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8EHQ 0197-13656

Health Sciences and Regulatory Programs

January 10, 1997

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PDCN: 8896000153 - 8EHQ-96-13673

TSCA Document Processing Center (7408)
Office of Pollution Prevention & Toxics
U. S. Environmental Protection Agency
401 M Street, S. W.
Washington, D. C.

8EHQ-96-13656

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Attention: 8(e) Coordinator

RE: 8EHQ-0596-13656
8EHQ-0696-13673
Follow-up Report

Dear Sir:

In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act, ARCO Chemical Company (ACC) on May 21, 1996 submitted information on preliminary results from a 90-day vapor inhalation toxicity study with ethyl-*tert*-butyl ether (ETBE), CAS #637-92-3 (8EHQ-0596-13656). This study was conducted in Fischer 344 rats and included neurotoxicity evaluations. From the preliminary findings, the study pathologist had identified a degenerative change of the seminiferous tubules from testes of the male rats examined after exposure to the high dose of 5000 ppm ETBE for 13 weeks. In the final report, this effect also was noted at the 1750 ppm mid dose. This effect was not seen at the 500 ppm low dose. These findings suggest a mild toxicity in rats in the 5000 and 1750 ppm exposure group with a no-observable-adverse-effect-level of 500 ppm for repeated exposure of ETBE in rats.

ARCO Chemical Company, on June 19, 1996, submitted a second 8(e) notice (8EHQ-0696-13673) on preliminary results from the same study where the study director identified alterations in the bone marrow of female rats exposed to the high dose (5000 ppm). Similar effects were not reported in the male rats and no associated hematologic changes were apparent. This effect also was noted at the 1750 ppm dose in the final report and also suggest a no-observable-adverse-effect-level of 500 ppm for repeated exposure of ETBE in rats.

ETBE does not appear to be a neurotoxicant. Although transient ataxia, believed to be solvent related, sometimes was observed in male rats in the 5000 ppm exposure group immediately after exposure and subtle and inconsistent changes in FOB and motor activity were noted throughout the study, none of the changes demonstrated either a dose-response relationship or a consistent pattern of neurologic dysfunction.

97 JAN 17 AM 8:26
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89970000047

A copy of the final report is enclosed.

Sincerely,

E. C. Capaldi
EH&S Advisor

CC: TSCA Document Processing Center (7407)
Attn: TSCA Section 8(d) Reporting

Study Title

Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats

Data Requirement

Not Applicable

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Study Initiation Date

August 20, 1995

Study Completion Date

December 20, 1996

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95029

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Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats

SUMMARY

The study consisted of 3 exposure groups and an air-exposed control group. Within each exposure group was a core group (11 rats/sex/group), a neurotoxicology subgroup (12 rats/sex/group), an interim clinical pathology subgroup (10 rats/sex/group) and a cell replication subgroup (15 rats/sex/group). Rats in the core group and the neurotoxicology subgroup were exposed by inhalation for 6 hours/day, 5 days/week, over a 90 day period to target concentrations 0, 500, 1750, or 5000 ppm of ethyl tertiary butyl ether (ETBE) vapor resulting in at least 65 ETBE exposures. Body weights and clinical signs were assessed at intervals throughout the study. A functional observational battery (FOB) was performed on rats in the neurotoxicology subgroup prior to exposure, after one exposure, and after 6, 10, 20, 42 and 65 exposures. Motor activity was assessed prior to exposure, and after 20, 42, and 65 exposures. Cell replication in kidneys was evaluated on 5 rats/sex/group from the cell replication subgroup during 1, 4, and 13 weeks of exposure. A subset of 6 rats assigned to the neurotoxicology subgroup were evaluated for evidence of neuropathology at the end of the exposures. Hematology and clinical chemistry were evaluated after at least 30 exposures in the interim clinical pathology subgroup and at termination of the exposure period in the core group. Ophthalmologic evaluations were performed pretest and after the sixty-fifth ETBE exposure.

Average ETBE exposure concentrations were: 505, 1748, and 4971 ppm of ETBE. Two female rats in the control group died during the study. The only noteworthy clinical finding was ataxia which was sometimes observed in male rats in the 5000 ppm exposure group during daily mortality checks made following the daily exposure during the duration of the study. Subtle and inconsistent changes in FOB and motor activity were noted throughout the study. However, none demonstrated a dose-response relationship or a consistent pattern of neurologic dysfunction. Male and female rats in the 1750 and 5000 ppm group had a 25% decrease in body weight gain compared with controls during the first week of the study suggesting a transitory adjustment to ETBE exposure. By study termination, the final body weights were either similar to controls (males) or significantly increased (females in the 5000 ppm group) relative to controls. The only significant

differences in hematology parameters for both the 6 and 13 week time points were an increase in platelets and MCHC in male rats and an increased MCV in females exposed to 5000 ppm. The relationship of these isolated changes to ETBE exposure is unclear. ETBE-associated changes in clinical chemistry parameters in male rats consisted of changes in chloride and total protein at the interim and final time points. No consistent alterations were seen in females at these two sample times. No changes were considered to be indicative of a disease process. Statistically significant increases in organ weights for liver, kidney, adrenal gland and heart (female only) were noted in male and female rats in the 5000 ppm group compared with controls. An increased number of regenerative foci were noted in the kidneys of male rats exposed to all three concentrations of ETBE compared with controls. An increased incidence of degenerative tubules was observed in the testes of male rats exposed to 1750 and 5000 ppm ETBE. No treatment effect was seen at 500 ppm ETBE. Results of the cell proliferation evaluations indicated that kidneys of male rats exhibited time and concentration dependent increases in the labeling index of the proximal tubule cells. An increase in protein droplet accumulation and evidence of α2u-globulin immunoreactivity was noted in these animals. No evidence of treatment related nephropathy, protein droplet accumulation or α2u-globulin immunoreactivity was observed in female rats. An increased incidence of congestion was noted in bone marrow of female rats exposed to 1750 and 5000 ppm ETBE.

These findings suggest a mild toxicity in rats in the 5000 and 1750 ppm exposure group. ETBE does not appear to be a neurotoxicant apart from the solvent related ataxia observed immediately after exposure. Renal lesions, increased cell proliferation, and presence of α2u-globulin in kidney cells of male rats only is consistent with the male rat specific syndrome of α2u nephropathy. The lesions noted in the testes of male rats suggest a no-observable-adverse-effect-level of 500 ppm for repeated exposure of ETBE in rats.

PURPOSE

The objective of this study was to evaluate the toxic effects in Fischer 344 rats resulting from at least 65 inhalation exposures to ethyl tertiary butyl ether (ETBE) over a 90 day period. Inhalation is a likely route for human exposure to ETBE. Neurotoxicological effects and cell replication in kidneys were also investigated. These data as well as data

from ETBE pharmacokinetic studies in Fischer 344 rats, will be used to assess the effects of repeated exposures to ETBE.

STATEMENT OF COMPLIANCE

This study was conducted at the Chemical Industry Institute of Toxicology in accordance with the following guidelines and standards:

U.S. Environmental Protection Agency (EPA), Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards, 40 CFR Part 792

U.S. Environmental Protection Agency (EPA), Toxic Substances Control Act (TSCA) Health Effects Testing Guidelines, 40 CFR Part 798, Subpart C, Section 798.2450, and Subpart G, Sections 798.6050, 798.6200, and 798.6400 with the following exceptions:

Raw data records, in some cases, did not follow 792.130(e). Appropriate corrections were made to the raw data. Some Standard Operating Procedures were in draft form but were issued before study completion. Validation efforts for Path-Tox, CHRIS, and Andover Infinity systems were in progress and have been completed.

Study Director James A. Bond 12/20/96
James A. Bond, Ph.D., D.A.B.T Date

QUALITY ASSURANCE UNIT INSPECTION AND REPORTING DATES

Study Title: Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluation in Fischer 344 Rats

Protocol No.: 95029 Study Director: Dr. James Bond

Sponsor: ARCO Chemical Company

Phase(s)	Quality Assurance Unit (QAU) Inspection Date(s)	Date of QAU Report to Study Director	Date of QAU Report to Management
Protocol Review	05/19 & 05/22/95	05/22/95	05/22/95
Protocol Review	05/24/95	05/25/95	05/25/95
Protocol Review	08/03/95	08/03/95	08/03/95
Protocol Review	08/10/95	08/10/95	08/10/95
Pre-Experimental Period	10/02/95	10/03/95	10/03/95
Pre-Experimental Period	10/03/95	10/03/95	10/03/95
Pre-Experimental Period	10/04/95	10/04/95	10/04/95
Protocol Amendment	10/06/95	10/06/95	10/06/95
Pre-Experimental Period	10/09/95	10/11/95	10/11/95
Pre-Experimental Period	10/09/95	10/13/95	10/13/95
Pre-Experimental Period	10/12/95	10/13/95	10/13/95
Experimental Period	10/16/95	10/20/95	10/20/95
Experimental Period	10/18/95	10/18/95	10/18/95
Experimental Period	10/19/95	10/31/95	10/31/95
Experimental Period	10/20/95	10/23/95	10/23/95
Experimental Period	10/25/95	10/27/95	10/27/95
Experimental Period	11/16/95	11/27/95	11/27/95
Protocol Amendments	12/01/95	12/08/95	12/08/95
Experimental Period	11/30/95 & 12/07/95	12/11/95	12/11/95
Experimental Period	12/12/95	12/13/95	12/13/95
Experimental Period	01/15/96	01/15/96	01/15/96
Experimental Period	01/15/96	01/15/96	01/15/96
Experimental Period	01/17/96	01/17/96	01/17/96

Phase(s)	Quality Assurance Unit (QAU) Inspection Date(s)	Date of QAU Report to Study Director	Date of QAU Report to Management
Experimental Period	01/18/96	01/18/96	01/18/96
Experimental Period	01/24/96	01/29/96	01/29/96
Experimental Period	01/25/96	01/29/96	01/29/96
Experimental Period	02/09/96	02/15/96	02/15/96
Experimental Period	03/14/96	03/14/96	03/14/96
Protocol Amendment	04/01/96	04/01/96	04/01/96
Experimental Period	05/09/96	05/15/96	05/15/96
Raw Data, Draft Final Report	04/25-26/96, 05/01/96 & 05/13-14/96	05/15/96	05/15/96
Raw Data, Draft Final Report	05/24/96	05/24/96	05/24/96
Protocol Amendment	06/18/96	06/18/96	06/18/96
Raw Data, Draft Final Report	07/03/96 & 07/09/96	07/09/96	07/09/96
Raw Data, Draft Final Report	06/28/96, 07/02/96 & 07/08/96	07/10/96	07/10/96
Raw Data	07/10/96	07/10/96	07/10/96
Raw Data, Draft Final Report	06/20/96 & 07/10/96	07/19/96	07/19/06
Raw Data, Draft Final Report	07/19/96	07/19/96	07/19/96
Raw Data, Draft Final Report	07/10/96, 07/11/96 & 07/19/96	07/22/96	07/22/96
Draft Final Report	07/26/96	07/26/96	07/26/96
Protocol Amendment	08/16/96	08/16/96	08/16/96
Raw Data, Draft Final Report	08/15/96 & 08/19-20/96	08/23/96	08/23/96
Protocol Amendment	12/12/96	12/12/96	12/12/96
Raw Data, Draft Final Report	12/12-13/96 12/15-16/96	12/17/96	12/17/96
Final Report	12/19/96	12/19/96	12/19/96

Phase inspections, raw data and final report reviews were performed by the CIIT Quality Assurance Unit in accordance with the U.S. Environmental Protection Agency's Toxic Substances Control Act (TSCA) (40 CFR Part 792). The dates of the QAU inspections and dates results were reported to the Study Director and Management are noted in the above list.

CIIT's Quality Assurance Unit has reviewed the final report to help assure that the report describes the methods and that the results reflect the raw data.

Patricia O'Brien Pomerleau 12/20/96

Patricia O'Brien Pomerleau, M.S. Date
Quality Assurance Manager, CIIT

MATERIALS AND METHODS

This study was conducted according to the protocol entitled Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats (CIIT Protocol 95029 dated August 10, 1995, and protocol amendments dated October 4, 1995, October 30, 1995, November 13, 1995, November 22, 1995, January 22, 1996, May 30, 1996, August 15, 1996, and December 12, 1996 (Appendix 1). Study deviations are listed in Appendix 2.

Experimental Design

The experimental design is presented in Table 1. This study consisted of 3 exposure groups and an air-exposed control group. Within each exposure group there was a core group (11 rats/sex/group), a neurotoxicology subgroup (12 rats/sex/group), an interim clinical pathology subgroup (10 rats/sex/group) and a cell replication subgroup (15 rats/sex/group). Rats were exposed to ETBE vapor for 6 hours/day, 5 days/week, over a 90 day period for a total of at least 65 exposures with the exception of the rats in the cell replication and interim clinical pathology subgroups. Body weights and clinical signs were assessed prior to the start of the study and weekly throughout the study. Ophthalmologic evaluations were performed pretest and after the sixty-fifth exposure. A functional observational battery (FOB) was conducted on rats assigned to the neurotoxicology subgroup prior to exposure, after one exposure, and after 6, 10, 20, 42 and 65 exposures. Motor activity was assessed on the same rats prior to exposure, and after 20, 42, and 65 exposures. Cell replication in kidneys was evaluated in 5 rats/sex/group from the cell replication subgroup during the first, fourth, and thirteenth weeks of exposure. Tissues from a subset of 6 rats assigned to the neurotoxicology subgroup were evaluated microscopically for evidence of neuropathology at the end of the exposures. Hematology and clinical chemistry were evaluated after at least 30 exposures in an interim subgroup of rats and at termination of the exposure period in the core group. At the termination of the study all surviving animals assigned to the core group received a complete necropsy. Selected organs were weighed and tissues were taken for microscopic examination.

