEB, at present, represents the only viable recourse due to the paucity of end point specific toxicity data (Marzulli, 1985). Previously, the only available acute inhalation toxicity data for TEB, on which current limit standards are based, were provided by Rinehart (1960) who reported a 4-h LC<sub>50</sub> for TEB of 700 ppm or 2.8 mg/L. The present investigation provides important information for consideration of a reevaluation of these standards, based upon advanced technological approaches to the quantitative and qualitative characterization of TEB atmospheres and a revised evaluation of the acute inhalation toxicity of TEB. Superficially, the overall LC<sub>50</sub> of 738 ppm for TEB observed in the present investigation agrees well with Rinehart's findings; however, considering several factors (primarily exposure duration), it is apparent that the results of the present investigation imply that TEB is more toxic than previous data indicate. Rinehart's experiments were conducted exclusively on male rats of unspecified strain weighing between 200 and 300 g. Therefore an LC<sub>50</sub> of 676 ppm (males only) observed in the present investigation may serve as a more appropriate basis of comparison. Assuming that Haber's principle (Haber, 1924; MacFarland, 1976) applies to TEB, then a 4-h LC<sub>50</sub> of 169 ppm (for males) would be expected, which corresponds to slightly over 24% of the value estimated by Rinehart. Marzulli (1985) points out that Rinehart's (1960) use of nominal (calculation of weight loss from bubbling N<sub>2</sub> through a 9% mixture of TEB in mineral oil) and not analytical methods to determine exposure concentration undoubtedly resulted in an overestimation of the true exposure concentration, due to a loss of TEB from adsorption on exposure chamber surfaces. Several other factors, such as weight loss due to evaporation of materials other than TEB from the dispersion apparatus, including mineral oil, may have contributed to the overestimation of TEB concentration. Standing mineral oil (passive air/liquid interface) has been shown to lose about 2.0% of its mass in 24 h and up to 4.5% in approximately 36 h (unpublished observation). Even if there was not a significant overestimation of TEB exposure concentration due to these factors, it is well known that vapor concentration of a material decreases exponentially as a result of vaporization from a fixed quantity reservoir or from another media via dispersive separation by a continuous carrier gas flow (Nelson, 1971; Barrow and Blehm, 1990). Thus, it is possible that in Rinehart's apparatus, animals were exposed to higher initial TEB concentrations followed by low concentrations during the latter part of the exposure period. It is feasible that the total 700 ppm of TEB may have been delivered within the first hour or so of the 4-h exposure period. Because of the

suspected overestimation of the exposure concentration, Marzulli (1985) postulated that a more appropriate index of the acute inhalation toxicity of TEB should be based on calculation of the LC<sub>50</sub> from the ip LD<sub>50</sub> (22.7 mg/kg) observed by Rinehart (1960). Marzulli predicted that the 4-h LC<sub>50</sub> for a 133-g rat inhaling 73 mL/min would be 0.147 mg/L (22.7 mg/kg × 0.113 kg/17.52 L, sic.) or 37 ppm, which was approximately 1/20th of the LC<sub>50</sub> estimated from the nominal data. A corresponding 1-h predicted LC<sub>50</sub> was 0.586 mg/L (22.7 mg/kg × 0.113 kg/4.38 L). These derived LC<sub>50</sub> values were used as a basis for recommending 1-, 12-, and 24-h EELs of 0.6, 0.05, and 0.03 ppm, respectively. These predicted LC<sub>50</sub>s assume that all of the inhaled TEB is absorbed and that the mechanism(s) of acute TEB toxicity for the inhalation and parenteral routes of exposure are not substantially different. A more appropriate predicted 4-h LC<sub>50</sub>, based on TEB inhalation by a 250-g rat (midrange weight of animals in Rinehart's investigation and comparable to 244-g mean body weight of males exposed in this investigation) with a minute ventilation (V<sub>E</sub>) of 125 mL/min (V<sub>E</sub> = 379 BW<sup>0.80</sup>; Stahl, 1967), would be 0.189 mg/L (22.7 mg/kg × 0.250 kg/30 L) or 49 ppm (using Marzulli's methods). A derived LC<sub>50</sub> of 49 ppm is approximately one-third (29%, 49/169) of that expected from the present investigation. This suggests that the present EELs, based on a 1:20 predicted vs. observed LC<sub>50</sub> ratio, require revision. A proportional reduction (1:3 vs. 1:20, 3/20 factor) would suggest revised 1-, 12-, and 24-h EELs of 0.09, 0.0075, and 0.0045 ppm until additional data are available.

The perivascular edema may have been due to an increased pulmonary vascular permeability. The ventral orientation of the tracheal lesions suggests that TEB may have dissolved in tracheal mucus, which is more abundant ventrally in a prone rat due to gravitational forces. The pathophysiological significance of the liver alteration was undetermined. The significance of the myocardial lesions observed is unknown; however, these lesions were not considered attributable to TEB exposure or influential on TEB exposure-related effects.

The apparent gender-related response difference to inhaled TEB is not thoroughly understood; however, it is possible that the larger size and subsequent larger V<sub>E</sub> of males vs. females may have resulted in a larger TEB "dose" during the exposures, hence a greater mortality rate. The present investigation does not provide sufficient information to determine the mechanism(s), threshold levels, or etiology of TEB-induced pulmonary or hepatic toxicity. The lack of studies investigating the potential CNS toxicity of TEB (indicated by proven CNS toxicity of related organoboranes), as well as the need to refine guidelines (based on CNS, mechanistic, and end point specific studies) for the safe use of TEB suggests a need for the conduct of additional investigations of the toxicity of this material.

## SECTION 5

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## QUALITY ASSURANCE

The study, 'Toxicity Evaluations of Air Force Fuels: The Acute Inhalation Toxicity of Triethylborane Spontaneous Oxidation Products: Immediate and Delayed Exposure,' was conducted by the NSI Technology Services Corporation, Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. No claim will be made that this was a "GLP" study as no attempt was made to adhere to the strict requirements of these guidelines. The various phases of this study were inspected by members of the Quality Assurance Unit. Results of these inspections were reported directly to the Study Director at the close of each inspection.

**DATE OF INSPECTION:**

August 23, 1988

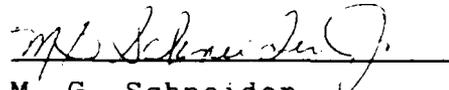
November 28 -  
December 7, 1990

**ITEM INSPECTED:**

Data audit.

Final report audit.

The Quality Assurance Unit has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretation presented in this Final Report.



M. G. Schneider  
QA Coordinator  
Toxic Hazards Research Unit

Date December 5, 1990