Table 1.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Experimental Design

Group		Number of Rats Male	Number of Rats Female	Target Exposure Concentrations (ppm)
Control	Core	11	11	0
	Neurotoxicology (NT)[1]	12	12	0
	Cell Replication (CR)	15	15	0
	Clinical Pathology (CP)	10	10	0
	Total	48	48	
Low	Core	11	11	500
	Neurotoxicology (NT)[1]	12	12	500
	Cell Replication (CR)	15	15	500
	Clinical Pathology (CP)	10	10	500
	Total	48	48	
Mid	Core	11	11	1750
	Neurotoxicology (NT)[1]	12	12	1750
	Cell Replication (CR)	15	15	1750
	Clinical Pathology (CP)	10	10	1750
	Total	48	48	
High	Core	11	11	5000
	Neurotoxicology (NT)[1]	12	12	5000
	Cell Replication (CR)	15	15	5000
	Clinical Pathology (CP)	10	10	5000
	Total	48	48	

[1]Animals assigned to the Neurotoxicology (NT) Subgroup were divided into two units NT-1 and NT-2 in order to facilitate the required neurotoxicology assessments which were conducted over a consecutive two day period.

Schedule

The experimental schedule is presented in Table 2. The exposures began on October 16, 1995 and the animals in the core group were euthanized for necropsy and clinical pathology on January 16-18, 1996. Animals in the Neurotoxicology subgroup were euthanized and selected rats necropsied on January 24-25, 1996.

Table 2.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Experimental Schedule

Date			Event
Year	Month	Day(s)	
1995	October	2	Starting date of animal acclimation
		3,6	Ophthalmic exam
		5	Pretest health screen
		9,10,11	Rats weighed, randomized and transponders implanted
		12,13	FOB/Motor Activity Assessment
		16	Experiment Start Date
		16	Exposures of core, CR, and CP subgroups begun
		16	Exposures of NT-1 subgroup begun
		16	BRDU pumps implanted
		17	Exposures of NT-2 subgroup begun
		17	FOB Assessment of NT-1 subgroup
		18	FOB Assessment of NT-2 subgroup
		20	Interim necropsy of CR subgroup
		25	FOB Assessment of NT-1 subgroup
		26	FOB Assessment of NT-2 subgroup
	November	1	FOB Assessment of NT-1 subgroup
		2	FOB Assessment of NT-2 subgroup
		6	BRDU pumps implanted
		10	Interim necropsy of CR subgroup
		16	FOB/Motor activity Assessment of NT-1 subgroup
		17	FOB/Motor activity Assessment of NT-2 subgroup
		29,30	Interim necropsy of CP subgroup
	December	19	FOB/Motor activity Assessment of NT-1 subgroup
		20	FOB/Motor activity Assessment of NT-2 subgroup
1996	January	8	BRDU pumps implanted
		12	Interim necropsy CR subgroup
		15	Ophthalmic exam of Core group
		16,17,18	Necropsy Core group
		23	FOB/Motor activity Assessment of NT-1 subgroup
		23	Ophthalmic examination of NT subgroups
		23	Final exposure day
		24	Necropsy NT-1 subgroup
		24	FOB/Motor activity Assessment of NT-2 subgroup

Table 2. (cont'd)
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Experimental Schedule

Date			Event
Year	Month	Day(s)	
1996	January	25	Necropsy NT-2 subgroup
		25	Completion of in-life phase
		25	Experiment Termination Date

Test Material

One shipment of 4, 55-gallon drums of ethyl tertiary butyl ether (ETBE; Manufacturer: Phillips 66; LOT #11; CAS Registry Number 637-92-3) was received from Pittsburgh Applied Research Corporation, Pittsburgh, PA on August 1, 1995 and assigned CIIT sample numbers T00997, T00998, T00999, and T01000. ETBE was considered to be stable at normal temperatures for the duration of the study and was therefore stored in a well-ventilated area under ambient conditions. A reserve sample of approximately 5 to 10 g was retained in an amber bottle with a Teflon-lined cap. A sample was analyzed at the beginning and end of the study to confirm stability. Information on identity, strength, purity, stability and composition are presented in Appendix 3 and Appendix 4.

Test Animal Species, Source, and Pretest Evaluation

A total of 250 male and 251 female Fischer 344 rats, 5 weeks of age, were received on October 2, 1995 from Charles River Laboratories, Inc. Raleigh, NC. A pretest health screen was performed on 3 animals/sex. These animals were subjected to a complete necropsy following a thorough clinical examination and were examined serologically for mycoplasmal, bacterial, and viral pathogens (CARB, H-1, KRV, *M. pulmonis*, parvovirus, PVM, RCV/SDA, Sendai). Sera were prepared and sent to Microbiological Associates Inc. (Rockville, MD) for serologic screening (Rat Level II Complete Antibody Profile). Fecal culture was conducted for aerobic pathogens and oropharyngeal swabs were performed for bacterial pathogens. Wet mounts of ileal and cecal contents were examined for enteric parasites and a cellophane tape test was conducted for *Syphacia sp.* ova. Histologic examination was conducted on selected target organs including respiratory tract, heart, liver, kidney, spleen, small and large intestine, cecum, brain with middle ear, nasal

turbinates and submandibular lymph node. Pretest physical examination, parasitology, serology, and histology were unremarkable, and the animals were determined to be suitable for use on the study. Body weight determinations and clinical and ophthalmic examinations of all animals were performed prior to the initiation of exposures.

Each animal was assigned a unique identification number prior to the start of the exposures as noted in Table 3. Animals considered for assignment to the study were identified using transponders and cage assignment charts. Animals designated for the neurotoxicity subgroup were also randomly assigned unique ear tag numbers. Based on the pretest body weight, 192 males and 192 females were selected for the study. These animals were divided into 3 experimental groups and 1 control group. Each exposure group consisted of 48 male and 48 female animals. Assignment to exposure groups was based on a weight randomization procedure using the Path-Tox computer software (Xybion Corp). Statistical equivalence and homogeneity were examined using the Path-Tox randomization procedure. After the selected animals received their first exposure, all animals not selected for the study were reassigned to other studies, training of staff, methods development, or euthanized. The fate of all animals not selected for use in this study was documented in the study file.

Table 3.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Animal Assignments

Exposure Level	Path-Tox Study Number	Animal Number	
		Males	Females
Control	95029	001 to 048	193 to 240
Low	95029	049 to 096	241 to 288
Mid	95029	097 to 144	289 to 336
High	95029	145 to 192	337 to 384

Animal Husbandry

All animals were housed one per cage in stainless steel, wire-mesh cages with an automatic water system in Room 291A from October 2, 1995 until October 11, 1995. At

that time, rats assigned to study were housed in 1 m^3 chambers with stainless steel cages and wire mesh floors in Rooms 297, 299, 301, and 303 until the end of the study. A layer of animal cage board (TECHBOARD) placed under each cage was changed daily. Cages were changed and sanitized once every week. Environmental conditions were monitored and recorded by the computerized Andover Infinity System. Temperature range of 66-76°F, relative humidity of 40-60%, and an air flow of 200-250 liters per minute were maintained to the extent possible. Deviations from these ranges were recorded in the study file and are noted in Appendix 2 as protocol deviations. Automatic light controls were set to provide fluorescent lighting for a 12 hr photo period (0700-1900 hours for light phase).

NIH-07 certified diet from Zeigler Brothers, Inc. was available ad libitum except during exposures, motor activity evaluations, and on the day of sacrifice after removal of the animals from the inhalation chambers. Deionized water was available ad libitum, by an automatic watering system during the entire period the animals were in the wire-mesh cages including during the inhalation exposures. Water was not available on the day of necropsy after removal of the animals from the inhalation chambers. Results of analysis of water and diet samples are provided in Appendix 3.

Target Exposure Concentrations and Exposure Schedule

The target ETBE exposure concentrations were 500, 1750, and 5000 ppm. The control group was exposed to filtered air only. The highest level, 5000 ppm, is 50% of the lower explosive limit. Rats selected for the study were exposed for 6 hours/day, 5 consecutive days/week, for at least 13 weeks for a total of at least 65 exposure days, with the following exceptions. Five rats/sex/group assigned to the cell replication subgroup were exposed for 6 hours/day, 5 consecutive days/week, for 4, 19, or 63 exposures (approximately 1, 4, or 13 weeks). The 10 rats/sex/group assigned to the clinical pathology interim subgroup were exposed for 32 (male) or 33 (female) exposures. Rats in the neurotoxicology subgroup were not exposed on the day of neurobehavioral test; additional exposure days were added at the end of the study to result in no less than 65 days of exposure. For rats in the neurotoxicology subgroup, the start of the inhalation exposures was staggered by one day for one-half of the animals. No animals were exposed on December 25, 1995.

Inhalation Exposure Chambers Description and Operation

Details regarding the inhalation exposure concentrations are presented in Appendix 4. Exposures were conducted in 1000 L Hazelton H-1000 (Lab Products, Inc., Maywood, NJ) stainless steel chambers with glass doors and windows for animal observations. Animals were housed in the chambers during the entire 90-day study duration. The H-1000 chambers were contained inside an $8m^3$ Hinners-style exposure chamber. Animal cage positions were rotated weekly. Mice were exposed to identical target ETBE vapor concentrations in separate chambers concurrently with this study (CIIT Study No. 95030). The total volume of the animals in the chambers was less than 5% of the volume of the chambers. Four chambers were used, one for each exposure level, including controls. Airflow rates were maintained between 200 and 250 l/min thus maintaining 12 to 15 air changes per hour to ensure an adequate oxygen content of at least 19%. Oxygen content was measured during the study. Airflow measurements in the H-1000 chambers were monitored but not recorded for the first 18 days, except during the exposure period. No airflow alarms were noted during this time, and the 8 m^3 chamber airflow which controlled the air flow through the H-1000 remained stable, so adequate airflow was present at all times. Thus, the impact on the results of the study was negligible. After this 18 day period, airflow rates were monitored continuously and a 15 minute average recorded every 15 minutes.

The temperature and humidity of the air entering the chambers were also monitored continuously, and a 15 minute average recorded approximately every 15 minutes. A daily report was printed of the data collected over the previous 24 hours and the overall daily average for temperature and humidity was calculated and reported, except where noted in Appendix 4.

ETBE Vapor Generation

A liquid metering pump (Fluid Metering, Inc) was used to meter liquid ETBE from a stainless steel reservoir into a heated stainless steel J-tube. Nitrogen gas flowed through the J-tube and carried ETBE vapor into the exposure chamber. As there were two separate chambers (one for mice and one for rats) at each concentration, one generator was used to supply two chambers. The ETBE vapor mixed with the incoming air stream and then was

split between the two chambers. Concentration measurements in the chambers showed that there was typically less than 2% variation between the chambers.

Analysis of ETBE Vapor Concentrations

A gas chromatograph (Hewlett-Packard model 5890) was used to analyze the chamber atmosphere for ETBE. Chamber concentrations of ETBE and control chamber atmospheres were determined by gas chromatography at least 6 times during each exposure day. The gas chromatograph was programmed to sample all of the chambers in sequence once every half hour. Chamber probes for sampling were placed in the breathing zone of the animals. A sample line, located in the center of the chamber was used to continuously draw a sample of the atmosphere. From that sample line, a stream selector valve was used to draw samples from the sample lines one at a time, into the sample loop of the gas chromatograph. The daily nominal (estimated) chamber concentration was also determined. The nominal concentration was estimated based on the liquid ETBE flow rate and the chamber air flow rates. The uniformity of the ETBE concentration in each of the 3 exposure chambers was examined once prior to the start of the exposures without animals. For each individual distribution test, the concentration was measured at 5 positions throughout the chamber. The distribution of ETBE in the chamber atmosphere was determined to be uniform based on a within chamber variation of less than 8%. The stability of ETBE in the exposure chambers was determined periodically by analyzing the exposure atmosphere for ETBE content.

Animal Observations

On exposure days, all rats were observed for mortality and overt clinical signs of toxicity just prior to exposure and shortly following the exposure. On nonexposure days, all rats were observed for mortality and overt clinical signs of toxicity once during the day. Animals that showed signs of extreme distress or were moribund, were euthanized. During the exposure regimen a detailed examination for clinical signs of disease or abnormality, which involved animal handling, was performed on each animal once per week prior to the start of the daily exposure. Observations were recorded in the Path-Tox computer system. Cage-side observations included change in the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic, and central nervous systems,

somatomotor activity, and behavior patterns. Lack of clinical signs during these detailed physical examinations was recorded in Path-Tox computer system as "normal."

Ophthalmic Examinations

A complete ophthalmic examination was performed by Dr. Robert Peiffer (UNC-Chapel Hill, Chapel Hill, NC), a consulting veterinary ophthalmologist, on all rats prior to the start of the study and on all surviving rats after the sixty-fifth exposure to ETBE (Table 2). Both eyes were examined by indirect ophthalmoscopy and biomicroscopy.

Body Weight

The body weights of all rats were taken and recorded using the Path-Tox computer system prior to placement on the study, weekly during the exposure regimen, in conjunction with scheduled FOB evaluations, and preceeding necropsy.

Neurobehavioral Evaluations

Motor activity evaluations were performed on all rats assigned to the neurotoxicology subgroup using an automated recording apparatus designed to measure spontaneous motor activity in a novel environment (San Diego Instruments Inc.). The apparatus has the capacity to monitor up to 32 individual animals simultaneously. The 24 animals in each block were distributed with respect to test session, exposure group, and recording device to limit bias resulting from possible differences in recorded sensitivity. Motor activity evaluations were conducted in Room 273A which is specifically designed for such evaluations permitting control of environmental fluctuations in sound, light, temperature, and relative humidity. White noise levels of 68-72 dBA and mean motor activity enclosure illumination of approximately 1-3 foot candle were maintained during motor activity test sessions. Motor activity was evaluated during the week prior to exposure, and the day after 20, 42, and 65 exposures. Each test session was 60 minutes in duration and occurred on a non-exposure day. Missed exposure days due to motor activity or FOB measurements were made up at the end of the study. Animals were weighed on each test day.

Functional observation battery (FOB) evaluations designed to detect gross alterations in nervous system function were performed on all rats assigned to the

neurotoxicology subgroup during the week prior to exposure, and after 1, 6, 10, 20, 42 and 65 exposures. Additional details on the FOB and motor activity evaluations are presented in Appendix 5.

Clinical Pathology

Blood was taken for interim clinical pathology evaluations from surviving rats in the designated clinical pathology subgroup after 32 or 33 exposures for males or females respectively. Blood was obtained for clinical pathology evaluations conducted at the end of the study from all surviving animals from the core group. The provisional order of bleeding and analysis were determined using a randomization procedure to minimize bias (JMP®, SAS Inc., Cary, NC). All blood samples were obtained by cardiac puncture from sodium pentobarbital anesthetized animals. Food and water were supplied ad libitum to these rats from the end of the final inhalation exposure up until the time the rats were removed from the chamber the morning that the evaluations were made. Details on the clinical pathology evaluations can be found in Appendix 6 (Hematology) and Appendix 7 (Clinical Chemistry). Clinical pathology evaluations included:

Hematology (Determined on whole blood samples)

differential leukocyte count
hematocrit
hemoglobin
mean corpuscular hemoglobin (MCH)
mean corpuscular hemoglobin concentration (MCHC)
mean corpuscular volume (MCV)
platelet count
red blood cell count
white blood cell count

Clinical Chemistry (Determined on serum samples)

alanine aminotransferase (ALT)
albumin
alkaline phosphatase (ALP)
aspartate aminotransferase (AST)
bilirubin
calcium
chloride
glucose (nonfasting)
phosphorus
potassium
sodium
total protein
blood urea nitrogen (BUN)

creatinine
gamma-glutamyl transferase (GGT)

Cell Replication and Special Stains

Cell replication was evaluated during weeks 1, 4, and 13 in kidneys of male and female rats assigned to the cell replication subgroup. Three and one-half days prior to the scheduled necropsy, an Alzet micro-osmotic pump (Model 2ML1; 7-day pump, 10 µl/hr) containing a 16 mg/ml solution of 5-bromo-2'-deoxyuridine (BrdU) in phosphate buffered saline was surgically implanted subcutaneously in each of 5 rats/sex/group. Pumps were implanted after completion of the ETBE exposure on the Monday of the first, fourth, and thirteenth weeks of exposure. On Friday morning of that week rats were removed from the exposure chambers prior to the start of the daily exposure, anesthetized with sodium pentobarbital and perfused in situ by retrograde descending aortic perfusion with 10% buffered neutral formalin. The kidneys and a section of the duodenum from the rats were removed and fixed in 10% neutral buffered formalin. The BrdU labeled cells in paraffin embedded tissue sections were immunohistochemically identified. The duodenal tissue was used as control tissue for verification of BrdU incorporation. The labeling index for each rat was determined by counting a minimum of 1000 cortical epithelial cells/animal (2 kidneys). All exposure groups and controls were examined.

Four slides were prepared from the kidneys of male rats perfused for the cell proliferation studies from the control and each ETBE exposure group. One of the 4 slides was stained with hematoxylin and eosin (HE) and another using the Mallory Heidenhain technique and evaluated for the presence of hyaline droplets in the proximal tubules. Of the remaining 2 slides, 1 was immunohistochemically stained with an antibody to α2u-globulin, and the other slide was held in reserve. A positive control demonstrating α2u-globulin in kidneys, i.e., a kidney section from a male rat previously treated with 2,2,4-trimethyl pentane, was carried through the staining procedure at the same time. All tissues stained with HE, Mallory Heidenhain (MH) and the α2u-globulin antibody were evaluated by the study pathologist. HE stained slides were evaluated for the total number of aggregates of regenerating tubules in both sections of kidney for each rat. The MH stained slides were evaluated for amount of protein droplets in cortical tubules. MH grades were 1 = minimal, 2 = <10% of cortex involved, 3 = 10-25% cortex involved, 4 = 25-50% of

cortex involved, 5 = >50% of cortex involved. Immunoreactivity for α2u-globulin was assessed as either present in protein-droplets or absent.

Anatomic Pathology Evaluations

At the end of the exposure regimen, all surviving rats assigned to the core group were anesthetized with sodium pentobarbital and euthanized by exsanguination. These animals received a complete necropsy including an external exam. All retained tissues were fixed in 10% neutral buffered formalin. The order of necropsy was randomized in advance using JMP® statistical software (SAS Institute, Cary, NC) to reduce necropsy observation bias. The following tissues were collected from males or females as appropriate:

gross lesions
adrenals
aorta
bone marrow
brain (cerebral cortex, cerebellar cortex, medulla/pons)
cecum
colon
duodenum
epididymis
esophagus
eyes
exorbital lacrimal gland
femur (including articular surface)
heart
ileum
jejunum
kidneys
larynx
liver
lungs with mainstem bronchi
lymph nodes (mesenteric and nonmesenteric)
mammary gland (females)

nasopharyngeal tissues
ovaries
pancreas
parathyroid complex
pituitary
prostate
rectum
salivary glands
sciatic nerve
seminal vesicles
skeletal muscle (thigh)
skin
spinal cord (cervical, midthoracic, and lumbar)
spleen
sternum
stomach
testes
thymic region
thyroid
tongue
trachea
urinary bladder
uterus (corpus and cervix)
vagina
Zymbal's gland

Organ Weights

The following organs from all surviving core group animals were trimmed, blotted, and weighed:

adrenals
brain (including brain stem)
heart

kidneys
liver
lungs (weighed prior to inflation)
ovaries (females)
spleen
testes (males)

Histopathology

All tissues to be examined microscopically were processed at Experimental Pathology Laboratories (Research Triangle Park, NC) for paraffin embedding, sectioned at 5 microns, and stained with hematoxylin and eosin (H & E). Tissues from the control and high exposure groups were examined microscopically. In addition, the lungs, liver, kidneys, and all gross lesions were examined by light microscopy for all core group animals in the low and mid exposure groups. When necessary, lesions were graded as to severity. After the initial microscopic examination, the testes and female rat femoral bone marrow were identified as additional target tissues.

All testes were examined from all treatment groups. One hundred round cross sections of seminiferous tubules from each rat were examined and identified as to which group of developmental stages the cross-section belonged to based on the stage descriptions in Hess, 1990. Briefly the stages were separated based on 1 stage of spermatids present (stages IX-XIII), 2 stages of spermatids present mixed (stages I-V), 2 stages of spermatids present with elongated near lumen (stages VI-VIII), or meiotic divisions present (stage XIV). Additionally, the number of tubules that also contained lumenal debris or had necrotic or degenerated cells were counted. Degenerated cells were characterized by loss of differential cytoplasmic staining, clumped chromatin or nuclear pyknosis. The numbers were tabulated as percent of tubules counted.

Neuroanatomic Pathology

At the end of the exposure regime, 6 rats/sex/group randomly chosen from the neurotoxicology subgroup were anesthetized with sodium pentobarbital and perfused *in situ* by intracardiac perfusion with 1.5% buffered glutaraldehyde. After perfusion, the cranium and vertebral arches covering the brain and spinal cord were removed, and the

peripheral nerves in the hind legs were exposed. Any abnormal coloration was noted for the brain and cord. These tissues were collected and stored, until trimmed, in the same fixative as used for the perfusions. After removal, simple measurement of the size (length and width) and weight of the whole brain was made. Details of the neuropathology evaluation can be found in Appendix 5.

Tissues from animals in the high exposure and control groups were processed for evaluation by light microscopy. The tissues from animals in the low and mid exposure groups were stored in appropriate fixative for possible future microscopic examination. The following tissues were collected for neuroanatomic pathology:

gross lesions
center of the cerebrum
center of the midbrain
cerebellum and pons
dorsal and ventral root fibers
dorsal root ganglia
eye and optic nerve
forebrain
Gasserian ganglion
medulla oblongata
proximal sciatic nerve (above the knee)
spinal cord (cervical and lumbar)
sural (fibular) nerve (below the knee)
tibial nerve (below the knee)

These tissues were processed for histology and examined by light microscopy. Brains and spinal cords, along with ganglia and spinal root fibers to be examined by light microscopy, were processed for paraffin embedding, sectioned at 5-6 microns, and stained with hematoxylin and eosin. From 1 hind leg, the proximal sciatic nerve from just proximal to the knee, as well as the tibial and sural nerves from just distal to the knee, were embedded in glycol methacrylate, sectioned at 2 microns, and stained with hematoxylin and eosin. Lesions were graded as to severity, and assigned where possible, into 4 categories (no abnormalities, minimal, moderate, and severe).

Statistical Evaluations

Statistical analyses were performed using either SAS statistical software or JMP® statistical software. The probability value of less than 0.05 was used as the critical level of significance for each statistical test, except where noted. However, the critical level of overall significance (alpha level) for each test domain was adjusted to correct for multiple evaluations. All uncorrected probability values of less than 0.05 are listed in the report.

Terminal body weight data taken prior to necropsy were analyzed separately by sex and by necropsy date for each subgroup (e.g., Cell Replication after 1 week exposure). For a given analysis the data were tested for normality by the Shapiro-Wilk test, and for homogeneity of variance by the O'Brien and Brown-Forsythe tests. The significance level for these tests was 0.01. A one-way analysis of variance (ANOVA) was performed to assess the equality of the treatment groups at two time points: day of randomization to study and treatment group (Pretest Weight), and at time of sacrifice (different depending on study group). If the ANOVA was statistically significant, Dunnett's test was performed to determine which treatment groups were different from the Control group. The significance level for these latter tests was set at 0.05.

The data for individual organ weights for male and female rats obtained at the final necropsy were tested for normality and homogeneity using a significance level of 0.01. A one-way analysis of variance (ANOVA) was conducted to test the equality of the group means. When this test rejected the equality of all groups, Dunnett's test was used to determine which groups differed from control. When the normality condition was not met, the non-parametric Kruskal-Wallis test was used to determine whether the group means were equal. The significance level for each test was 0.05, except where noted.

The data for quantitative, continuous variables were intercompared for the exposure and control groups by tests for homogeneity of variance, 1-way fixed effects ANOVA, and Dunnett's multiple comparison procedure for significant ANOVA. Where the ANOVA indicated statistical significance among experimental groups, Dunnett's test was used to delineate which groups differed from the control group. Williams' test was used to determine the lowest dose group which was significantly different from the control group. When the assumptions for a parametric ANOVA were not met, nonparametric procedures were used. Other statistical tests for additional endpoints are described in the appropriate appendices.

RESULTS

Test Article Purity and Stability

The identity of ETBE was confirmed by nuclear magnetic resonance spectroscopy and gas chromatography mass spectroscopy (Appendix 3). Analyses of the ETBE test material performed prior to and following the exposure regimen showed no significant compositional change (Appendix 4). The test material was approximately 97 percent ETBE.

Chamber Concentration, Temperature, and Relative Humidity

The target chamber concentrations were 500, 1750, and 5000 ppm of ETBE vapor. Control animals were exposed to filtered air only. Gas chromatographic analysis of the chamber atmospheres resulted in mean ± SD concentrations of 505±13, 1748±59, and 4971±155 ppm, respectively. No ETBE was detected in the control chamber. Additional details regarding the inhalation exposure concentrations are presented in Appendix 4.

The overall average temperature and relative humidity in the exposure chambers are summarized in Table 4 for each exposure chamber averaged over the duration of the study. These data include the temperatures during the six-hour exposures. For all four chambers the overall average temperature was 71.9°F. Average relative humidities ranged from 49.7 to 50.1%. During the duration of the study, the mean daily chamber temperatures for all exposure groups ranged from 71.0 to 72.1°F, and the relative humidity ranged from 48 to 53 percent. The daily average temperature and relative humidity in the exposure chambers during the duration of the study are presented in Appendix 4 for each exposure chamber.

In order to maintain control over environmental conditions (air flow, temperature, and relative humidity), chambers were sampled continuously by the Andover Infinity System and when necessary adjustments were made. This system enabled maintenance of average environmental conditions within a 24-hour period within very narrow limits. Continuous monitoring of conditions also documents instantaneous and short-term (15 min) excursions from the target ranges. These excursions are noted in the Inhalation Appendix (Appendix 4). Instantaneous temperature readings outside the Protocol limits

(66–76°F) were rare. No 15-min period was outside the temperature range specified by the Protocol. The maximum instantaneous excursion noted, 81.4°F, is just outside the temperature range for rodents (64–79°F) suggested by the ILAR (1996). Thus, these instantaneous excursions in temperature are judged not to have impacted adversely on the study.

Excursions out of the range for percent relative humidity (40–60%) specified by the Protocol were more frequent. In no case were the 15-minute averages below that specified in the protocol. There were 12 instances in which the 15-minute average maximum was above the range recommended by ILAR (10–70%) with the highest being 84%. These excursions occurred during the periods in the morning or afternoon when the inhalation chambers were being serviced by the animal care staff. When the chamber doors are opened, and the cage racks housing the animals are rolled out into the room, relative humidity is no longer controlled by air flow into the chamber. Because twice-daily care of the animals is of highest priority, these disturbances in environmental conditions are unavoidable. They are not of sufficient frequency or duration to adversely impact the study.

Table 4
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Overall Average Temperature (°F) and Relative Humidity (%) During the Duration of the Study[a]

	Individual Chamber (Exposure Level)			
	Chamber 303	Chamber 301	Chamber 299	Chamber 297
	Control	500 ppm	1750 ppm	5000 ppm
Temperature				
Average	71.9	71.9	71.9	71.9
Std. Dev.	0.1	0.1	0.03	0.02
N	100	100	100	100
Relative Humidity				
Average	50.1	49.7	50.1	50.0
Std. Dev.	0.5	0.4	0.1	0.1
N	100	100	100	100

[a]Overall averages were computed by taking the mean of the daily averages for each chamber. Daily averages include both exposure and non-exposure periods and are computed for both exposure and non-exposure days.

Animal Observations and Mortality

Exposure to ETBE had no apparent effect on mortality in male or female rats. Table 5 summarizes unscheduled deaths during the study. The fate of individual animals on study is reported in Appendix 8. No male rats died or were euthanized during the study. Two female rats in the control group died during the study. One death was an accidental trauma. The other female was euthanized due to moribund condition.

Table 5.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Unscheduled Deaths

Animal Number	Sex	Exposure Level	Experimental Group or Subgroup	Date of Death	Type of Death
209	F	Control	Clinical Pathology	Oct-19-95	Found Moribund
224	F	Control	Core	Nov-3-95	Accidental Trauma

The only noteworthy ETBE-related clinical finding was ataxia which was sometimes observed in male rats of the 5000 ppm group during mortality checks made following the daily exposures. Since detailed clinical observations were not scheduled at this time, these findings were noted in the animal room logbooks and are of a qualitative nature without notations regarding incidence or severity.

Tables 6 and 7 summarize the findings of abnormal signs for the weekly clinical observations made on male and female rats, respectively, during the course of the study. No observations were made that could be attributed to ETBE exposure. Since the observations were made in the morning, prior to the onset of exposures, no ataxia due to ETBE exposure was noted. Individual animal clinical signs are presented in Appendix 9.

Table 6.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Clinical Observations for Male Rats[a]

Category/ Clinical Observation	Exposure Group[b]:	1	2	3	4
	No. In Group:	48	48	48	48
Eyes/					
Discharge -Unilateral (One Eye)		-	-	1	-
Animals With Observations For This Category:		-	-	1	-
Ano-Genital/					
Diarrhea		-	-	1	-
Animals With Observations For This Category:		-	-	1	-
Animal Identification/					
Transponder Re-Implanted & Read		-	-	1	-
Transponder Will Not Read		-	1	1	-
Animals With Observations For This Category:		-	1	2	-

[a]Number of rats with an observation in the respective category any time during the study.
[b]1 = control, 2 = 500 ppm, 3 = 1750 ppm, 4 = 5000 ppm

Table 7.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Clinical Observations for Female Rats[a]

Category/ Clinical Observation	Exposure Group[b]:	1	2	3	4
	No. In Group:	48	48	48	48
Eyes/					
Discharge -Unilateral (One Eye)		1	2	2	-
Discharge -Bilateral (Both Eyes)		-	1	-	-
Animals With Observations For This Category:		1	2	2	-
Ano-Genital/					
Urine Staining		-	2	-	-
Animals With Observations For This Category:		-	2	-	-
Animal Identification/					
Transponder Failure		-	1	-	-
Transponder Re-Implanted & Read		-	-	-	1
Transponder Will Not Read		-	-	1	-
Animals With Observations For This Category:		-	1	1	1

[a]Number of rats with an observation in the respective category any time during the study.
[b]1 = control, 2 = 500 ppm, 3 = 1750 ppm, 4 = 5000 ppm

Table 8
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Motor Activity and Functional Observation Battery Data for Male and Female Rats Combined[a]

Neurologic Functional Domain	Test Session (days after start of exposure, approximate) Pre	1	7	14	30	60	90
Autonomic							
Excessive lacrimation	NO	NO	NO	NO	NO	NO	NSD
Excessive salivation	NO	NO	NO	NO	NO	NO	NO
Abnormal pupil reflex	NO	NO	NO	NO	NO	NO	NO
Abnormal respiration	NO	NO	NO	NO	NO	NO	NO
Palpebral closure	NSD	NSD	NSD	NSD	NSD	NSD	NSD
Stool quality	NSD	NSD	NSD	NSD	NSD	NSD	s[b]
Defecation (# fecal boluses)	NSD	NSD	NSD	NSD	NSD	NSD	NSD
Urination (# urine pools)	NSD	NSD	NSD	NSD	NSD	NSD	NSD
Neuromuscular							
Abnormal muscle tone	NO	NO	NO	NO	NO	NO	NO
Abnormal gait	NO	NO	N[b]	NSD	NO	NO	N[c]
Ataxia	NO	NO	NO	NO	NO	NO	NO
Delayed surface righting	NO	NSD	NO	NO	NO	NSD	NSD
Landing foot splay	NSD	NSD	NSD	NSD	NSD	NSD	NSD
Grip strength	NSD	s[d]	NSD	s[d]	NSD	NSD	s[d]

[a]s: Significant ($P \leq 0.05$) statistical difference observed
NSD: No significant difference observed
NO: Abnormal clinical sign not observed in any rat
N: Abnormal clinical sign not observed in any ETBE-exposed rat

[b]Stool quality alteration was not toxicologically significant.

[c]Abnormal gait observed in one control rat.

[d]Toxicologically insignificant changes in grip strength occurred during the study. None of the grip strength changes observed demonstrated a dose-response relationship or a consistent pattern of neurologic dysfunction throughout the study.

Table 8 (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Motor Activity and Functional Observation Battery Data for Male and Female Rats Combined[a]

Neurologic Functional Domain	Test Session (days after start of exposure, approximate) Pre	1	7	14	30	60	90
CNS Excitability							
Abnormal handling reactivity	NO	NO	NO	NO	NO	NO	NO
Abnormal arousal	NO	NO	NO	NO	NO	NO	NO
Tremors	NO	NO	NO	NO	NO	NO	NO
Spasms	NO	NO	NO	N[e]	NO	NO	NO
Seizures	NO	NO	NO	NO	NO	NO	NO
Clonic convulsions	NO	NO	NO	NO	NO	NO	NO
Excessive vocalization	NO	NO	NO	NO	NO	NO	NO
Physiological							
Altered skin temperature or color	NSD	NSD	NSD	NSD	NO	NO	NO
Piloerection	NO	NO	NO	NO	NO	NO	NO
Body weight	NSD	NSD	NSD	NSD	NSD	s[f]	s[f]
Sensorimotor							
Acoustic response	NO	NO	NO	NO	NO	NO	NO
Tail pinch response	NO	NO	NO	NO	NO	NO	NO
Approach response	NO	NO	NO	NO	NO	NO	NO
Visual placing	NO	NO	NO	NO	NO	NO	NO
General Appearance							
Facial crust	NSD	NSD	NSD	NSD	NSD	NSD	NSD
Altered fur appearance	NSD	NSD	NSD	NSD	NSD	NSD	NSD

[a]s: Significant ($P \leq 0.05$) statistical difference observed
NSD: No significant difference observed
NO: Abnormal clinical sign not observed in any rat
N: Abnormal clinical sign not observed in any ETBE-exposed rat

[e]Mild muscle spasms observed in one control rat.

[f]A statistically significant increase in body weight was observed in ETBE-exposed female rats (vs. control female rats) following 42 and 65 exposure days to 5000 ppm ETBE. No statistically significant changes in body weight were observed in the ETBE-exposed male rats.

Table 8 (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Motor Activity and Functional Observation Battery Data for Male and Female Rats Combined[a]

Neurologic Functional Domain	Test Session (days after start of exposure, approximate) Pre	1	7	14	30	60	90
CNS Activity							
Altered posture	NSD	NO	NSD	NSD	NO	NSD	s[g]
Altered body position	NO	NO	NO	NO	NO	NO	NO
Activity in FOB open field	NSD	NSD	NSD	NSD	NSD	NSD	NSD
Unusual behaviors	NO	NO	NO	NSD	NO	N[h]	NO
Rearing (# rears)	NSD	NSD	NSD	NSD	NSD	NSD	NSD
Spontaneous motor activity	NSD	NSD	NSD	NSD	s[i]	NSD	s[i]

[a]s: Significant ($P \leq 0.05$) statistical difference observed
NSD: No significant difference observed
NO: Abnormal clinical sign not observed in any rat
N: Abnormal clinical sign not observed in any ETBE-exposed rat

[g]Normal posture, asleep. Not toxicologically significant.

[h]Hiccuping motion observed in one control rat

[i]Statistically significant changes indicate altered motor activity observed during only a portion of the 1 hour test session. None of the described motor activity alterations were consistent throughout a motor session nor did they demonstrate a dose response. These subtle changes in motor activity are therefore not consistent with ETBE-induced neurotoxicity and are likely a statistical artifact due to a lack of a statistical correction for multiple comparisons.

Ophthalmic Examination

There were no exposure-related eye abnormalities following the 13-week exposure regimen.

Neurobehavioral Evaluations

Table 8 summarizes results of neurobehavioral tests conducted during the study. Total motor activity and total ambulations were determined during individual one hour motor activity test sessions using a commercially available motor activity system (San

Diego Instruments, San Diego, CA). Motor activity was initially determined in naive (untreated) male and female rats 4 days prior to the beginning of ETBE exposure. The activity of the naive rats approached an asymptote during the last 10-15 minutes of the 60-minute testing session indicating that the 60 minute test period was long enough to meet the criteria detailed by the U.S. EPA testing guideline for motor activity. Motor activity was reassessed following 20, 42, and 65 exposure days corresponding to approximately 30-, 60-, and 90-days after the start of the exposure. Only minor changes in either total motor activity or ambulation were observed. No statistically significant overall treatment effects for ETBE on either motor activity or ambulations were observed during any motor activity test session (Table 8).

The effect of ETBE on motor activity during discrete portions of the test session (e.g., during initial exploration) was also evaluated. Comparisons of each 6-minute motor activity test interval (ANOVA; $p \leq 0.05$) were performed. Not unexpectedly, several individual test sessions demonstrated statistically significant effects during single time intervals and are detailed in Appendix 5. None of the motor activity alterations were consistent throughout a motor session nor did they demonstrate a dose response. These subtle changes in motor activity are therefore not consistent with ETBE-induced neurotoxicity and are likely a statistical artifact due to a lack of a statistical correction for multiple comparisons.

In addition to motor activity, a blinded functional observation battery (FOB) evaluation was also completed. All FOB assessments were performed by the same technician throughout the study. Pre-exposure FOBs were performed 4 days prior to ETBE exposure. One male rat was replaced with another male rat of similar body weight, which had previously not been selected for the study, during the pre-exposure testing due to a corneal abnormality. The FOB was reassessed following 1, 6, 10, 20, 42, and 65 ETBE exposures corresponding to approximately 1-, 7-, 14-, 30-, 60-, and 90-days after the start of the exposure, respectively. No statistically significant changes in clinically relevant qualitative FOB endpoints were observed in ETBE-exposed rats (Table 8). Only minor changes in the quantitative FOB data were observed which are detailed in Appendix 5. None of the FOB changes demonstrated a dose-response relationship or a consistent pattern of neurologic dysfunction throughout the study.

Rats were killed by perfusion for neuropathology at the termination of the study. Only minor gross lesions were observed and are detailed in Appendix 5. Only the control and high dose groups were examined microscopically. No gross or microscopic abnormalities were observed in the central, peripheral or autonomic nervous systems of rats exposed to 5000 ppm ETBE. No statistically significant difference in brain weight or size were observed in ETBE-exposed rats.

Body Weights

Average body weights for all rats in each exposure group measured weekly throughout the study are summarized in Tables 9 (male rats) and 10 (female rats). Figures 1 and 2 present the average group body weight data with time over the course of the study for males and females respectively. Individual animal body weight data are presented in Appendix 10.

There was an approximate 25% decrease in body weight gain compared with controls during the first week for males exposed to 1750 or 5000 ppm ETBE. This probably represents a transitory adjustment to ETBE treatment. After the third week there were no differences in the body weights among the 4 groups for the remainder of the study. There were no exposure-related alterations in mean body weights for rats in the 500 ppm group.

There was an approximate 25% decrease in body weight gain compared with controls during the first week for females exposed to 1750 or 5000 ppm ETBE. Similar to what was observed for males, this probably represents a transitory adjustment to ETBE treatment. After the third week there were no differences in the body weights among the 4 groups until weeks 13 and 14 when the body weights of the female rats in the 5000 ppm group were significantly increased compared with controls.

Table 9.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Mean Weekly Body Weights for All Surviving Male Rats[a]

Body Weight Statistic	Exposure Group (ppm)			
	Control	500	1750	5000
Pretest				
Number	48	48	48	48
Mean Weight	119.70	119.72	119.75	119.71
Standard Deviations	4.97	4.99	4.96	4.96
Week 1				
Number	43	48	48	48
Mean Weight	163.20	160.22	152.96[b]	152.70[b]
Standard Deviations	10.19	8.84	7.93	6.75
Week 2				
Number	43	43	43	43
Mean Weight	183.81	185.21	173.12[b]	175.58[b]
Standard Deviations	13.33	11.53	11.83	8.45
Week 3				
Number	43	43	43	43
Mean Weight	199.98	200.61	201.04	192.86[c]
Standard Deviations	15.32	13.21	12.69	8.85
Week 4				
Number	38	43	43	43
Mean Weight	211.47	215.70	210.20	212.85
Standard Deviations	17.50	15.07	13.71	10.18

[a]Body weight data are express in grams
[b]Group mean different from control at a significance level of 0.01
[c]Group mean different from control at a significance level of 0.05

Table 9. (cont'd.)
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Mean Weekly Body Weights for All Surviving Male Rats[a]

	Exposure Group (ppm)			
Body Weight Statistic	Control	500	1750	5000
Week 5				
Number	38	38	38	38
Mean Weight	223.35	225.99	224.24	221.97
Standard Deviations	20.68	14.59	14.71	10.43
Week 6				
Number	38	38	38	38
Mean Weight	230.56	233.33	231.66	229.10
Standard Deviations	21.43	16.53	15.22	11.63
Week 7				
Number	28	28	28	38
Mean Weight	243.35	243.31	245.71	244.10
Standard Deviations	25.43	16.87	16.53	10.89
Week 8				
Number	28	28	28	28
Mean Weight	255.53	255.77	257.49	252.87
Standard Deviations	26.37	17.91	17.58	11.77
Week 9				
Number	28	28	28	28
Mean Weight	264.47	265.07	267.86	262.71
Standard Deviations	26.15	18.40	18.67	13.20

[a]Body weight data are express in grams
[b]Group mean different from control at a significance level of 0.01
[c]Group mean different from control at a significance level of 0.05

Table 9. (cont'd)
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Mean Weekly Body Weights for All Surviving Male Rats[a]

Body Weight Statistic	Exposure Group ppm			
	Control	500	1750	5000
Week 10				
Number	28	28	28	28
Mean Weight	270.92	273.05	274.87	271.31
Standard Deviations	27.76	18.57	19.29	12.51
Week 11				
Number	28	28	28	28
Mean Weight	280.19	281.86	281.88	281.15
Standard Deviations	29.03	17.89	19.40	12.57
Week 12				
Number	28	28	28	28
Mean Weight	286.02	287.70	289.52	292.01
Standard Deviations	29.55	20.57	20.22	13.07
Week 13				
Number	23	28	28	28
Mean Weight	288.23	294.65	298.00	295.97
Standard Deviations	30.62	18.47	20.17	14.00
Week 14				
Number	12	12	12	23
Mean Weight	295.66	301.98	308.85	301.88
Standard Deviations	32.20	16.25	28.63	16.38

[a]Body weight data are express in grams
[b]Group mean different from control at a significance level of 0.01
[c]Group mean different from control at a significance level of 0.05

Table 10.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Mean Weekly Body Weights for All Surviving Female Rats[a]

Body Weight Statistic	Exposure Group (ppm)			
	Control	500	1750	5000
Pretest				
Number	48	48	48	48
Mean Weight	84.13	84.19	84.15	84.13
Standard Deviation	4.05	3.93	3.99	4.01
Week 1				
Number	42	48	48	48
Mean Weight	110.35	107.59[c]	105.52[b]	104.44[b]
Standard Deviation	6.36	5.49	4.22	4.39
Week 2				
Number	42	43	43	43
Mean Weight	122.70	120.55	118.88[b]	116.11[b]
Standard Deviation	6.04	5.94	5.24	4.34
Week 3				
Number	42	43	43	43
Mean Weight	129.25	128.62	130.49	126.18[c]
Standard Deviation	7.42	7.26	5.79	5.05

[a]Body weight data are express in grams
[b]Group mean different from control at a significance level of 0.01
[c]Group mean different from control at a significance level of 0.05

Table 10 (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Mean Weekly Body Weights for All Surviving Female Rats[a]

	Exposure Group (ppm)			
Body Weight Statistic	Control	500	1750	5000
Week 4				
Number	36	43	43	43
Mean Weight	137.72	136.16	134.93	135.28
Standard Deviation	7.27	7.08	6.32	5.96
Week 5				
Number	36	38	38	38
Mean Weight	143.69	142.63	141.01	143.50
Standard Deviation	7.89	7.79	7.50	6.42
Week 6				
Number	36	38	38	38
Mean Weight	146.60	145.40	143.85	145.95
Standard Deviation	8.40	7.97	7.77	6.32
Week 7				
Number	27	28	38	38
Mean Weight	150.27	148.63	150.07	153.36
Standard Deviation	8.94	7.48	8.29	7.72
Week 8				
Number	27	28	28	28
Mean Weight	155.09	153.08	154.50	158.57
Standard Deviation	9.73	9.15	8.32	8.03
Week 9				
Number	27	28	28	28
Mean Weight	159.76	156.15	159.05	160.18
Standard Deviation	10.37	8.81	9.61	7.68

[a]Body weight data are express in grams
[b]Group mean different from control at a significance level of 0.01
[c]Group mean different from control at a significance level of 0.05

Table 10 (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Mean Weekly Body Weights for All Surviving Female Rats[a]

Body Weight Statistic	Exposure Group (ppm) Control	500	1750	5000
Week 10				
Number	27	28	28	28
Mean Weight	161.96	157.89	160.92	165.07
Standard Deviation	11.53	9.64	9.47	8.04
Week 11				
Number	27	28	28	28
Mean Weight	165.08	161.99	164.41	169.93
Standard Deviation	10.91	9.65	10.50	8.12
Week 12				
Number	27	28	28	28
Mean Weight	168.13	164.52	168.44	174.71
Standard Deviation	11.04	9.61	10.67	9.33
Week 13				
Number	22	28	28	28
Mean Weight	168.40	167.11	171.47	177.71[b]
Standard Deviation	11.66	10.08	11.21	9.67
Week 14				
Number	12	12	19	23
Mean Weight	170.16	165.41	174.57	180.95[b]
Standard Deviation	7.59	11.29	9.76	8.82

[a]Body weight data are express in grams
[b]Group mean different from control at a significance level of 0.01
[c]Group mean different from control at a significance level of 0.05

Figure 1.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Change in Mean Body Weights of All Surviving Male Rats with Time During the Exposure Period

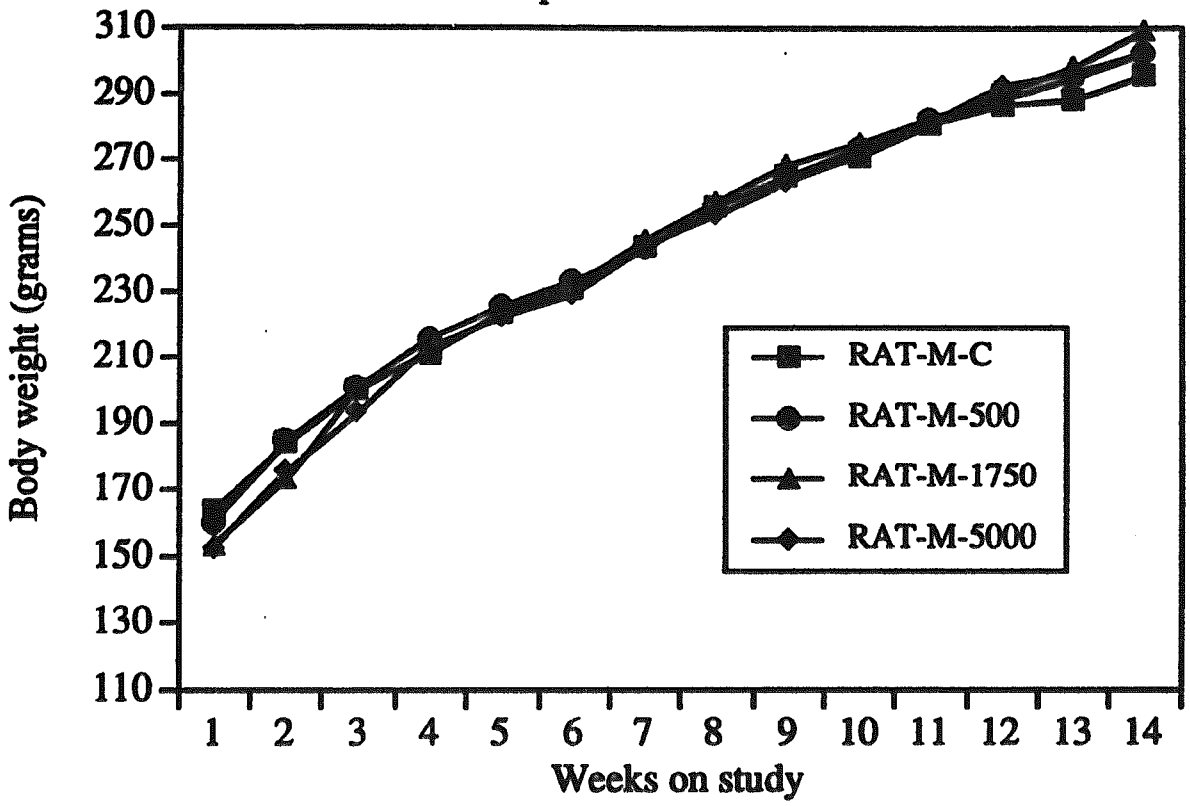
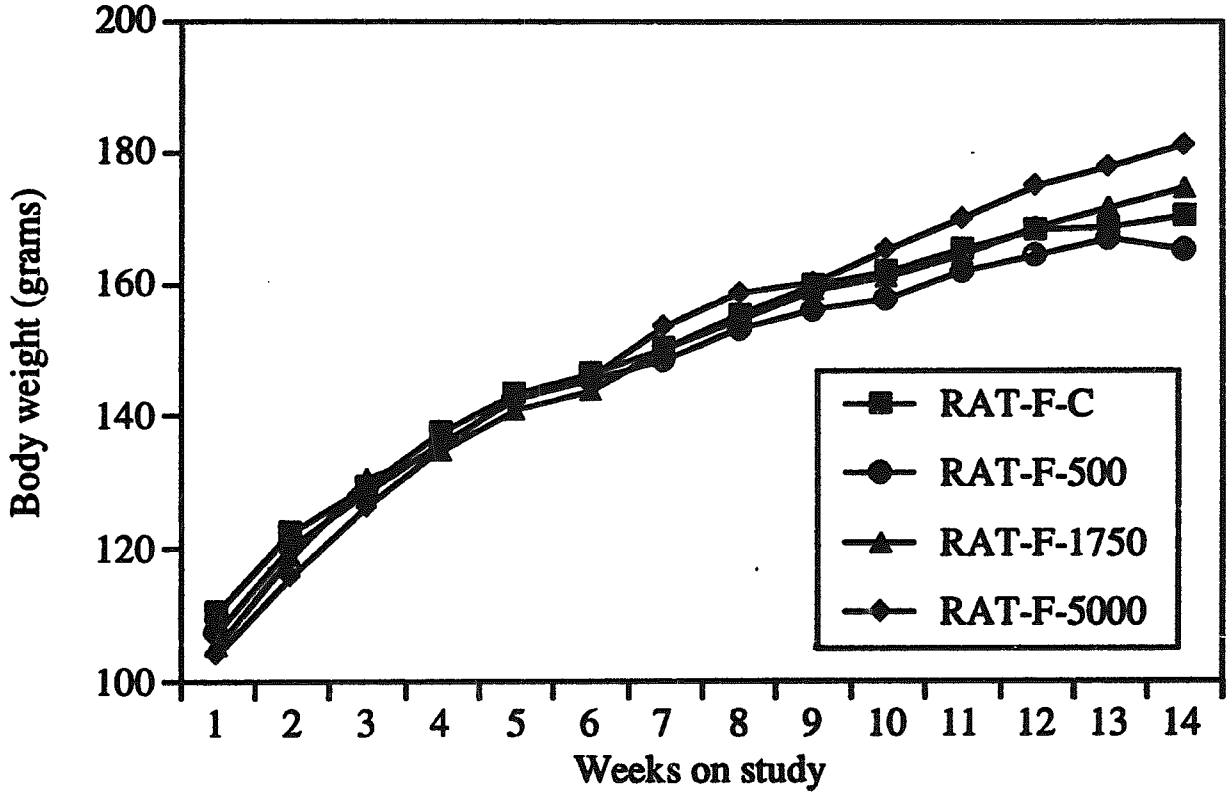


Figure 2.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Change in Mean Body Weights of All Surviving Female Rats with Time During the Exposure Period



Terminal body weights were taken at necropsy for all rats on study. Data from rats which survived to the scheduled necropsy date were analyzed across exposure groups for the core group and for each subgroup (Clinical Pathology, Neurotoxicity, and Cell Replication). Mean terminal body weights for each subgroup are summarized in Tables 11 and 12. There were no significant differences in terminal body weights among male rats for any of the subgroups for any exposure group. Female rats in the Core and Neurotoxicity Subgroups had significantly increased body weights at the 5000 ppm exposure group. Data for terminal body weights for individual animals is presented in Appendix 11.

Table 11.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Pretest and Terminal Body Weights for Male Rats[a]

Group or Subgroup	Body Weight Statistic	Exposure Group (ppm) Control	500	1750	5000
CR 1	Mean (Pretest)	121.0	121.0	121.0	120.9
CR 1	Std dev (Pretest)	6.2	6.1	6.1	6.1
CR 1	N (Pretest)	5	5	5	5
CR 1	Mean(study day 5)	166.1	166.7	162.7	160.4
CR 1	Std dev(study day 5)	7.6	4.7	8.9	6.4
CR 1	N(study day 5)	5	5	5	5
CR 2	Mean (Pretest)	120.3	120.4	120.4	120.4
CR 2	Std dev (Pretest)	6.0	6.1	6.0	6.2
CR 2	N (Pretest)	5	5	5	5
CR 2	Mean(study day 26)	227.6	218.0	209.2	217.2
CR 2	Std dev(study day 26)	9.2	17.1	15.5	7.8
CR 2	N(study day 26)	5	5	5	5
CR 3	Mean (Pretest)	120.2	120.2	120.2	120.1
CR 3	Std dev (Pretest)	3.0	3.0	3.0	2.9
CR 3	N (Pretest)	5	5	5	5
CR 3	Mean(study day 89)	290.9	310.2	292.1	293.5
CR 3	Std dev(study day 89)	25.6	5.9	16.0	12.6
CR 3	N(study day 89)	5	5	5	5
Core	Mean (Pretest)	117.3	117.3	117.3	117.4
Core	Std dev (Pretest)	3.8	4.0	4.0	3.9
Core	N (Pretest)	11	11	11	11
Core	Mean(study day 93-94)	283.8	285.8	293.0	292.0
Core	Std dev(study day 93-94)	30.4	20.6	9.9	14.0
Core	N(study day 93-94)	11	11	11	11

[a]Body weight data are expressed in grams.

Table 11. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Pretest and Terminal Body Weights for Male Rats[a]

Group or Subgroup	Body Weight Statistic	Exposure Group (ppm) Control	500	1750	5000
CP	Mean (Pretest)	121.5	121.5	121.7	121.6
CP	Std dev (Pretest)	6.7	6.6	6.6	6.6
CP	N (Pretest)	10	10	10	10
CP	Mean(study day 45)	235.6	244.9	240.1	247.2
CP	Std dev(study day 45)	17.2	20.1	21.1	11.9
CP	N(study day 45)	10	10	10	10
NT	Mean (Pretest)	119.4	119.3	119.4	119.4
NT	Std dev (Pretest)	3.9	3.9	3.8	3.9
NT	N (Pretest)	12	12	12	12
NT	Mean(study day 101-102)	299.3	306.2	309.0	316.9
NT	Std dev(study day 101-102)	30.7	17.6	40.6	15.3
NT	N(study day 101-102)	12	12	12	12

[a]Body weight data are expressed in grams.

Table 12.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Pretest and Terminal Body Weights for Female Rats[a]

Group or Subgroup	Body Weight Statistic	Exposure Group (ppm) Control	500	1750	5000
CR 1	Mean (Pretest)	85.3	85.2	85.3	85.2
CR 1	Std dev (Pretest)	4.1	4.0	4.0	4.0
CR 1	N (Pretest)	5	5	5	5

[a]Body weight data are expressed in grams.

Table 12. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Pretest and Terminal Body Weights for Female Rats[a]

Group or Subgroup	Body Weight Statistic	Exposure Group (ppm) Control	500	1750	5000
CR 1	Mean(study day 5)	114.0	112.9	110.5	113.3
CR 1	Std dev(study day 5)	3.7	5.2	5.7	4.8
CR 1	N(study day 5)	5	5	5	5
CR 2	Mean (Pretest)	83.3	83.5	83.3	83.4
CR 2	Std dev (Pretest)	5.4	5.2	5.5	5.2
CR 2	N (Pretest)	5	5	5	5
CR 2	Mean(study day 26)	141.9	139.5	139.8	138.3
CR 2	Std dev(study day 26)	3.2	4.6	6.9	3.5
CR 2	N(study day 26)	5	5	5	5
CR 3	Mean (Pretest)	83.0	83.1	83.2	83.0
CR 3	Std dev (Pretest)	3.3	3.1	3.1	3.3
CR 3	N (Pretest)	5	5	5	5
CR 3	Mean(study day 89)	175.6	174.1	168.6	169.6
CR 3	Std dev(study day 89)	10.5	10.2	16.3	10.7
CR 3	N(study day 89)	5	5	5	5

[a]Body weight data are expressed in grams.

Table 12. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Pretest and Terminal Body Weights for Female Rats[a]

Group or Subgroup	Body Weight Statistic	Exposure Group (ppm) Control	500	1750	5000
Core	Mean (Pretest)	82.9	82.9	82.9	82.8
Core	Std dev (Pretest)	4.8	4.7	4.7	4.8
Core	N (Pretest)	11	11	11	11
Core	Mean(study day 94-95)	164.3	165.0	169.6	175.5[b]
Core	Std dev(study day 94-95)	12.4	7.3	9.8	7.5
Core	N(study day 94-95)	10	11	11	11
CP	Mean (Pretest)	84.9	84.9	84.8	84.9
CP	Std dev (Pretest)	2.9	2.8	2.8	2.8
CP	N (Pretest)	10	10	10	10
CP	Mean(study day 46)	150.5	151.7	146.2	152.9
CP	Std dev(study day 46)	8.8	8.9	5.2	7.2
CP	N(study day 46)	9	10	10	10
NT	Mean (Pretest)	85.0	85.1	85.0	85.0
NT	Std dev (Pretest)	4.1	4.1	4.1	4.1
NT	N (Pretest)	12	12	12	12
NT	Mean(study day 101-102)	171.0	167.2	180.0	184.0[b]
NT	Std dev(study day 101-102)	9.7	12.5	11.1	10.0
NT	N(study day 101-102)	12	12	12	12

[a]Body weight data are expressed in grams.
[b] Different from control by Dunnett's test at $p < 0.05$

Hematology

Tables 13 through 16 summarize hematology data for male and female rats. Hematology data for individual animals is presented in Appendix 6. Rats were euthanized and blood taken at an interim point from the Clinical Pathology subgroup after approximately 6 weeks of exposure (Tables 13 and 14) or at final necropsy from the Core group after approximately 13 weeks of exposure (Tables 15 and 16). For male rats the only hematology parameters that were statistically different from controls at both the interim (week 6) and final (week 13) time points were an increase in platelets and a decrease in

MCHC in rats exposed to 5000 ppm ETBE. The relationship of these isolated changes to ETBE exposure is not clear.

For female rats the only hematology parameter that was statistically different from controls at both week 6 and week 13 was an increase in MCV in rats exposed to 5000 ppm. MCV is a calculated value based on the RBC count and hematocrit. The change in MCV without a reduction in RBC count indicates that there is not a regenerative response to anemia which would commonly cause an increased MCV.

Clinical Chemistry

Tables 17–20 summarize clinical chemistry data for male and female rats at the interim and final time points. Clinical chemistry data for individual animals is presented in Appendix 7. ETBE-exposed male rats had slight but significant changes in levels of chloride and total protein compared with controls at both the interim and final sampling interval (Tables 17 and 19). ETBE-exposed female rats had no consistent changes in clinical chemistry parameters compared with controls for both the interim and final time points (Tables 18 and 20). Other slight changes observed in ETBE-exposed male (albumin, alkaline phosphatase, calcium, creatinine, sodium) and female (bilirubin, phosphorus) rats were inconsistent with respect to sampling interval or level of exposure. All changes were minimal in extent and could not be considered definitive for any disease process in ETBE-exposed rats.

Table 13.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Interim Hematology Results in Male Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Red Blood Cells ($10^6/\mu l$)	Mean(RBC)	7.85	7.92	7.81	7.67
	Std Dev(RBC)	0.34	0.38	0.40	0.16
	N(RBC)	9	10	9	10
Hemoglobin (g/dl)	Mean(HGB)	15.16	15.17	14.93	14.73
	Std Dev(HGB)	0.46	0.53	0.62	0.23
	N(HGB)	9	10	9	10
Hematocrit (%)	Mean(HCT)	41.61	41.91	41.67	41.92
	Std Dev(HCT)	1.89	1.92	2.03	0.84
	N(HCT)	9	10	9	10
Mean Corpuscular Volume (fl)	Mean(MCV)	52.99	52.92	53.32	54.67 [a]
	Std Dev(MCV)	0.51	0.43	0.26	0.26
	N(MCV)	9	10	9	10
Mean Corpuscular Hemoglobin (pg)	Mean(MCH)	19.31	19.17	19.12	19.20
	Std Dev(MCH)	0.55	0.34	0.37	0.22
	N(MCH)	9	10	9	10
Mean Corpuscular Hemoglobin Concentration (%)	Mean(MCHC)	36.46	36.21	35.86	35.13 [a]
	Std Dev(MCHC)	1.09	0.61	0.71	0.38
	N(MCHC)	9	10	9	10
Platelets ($10^3/\mu l$)	Mean(PLAT)	610.3	667.4	670.1	698.6 [a]
	Std Dev(PLAT)	78.2	37.9	50.7	49.3
	N(PLAT)	9	10	9	10
White Blood Cells ($10^3/\mu l$)	Mean(WBC)	3.06	4.14	3.48	3.48
	Std Dev(WBC)	0.77	0.88	1.09	0.65
	N(WBC)	9	10	9	10

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 13. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Interim Hematology Results in Male Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Banded Neutrophils ($10^3/\mu l$)	Mean(BAND)	0.00	0.00	0.00	0.00
	Std Dev(BAND)	0.00	0.00	0.00	0.00
	N(BAND)	9	10	9	10
Segmented Neutrophils ($10^3/\mu l$)	Mean(SEG)	0.66	0.73	0.78	1.02 [a]
	Std Dev(SEG)	0.20	0.33	0.30	0.27
	N(SEG)	9	10	9	10
Lymphocytes ($10^3/\mu l$)	Mean(LYMPH)	2.36	3.37 [a]	2.64	2.43
	Std Dev(LYMPH)	0.62	0.84	0.87	0.51
	N(LYMPH)	9	10	9	10
Monocytes ($10^3/\mu l$)	Mean(MONO)	0.01	0.03	0.02	0.01
	Std Dev(MONO)	0.02	0.05	0.04	0.02
	N(MONO)	9	10	9	10
Eosinophils ($10^3/\mu l$)	Mean(EO)	0.03	0.01	0.03	0.03
	Std Dev(EO)	0.02	0.02	0.02	0.03
	N(EO)	9	10	9	10
Basophils ($10^3/\mu l$)	Mean(BASO)	0.00	0.00	0.00	0.00
	Std Dev(BASO)	0.00	0.00	0.00	0.00
	N(BASO)	9	10	9	10
Atypical Lymphocytes ($10^3/\mu l$)	Mean(ATLYMPH)	0.00	0.00	0.00	0.00
	Std Dev(ATLYMPH)	0.00	0.00	0.00	0.00
	N(ATLYMPH)	9	10	9	10
Metamyelocytes ($10^3/\mu l$)	Mean(META)	0.00	0.00	0.00	0.00
	Std Dev(META)	0.00	0.00	0.00	0.00
	N(META)	9	10	9	10
Myelocytes ($10^3/\mu l$)	Mean(MYELO)	0.00	0.00	0.00	0.00
	Std Dev(MYELO)	0.00	0.00	0.00	0.00
	N(MYELO)	9	10	9	10
Nucleated Red Blood Cells (cells/100 WBC)	Mean(NRBC)	0.00	0.10	0.00	0.10
	Std Dev(NRBC)	0.00	0.32	0.00	0.32
	N(NRBC)	9	10	9	10

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 14.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Interim Hematology Results in Female Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Red Blood Cells ($10^6/\mu l$)	Mean(RBC)	6.93	6.95	6.96	6.92
	Std Dev(RBC)	0.35	0.33	0.30	0.14
	N(RBC)	8	9	10	10
Hemoglobin (g/dl)	Mean(HGB)	14.35	14.52	14.49	14.41
	Std Dev(HGB)	0.57	0.50	0.56	0.32
	N(HGB)	8	9	10	10
Hematocrit (%)	Mean(HCT)	39.91	40.13	40.33	40.34
	Std Dev(HCT)	2.02	1.89	1.78	0.79
	N(HCT)	8	9	10	10
Mean Corpuscular Volume (fl)	Mean(MCV)	57.62	57.71	57.92	58.33 [a]
	Std Dev(MCV)	0.40	0.56	0.36	0.31
	N(MCV)	8	9	10	10
Mean Corpuscular Hemoglobin (pg)	Mean(MCH)	20.74	20.90	20.81	20.84
	Std Dev(MCH)	0.40	0.46	0.24	0.29
	N(MCH)	8	9	10	10
Mean Corpuscular Hemoglobin Concentration (%)	Mean(MCHC)	35.96	36.21	35.93	35.72
	Std Dev(MCHC)	0.73	0.75	0.35	0.43
	N(MCHC)	8	9	10	10
Platelets ($10^3/\mu l$)	Mean(PLAT)	686.5	631.1	597.5 a	667.4
	Std Dev(PLAT)	32.8	62.6	69.2	86.2
	N(PLAT)	8	9	10	10
White Blood Cells ($10^3/\mu l$)	Mean(WBC)	3.10	2.44	2.48	1.89 [a]
	Std Dev(WBC)	0.79	0.89	0.56	0.58
	N(WBC)	8	9	10	10

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 14. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Interim Hematology Results in Female Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Banded Neutrophils ($10^3/\mu l$)	Mean(BAND)	0.00	0.00	0.00	0.00
	Std Dev(BAND)	0.00	0.00	0.00	0.00
	N(BAND)	8	9	10	10
Segmented Neutrophils ($10^3/\mu l$)	Mean(SEG)	0.57	0.47	0.51	0.59
	Std Dev(SEG)	0.23	0.16	0.18	0.22
	N(SEG)	8	9	10	10
Lymphocytes ($10^3/\mu l$)	Mean(LYMPH)	2.49	1.96	1.96	1.28 [a]
	Std Dev(LYMPH)	0.68	0.78	0.51	0.46
	N(LYMPH)	8	9	10	10
Monocytes ($10^3/\mu l$)	Mean(MONO)	0.02	0.02	0.00	0.01
	Std Dev(MONO)	0.04	0.03	0.00	0.01
	N(MONO)	8	9	10	10
Eosinophils ($10^3/\mu l$)	Mean(EO)	0.02	0.01	0.01	0.02
	Std Dev(EO)	0.02	0.01	0.01	0.02
	N(EO)	8	9	10	10
Basophils ($10^3/\mu l$)	Mean(BASO)	0.00	0.00	0.00	0.00
	Std Dev(BASO)	0.00	0.00	0.00	0.00
	N(BASO)	8	9	10	10
Atypical Lymphocytes ($10^3/\mu l$)	Mean(ATLYMPH)	0.00	0.00	0.00	0.00
	Std Dev(ATLYMPH)	0.00	0.00	0.00	0.00
	N(ATLYMPH)	8	9	10	10
Metamyelocytes ($10^3/\mu l$)	Mean(META)	0.00	0.00	0.00	0.00
	Std Dev(META)	0.00	0.00	0.00	0.00
	N(META)	8	9	10	10
Myelocytes ($10^3/\mu l$)	Mean(MYELO)	0.00	0.00	0.00	0.00
	Std Dev(MYELO)	0.00	0.00	0.00	0.00
	N(MYELO)	8	9	10	10
Nucleated Red Blood Cells (cells/100 WBC)	Mean(NRBC)	0.38	0.56	0.00	0.00
	Std Dev(NRBC)	0.74	0.53	0.00	0.00
	N(NRBC)	8	9	10	10

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 15.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Final Hematology Results in Male Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Red Blood Cells ($10^6/\mu l$)	Mean(RBC)	7.87	7.99	8.04	7.80
	Std Dev(RBC)	0.21	0.33	0.46	0.42
	N(RBC)	11	11	10	11
Hemoglobin (g/dl)	Mean(HGB)	15.16	15.35	15.28	14.85
	Std Dev(HGB)	0.36	0.57	0.59	0.58
	N(HGB)	11	11	10	11
Hematocrit (%)	Mean(HCT)	42.24	43.02	43.67	42.42
	Std Dev(HCT)	1.24	1.87	1.65	2.01
	N(HCT)	11	11	10	11
Mean Corpuscular Volume (fl)	Mean(MCV)	53.68	53.85	54.38	54.43
	Std Dev(MCV)	0.69	0.93	1.64	0.63
	N(MCV)	11	11	10	11
Mean Corpuscular Hemoglobin (pg)	Mean(MCH)	19.28	19.22	19.05	19.06
	Std Dev(MCH)	0.43	0.31	0.53	0.45
	N(MCH)	11	11	10	11
Mean Corpuscular Hemoglobin Concentration (%)	Mean(MCHC)	35.91	35.71	35.00 a	35.01 a
	Std Dev(MCHC)	0.67	0.50	0.30	0.65
	N(MCHC)	11	11	10	11
Platelets ($10^3/\mu l$)	Mean(PLAT)	583.6	589.4	587.6	678.8 a
	Std Dev(PLAT)	68.4	56.3	94.2	81.1
	N(PLAT)	11	11	10	11
White Blood Cells ($10^3/\mu l$)	Mean(WBC)	3.20	3.07	3.19	3.07
	Std Dev(WBC)	0.57	0.88	1.04	0.75
	N(WBC)	11	11	10	11

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 15. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Final Hematology Results in Male Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Banded Neutrophils ($10^3/\mu l$)	Mean(BAND)	0.00	0.00	0.00	0.00
	Std Dev(BAND)	0.00	0.00	0.00	0.00
	N(BAND)	11	11	10	11
Segmented Neutrophils ($10^3/\mu l$)	Mean(SEG)	0.91	0.79	0.94	0.82
	Std Dev(SEG)	0.31	0.23	0.44	0.27
	N(SEG)	11	11	10	11
Lymphocytes ($10^3/\mu l$)	Mean(LYMPH)	2.26	2.26	2.21	2.21
	Std Dev(LYMPH)	0.50	0.84	0.72	0.69
	N(LYMPH)	11	11	10	11
Monocytes ($10^3/\mu l$)	Mean(MONO)	0.00	0.00	0.01	0.01
	Std Dev(MONO)	0.01	0.00	0.01	0.02
	N(MONO)	11	11	10	11
Eosinophils ($10^3/\mu l$)	Mean(EO)	0.03	0.02	0.03	0.03
	Std Dev(EO)	0.03	0.02	0.05	0.04
	N(EO)	11	11	10	11
Basophils ($10^3/\mu l$)	Mean(BASO)	0.00	0.00	0.00	0.00
	Std Dev(BASO)	0.00	0.00	0.00	0.00
	N(BASO)	11	11	10	11
Atypical Lymphocytes ($10^3/\mu l$)	Mean(ATLYMPH)	0.00	0.00	0.00	0.00
	Std Dev(ATLYMPH)	0.00	0.00	0.00	0.00
	N(ATLYMPH)	11	11	10	11
Metamyelocytes ($10^3/\mu l$)	Mean(META)	0.00	0.00	0.00	0.00
	Std Dev(META)	0.00	0.00	0.00	0.00
	N(META)	11	11	10	11
Myelocytes ($10^3/\mu l$)	Mean(MYELO)	0.00	0.00	0.00	0.00
	Std Dev(MYELO)	0.00	0.00	0.00	0.00
	N(MYELO)	11	11	10	11
Nucleated Red Blood Cells (cells/100 WBC)	Mean(NRBC)	0.09	0.27	0.10	0.09
	Std Dev(NRBC)	0.30	0.65	0.32	0.30
	N(NRBC)	11	11	10	11

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 16.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Final Hematology Results in Female Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Red Blood Cells ($10^6/\mu l$)	Mean(RBC)	7.73	7.64	7.72	7.63
	Std Dev(RBC)	0.45	0.44	0.25	0.39
	N(RBC)	9	11	11	11
Hemoglobin (g/dl)	Mean(HGB)	15.79	15.72	15.85	15.52
	Std Dev(HGB)	0.71	0.79	0.62	0.58
	N(HGB)	9	11	11	11
Hematocrit (%)	Mean(HCT)	44.28	44.02	44.77	44.32
	Std Dev(HCT)	2.52	2.33	1.42	2.32
	N(HCT)	9	11	11	11
Mean Corpuscular Volume (fl)	Mean(MCV)	57.26	57.61	57.97 [a]	58.11 [a]
	Std Dev(MCV)	0.73	0.59	0.49	0.60
	N(MCV)	9	11	11	11
Mean Corpuscular Hemoglobin (pg)	Mean(MCH)	20.42	20.59	20.52	20.35
	Std Dev(MCH)	0.53	0.39	0.23	0.37
	N(MCH)	9	11	11	11
Mean Corpuscular Hemoglobin Concentration (%)	Mean(MCHC)	35.69	35.71	35.39	35.04
	Std Dev(MCHC)	0.78	0.44	0.53	0.71
	N(MCHC)	9	11	11	11
Platelets ($10^3/\mu l$)	Mean(PLAT)	609.1	618.9	589.1	622.6
	Std Dev(PLAT)	62.9	83.2	48.8	42.9
	N(PLAT)	9	11	11	11
White Blood Cells ($10^3/\mu l$)	Mean(WBC)	1.91	2.20	2.48	2.23
	Std Dev(WBC)	0.71	0.81	0.55	1.11
	N(WBC)	9	11	11	11

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 16. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Final Hematology Results in Female Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Banded Neutrophils ($10^3/\mu l$)	Mean(BAND)	0.00	0.00	0.00	0.00
	Std Dev(BAND)	0.00	0.00	0.00	0.00
	N(BAND)	9	11	11	11
Segmented Neutrophils ($10^3/\mu l$)	Mean(SEG)	0.47	0.54	0.63	0.62
	Std Dev(SEG)	0.20	0.23	0.28	0.30
	N(SEG)	9	11	11	11
Lymphocytes ($10^3/\mu l$)	Mean(LYMPH)	1.43	1.64	1.85	1.60
	Std Dev(LYMPH)	0.54	0.64	0.59	0.85
	N(LYMPH)	9	11	11	11
Monocytes ($10^3/\mu l$)	Mean(MONO)	0.00	0.01	0.00	0.00
	Std Dev(MONO)	0.00	0.02	0.01	0.01
	N(MONO)	9	11	11	11
Eosinophils ($10^3/\mu l$)	Mean(EO)	0.01	0.01	0.00	0.00
	Std Dev(EO)	0.02	0.01	0.01	0.01
	N(EO)	9	11	11	11
Basophils ($10^3/\mu l$)	Mean(BASO)	0.00	0.00	0.00	0.00
	Std Dev(BASO)	0.00	0.00	0.00	0.00
	N(BASO)	9	11	11	11
Atypical Lymphocytes ($10^3/\mu l$)	Mean(ATLYMPH)	0.00	0.00	0.00	0.00
	Std Dev(ATLYMPH)	0.00	0.00	0.00	0.00
	N(ATLYMPH)	9	11	11	11
Metamyelocytes ($10^3/\mu l$)	Mean(META)	0.00	0.00	0.00	0.00
	Std Dev(META)	0.00	0.00	0.00	0.00
	N(META)	9	11	11	11
Myelocytes ($10^3/\mu l$)	Mean(MYELO)	0.00	0.00	0.00	0.00
	Std Dev(MYELO)	0.00	0.00	0.00	0.00
	N(MYELO)	9	11	11	11
Nucleated Red Blood Cells (cells/100 WBC)	Mean(NRBC)	0.33	0.00	0.09	0.09
	Std Dev(NRBC)	1.00	0.00	0.30	0.30
	N(NRBC)	9	11	11	11

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 17.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Interim Clinical Chemistry Results for Male Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Albumin (g/dl)	Mean(ALB)	3.5	3.6	3.5	3.8 [a]
	Std Dev(ALB)	0.2	0.2	0.2	0.1
	N(ALB)	10	10	10	10
Alkaline Phosphatase (U/L)	Mean(ALP)	254	235	226 [a]	228
	Std Dev(ALP)	34	19	14	15
	N(ALP)	10	10	10	10
Alanine Aminotransferase (U/L)	Mean(ALT)	51.0	48.2	50.6	48.7
	Std Dev(ALT)	16.4	13.4	13.9	8.9
	N(ALT)	10	10	10	10
Aspartate Aminotransferase (U/L)	Mean(AST)	123.6	104.7	120.6	98.3
	Std Dev(AST)	47.7	22.5	31.2	26.0
	N(AST)	10	10	10	10
Bilirubin (mg/dl)	Mean(BILI)	0.25	0.25	0.24	0.32
	Std Dev(BILI)	0.05	0.05	0.05	0.06
	N(BILI)	10	10	10	10
Blood Urea Nitrogen (mg/dl)	Mean(BUN)	21.4	21.2	19.9	21.6
	Std Dev(BUN)	3.2	2.1	1.3	1.5
	N(BUN)	10	10	10	10
Calcium (mg/dl)	Mean(CAL)	10.4	10.4	10.5	10.8
	Std Dev(CAL)	0.5	0.4	0.3	0.4
	N(CAL)	10	10	10	10
Chloride (mmol/L)	Mean(CL)	107	106	105	101 [a]
	Std Dev(CL)	1	1	2	8
	N(CL)	10	10	10	10
Creatinine (mg/dl)	Mean(CREA)	0.68	0.80	0.77	0.84 [a]
	Std Dev(CREA)	0.06	0.03	0.06	0.06
	N(CREA)	10	10	10	10
Gamma-Glutamyl Transferase (U/L)	Mean(GGT)	1.4	1.4	1.0	1.4
	Std Dev(GGT)	0.8	0.8	0.5	0.5
	N(GGT)	10	10	10	10
Glucose (mg/dl)	Mean(GLU)	210	235	213	216
	Std Dev(GLU)	19	35	43	27
	N(GLU)	10	10	10	10

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 17. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Interim Clinical Chemistry Results for Male Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Potassium (mmol/L)	Mean(K)	5.4	6.0	5.7	5.6
	Std Dev(K)	0.5	0.8	1.0	0.9
	N(K)	10	10	10	10
Sodium (mmol/L)	Mean(NA)	151	151	151	150 [a]
	Std Dev(NA)	1	1	1	10
	N(NA)	10	10	10	10
Phosphorus (mg/dl)	Mean(PHOS)	9.7	9.9	9.8	10.1
	Std Dev(PHOS)	0.9	1.2	0.8	0.7
	N(PHOS)	10	10	10	10
Total Protein (g/dl)	Mean(TPRO)	6.5	6.6	6.7	7.2 [a]
	Std Dev(TPRO)	0.3	0.2	0.3	0.2
	N(TPRO)	10	10	10	10

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 18.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Interim Clinical Chemistry Results for Female Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Albumin (g/dl)	Mean(ALB)	3.9	4.0	3.8	4.0
	Std Dev(ALB)	0.2	0.1	0.2	0.2
	N(ALB)	8	10	10	10
Alkaline Phosphatase (U/L)	Mean(ALP)	240	258	219	231
	Std Dev(ALP)	34	30	26	18
	N(ALP)	8	10	10	10
Alanine Aminotransferase (U/L)	Mean(ALT)	49.6	41.0	43.8	45.2
	Std Dev(ALT)	21.7	11.0	9.1	7.7
	N(ALT)	8	10	10	10
Aspartate Aminotransferase (U/L)	Mean(AST)	112.5	121.2	134.2	115.9
	Std Dev(AST)	40.2	38.4	30.4	41.8
	N(AST)	8	10	10	10
Bilirubin (mg/dl)	Mean(BILI)	0.33	0.31	0.27	0.20 [a]
	Std Dev(BILI)	0.09	0.06	0.08	0.05
	N(BILI)	8	10	10	10
Blood Urea Nitrogen (mg/dl)	Mean(BUN)	22.2	23.4	19.6	22.1
	Std Dev(BUN)	3.3	1.7	1.5	1.9
	N(BUN)	8	10	10	10
Calcium (mg/dl)	Mean(CAL)	11.0	11.0	10.8	10.9
	Std Dev(CAL)	0.7	0.4	0.5	0.4
	N(CAL)	8	10	10	10
Chloride (mmol/L)	Mean(CL)	108	108	108	108
	Std Dev(CL)	1	2	1	2
	N(CL)	8	10	10	10
Creatinine (mg/dl)	Mean(CREA)	0.68	0.64	0.61	0.64
	Std Dev(CREA)	0.06	0.07	0.08	0.06
	N(CREA)	8	10	10	10
Gamma-Glutamyl Transferase (U/L)	Mean(GGT)	0.1	0.3	0.1	0.3
	Std Dev(GGT)	0.4	0.5	0.3	0.5
	N(GGT)	8	10	10	10
Glucose (mg/dl)	Mean(GLU)	245	227	240	234
	Std Dev(GLU)	46	32	40	26
	N(GLU)	8	10	10	10

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 18. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Interim Clinical Chemistry Results for Female Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Potassium (mmol/L)	Mean(K)	5.5	4.9	5.6	5.3
	Std Dev(K)	1.5	1.0	1.1	1.0
	N(K)	8	10	10	10
Sodium (mmol/L)	Mean(NA)	150	150	149	151
	Std Dev(NA)	2	1	1	1
	N(NA)	8	10	10	10
Phosphorus (mg/dl)	Mean(PHOS)	9.1	8.7	7.7	7.5 [a]
	Std Dev(PHOS)	1.1	0.9	1.3	1.0
	N(PHOS)	8	10	10	10
Total Protein (g/dl)	Mean(TPRO)	6.8	6.8	6.6	6.9
	Std Dev(TPRO)	0.3	0.2	0.1	0.2
	N(TPRO)	8	10	10	10

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 19.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Final Clinical Chemistry Results for Male Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Albumin (g/dl)	Mean(ALB)	3.5	3.5	3.6	3.8
	Std Dev(ALB)	0.2	0.3	0.1	0.2
	N(ALB)	11	11	10	11
Alkaline Phosphatase (U/L)	Mean(ALP)	174	193	197	167
	Std Dev(ALP)	29	27	19	16
	N(ALP)	11	11	10	11
Alanine Aminotransferase (U/L)	Mean(ALT)	62.4	55.5	64.4	60.6
	Std Dev(ALT)	14.8	13.1	10.2	12.3
	N(ALT)	11	11	10	11
Aspartate Aminotransferase (U/L)	Mean(AST)	122.5	119.2	117.2	108.8
	Std Dev(AST)	23.9	21.0	18.7	20.7
	N(AST)	11	11	10	11
Bilirubin (mg/dl)	Mean(BILI)	0.23	0.25	0.27	0.29
	Std Dev(BILI)	0.05	0.05	0.08	0.05
	N(BILI)	11	11	10	11
Blood Urea Nitrogen (mg/dl)	Mean(BUN)	19.4	18.9	20.9	20.8
	Std Dev(BUN)	3.1	3.1	3.3	2.5
	N(BUN)	11	11	10	11
Calcium (mg/dl)	Mean(CAL)	11.2	11.3	11.4	11.6
	Std Dev(CAL)	0.3	0.4	0.4	0.5
	N(CAL)	11	11	10	11
Chloride (mmol/L)	Mean(CL)	105	104	104[a]	102[a]
	Std Dev(CL)	1	1	1	2
	N(CL)	11	11	10	11
Creatinine (mg/dl)	Mean(CREA)	0.73	0.79	0.78	0.79
	Std Dev(CREA)	0.07	0.10	0.09	0.11
	N(CREA)	11	11	10	11
Gamma-Glutamyl Transferase (U/L)	Mean(GGT)	0.6	0.6	0.7	1.1
	Std Dev(GGT)	0.5	0.5	0.5	0.3
	N(GGT)	11	11	10	11
Glucose (mg/dl)	Mean(GLU)	259	267	254	272
	Std Dev(GLU)	30	41	42	59
	N(GLU)	11	11	10	11

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 19. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Final Clinical Chemistry Results for Male Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Potassium (mmol/L)	Mean(K)	6.7	6.8	6.2	6.5
	Std Dev(K)	0.8	0.8	0.6	1.1
	N(K)	11	11	10	11
Sodium (mmol/L)	Mean(NA)	150	150	152	151
	Std Dev(NA)	1	1	1	2
	N(NA)	11	11	10	11
Phosphorus (mg/dl)	Mean(PHOS)	8.2	8.7	9.0	9.0
	Std Dev(PHOS)	1.1	1.0	1.0	0.9
	N(PHOS)	11	11	10	11
Total Protein (g/dl)	Mean(TPRO)	6.4	6.4	6.6	7.0 [a]
	Std Dev(TPRO)	0.4	0.3	0.2	0.3
	N(TPRO)	11	11	10	11

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 20.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Final Clinical Chemistry Results for Female Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Albumin (g/dl)	Mean(ALB)	3.8	4.0	3.8	3.9
	Std Dev(ALB)	0.3	0.2	0.3	0.2
	N(ALB)	10	11	11	11
Alkaline Phosphatase (U/L)	Mean(ALP)	173	163	160	158
	Std Dev(ALP)	31	24	29	26
	N(ALP)	10	11	11	11
Alanine Aminotransferase (U/L)	Mean(ALT)	57.8	60.6	66.4	52
	Std Dev(ALT)	8.5	15.1	16.6	14.8
	N(ALT)	10	11	11	11
Aspartate Aminotransferase (U/L)	Mean(AST)	130.1	124.4	131.4	108.4
	Std Dev(AST)	24.4	27.3	40.0	14.6
	N(AST)	10	11	11	11
Bilirubin (mg/dl)	Mean(BILI)	0.33	0.22[a]	0.23	0.25
	Std Dev(BILI)	0.08	0.11	0.06	0.08
	N(BILI)	10	11	11	11
Blood Urea Nitrogen (mg/dl)	Mean(BUN)	19.4	19.0	18.4	19.6
	Std Dev(BUN)	2.5	1.4	2.1	2.7
	N(BUN)	10	11	11	11
Calcium (mg/dl)	Mean(CAL)	11.8	11.6	11.6	11.8
	Std Dev(CAL)	0.8	0.4	0.6	0.6
	N(CAL)	10	11	11	11
Chloride (mmol/L)	Mean(CL)	107	107	106	106
	Std Dev(CL)	1	2	1	1
	N(CL)	10	11	11	11
Creatinine (mg/dl)	Mean(CREA)	0.75	0.70	0.71	0.71
	Std Dev(CREA)	0.07	0.07	0.08	0.09
	N(CREA)	10	11	11	11
Gamma-Glutamyl Transferase (U/L)	Mean(GGT)	1.2	1.1	1.2	1.1
	Std Dev(GGT)	0.6	0.3	0.4	0.3
	N(GGT)	10	11	11	11

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Table 20. (cont'd).
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Final Clinical Chemistry Results for Female Rats

		Exposure Group (ppm)			
Endpoint	Statistic	Control	500	1750	5000
Glucose (mg/dl)	Mean(GLU)	258	215	226	230
	Std Dev(GLU)	61	33	37	50
	N(GLU)	10	11	11	11
Potassium (mmol/L)	Mean(K)	6.6	5.9	6.3	6.5
	Std Dev(K)	1.1	1.2	1.5	1.4
	N(K)	10	11	11	11
Sodium (mmol/L)	Mean(NA)	152	152	152	151
	Std Dev(NA)	2	1	1	2
	N(NA)	10	11	11	11
Phosphorus (mg/dl)	Mean(PHOS)	8.6	9.2	9.1	9.4
	Std Dev(PHOS)	1.8	1.8	1.5	1.4
	N(PHOS)	10	11	11	11
Total Protein (g/dl)	Mean(TPRO)	6.8	6.9	6.7	6.9
	Std Dev(TPRO)	0.5	0.3	0.4	0.4
	N(TPRO)	10	11	11	11

[a]indicates statistically significant difference between exposure group and control detected by Dunnett's ANOVA, $p < 0.05$.

Gross Necropsy Evaluations

Gross lesions and organ weight data are reported for individual animals in Appendix 11. All gross lesions encountered at necropsy are summarized in Tables 21 and 22 for male and female rats, respectively. Weights of selected organs were taken at necropsy for both male and female rats at all dose groups. Mean organ weights are summarized in Tables 23 and 24 for male and female rats, respectively. For male rats significant increases in the mean kidney and liver weights were noted for the 1750 and 5000 ppm exposure groups. A significant increase in the mean adrenal weights was noted for male rats in the 5000 ppm exposure group.

For female rats significant increases in the mean adrenal and liver weights were noted for the 5000 ppm exposure groups. A significant increase in the mean kidney weights were noted for the 1750 and 5000 ppm exposure groups. A significant increase in the mean heart weights were noted for the 500 and 5000 ppm exposure groups.

Table 21.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Gross Observations for Male Rats

Tissue/Gross Observation	Exposure Group[b]:	1	2	3	4
	No. In Group:	11	11	11	11
Epididymis/					
Growth(s)/mass(es)		0	0	1	0
Animals With Observations For This Category:		0	0	1	0
Eyes & Optic Nerve					
Discoloration: Red		0	1	0	0
Animals With Observations For This Category:		0	1	0	0
Harderian Gland/					
Growth(s)/Mass(es)		1	0	0	1
Animals With Observations For This Category:		1	0	0	1
Kidney					
Cyst		0	0	1	0
Animals With Observations For This Category		0	0	1	0
Seminal Ves./CG					
Surface Texture: Abnormal		2	1	1	2
Mineralization		0	1	0	2
Animals With Observations For This Category		2	2	1	4
Urinary Bladder					
Surface Texture: Abnormal		1	0	0	0
Animals With Observations For This Category		1	0	0	0

[a]Number of rats with an observation in the respective category any time during the study.
[b]1 = control, 2 = 500 ppm, 3 = 1750 ppm, 4 = 5000 ppm

Table 22.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Gross Observations for Female Rats

Tissue/Gross Observation	Exposure Group[b]:	1	2	3	4
	No. In Group:	10	11	11	11
Brain					
Discoloration: Red		0	1	0	0
Animals With Observations For This Category		0	1	0	0
Cecum					
Discoloration: Red		1	0	0	1
Animals With Observations For This Category		1	0	0	1
Eyes & Optic Nerve					
Discoloration: Red		1	1	0	0
Animals With Observations For This Category		1	1	0	0
Pituitary Gland					
Large/Enlarged		0	0	0	1
Animals With Observations For This Category		0	0	0	1
Uterus					
Contents: Clear Fluid		1	1	1	0
Animals With Observations For This Category		1	1	1	0
Gross Lesion					
Body Fat: Abnormal		1	0	0	0
Animals With Observations For This Category		1	0	0	0

[a]Number of rats with an observation in the respective category any time during the study.
[b]1 = control, 2 = 500 ppm, 3 = 1750 ppm, 4 = 5000 ppm

Table 23.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Mean Organ Weights for Male Rats[a]

		Exposure Group (ppm)			
Organ	Statistic	Control	500	1750	5000
Adrenals	Mean	0.035	0.039	0.038	0.047[b]
	Std Dev	0.005	0.009	0.007	0.007
	N	11	11	11	11
Brain	Mean	1.871	1.846	1.835	1.825
	Std Dev	0.054	0.073	0.094	0.066
	N	11	11	11	11
Heart	Mean	0.804	0.788	0.820	0.830
	Std Dev	0.066	0.067	0.048	0.100
	N	11	11	11	11
Kidneys	Mean	1.733	1.850	1.903[b]	2.067[b]
	Std Dev	0.155	0.137	0.100	0.124
	N	11	11	11	11
Liver	Mean	8.857	9.377	10.118[b]	11.730[b]
	Std Dev	1.189	0.744	0.488	0.677
	N	11	11	11	11
Lungs	Mean	1.199	1.321	1.279	1.329
	Std Dev	0.071	0.159	0.141	0.187
	N	11	11	11	11
Spleen	Mean	0.579	0.611	0.597	0.606
	Std Dev	0.058	0.058	0.030	0.050
	N	11	11	11	11
Testes	Mean	2.816	2.780	2.834	2.824
	Std Dev	0.172	0.145	0.142	0.225
	N	11	11	11	11

[a]Organ weight data expressed in grams
[b]Different from control by Dunnett's test at $p < 0.05$

Table 24.
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Mean Organ Weights for Female Rats[a]

		Exposure Group (ppm)			
Organ	Statistic	Control	500	1750	5000
Adrenals	Mean	0.045	0.048	0.048	0.053[b]
	Std Dev	0.004	0.004	0.007	0.005
	N	10	11	11	11
Brain	Mean	1.679	1.688	1.639	1.668
	Std Dev	0.051	0.066	0.071	0.041
	N	10	11	11	11
Heart	Mean	0.495	0.545[b]	0.532	0.556[b]
	Std Dev	0.034	0.037	0.040	0.032
	N	10	11	11	11
Kidneys	Mean	1.077	1.125	1.208[b]	1.306[b]
	Std Dev	0.069	0.048	0.076	0.055
	N	10	11	11	11
Liver	Mean	5.190	5.295	5.637	6.529[b]
	Std Dev	0.440	0.397	0.519	0.519
	N	10	11	11	11
Lungs	Mean	0.960	1.001	0.977	1.002
	Std Dev	0.081	0.081	0.133	0.099
	N	10	11	11	11
Ovaries	Mean	0.085	0.095	0.088	0.090
	Std Dev	0.022	0.016	0.012	0.019
	N	10	11	11	11
Spleen	Mean	0.424	0.413	0.438	0.424
	Std Dev	0.036	0.025	0.052	0.02
	N	10	11	11	11

[a] Organ weight data expressed in grams
[b] Different from control by Dunnett's test at $p < 0.05$

Microscopic Evaluations

All organs taken at necropsy from high dose and control male rats were examined microscopically. Kidney, liver, lung, epididymis and testes from animals exposed to the

intermediate concentrations were examined. A summary of the microscopic findings for these tissues is presented in Table 25. No ETBE-exposure related lesions noted for livers, epididymis, or lung of male rats. The kidney had an increased incidence of nephropathy only in male rats exposed to ETBE. Nephropathy was characterized by an increased incidence in regenerative foci which was observed at all three ETBE exposure concentrations compared with controls. As noted below nephropathy was not noted in female rats similarly exposed to ETBE. The mechanism underlying this nephropathy was characterized and further described in special studies designed to determine the time course for cell replication and protein droplet accumulation. These studies are described in detail below (Renal Cell Proliferation and Special Stains of Kidneys).

Exposure to 1750 and 5000 ppm ETBE resulted in an increase in the incidence of degeneration in the seminiferous tubules of the testicles of male rats (Table 26). Degeneration was quantified by counting the number of affected tubules out of 100 total tubules in a cross section of a testes from each rat. The percent of tubules with debris in the lumen was not altered with ETBE exposure. Examination of the testes indicated that there was no effect of ETBE exposure on the percent of seminiferous tubules within various developmental stages (Table 27). Seminiferous tubules were staged based on groups described in Hess, 1990 in order to detect a potential maturation defect. In those testes with degenerated tubules, the majority of tubules with degenerated developing spermatids appeared to be in stages IX-XIII. The stage I-VIII seminiferous tubules from rats exposed to 1750 or 5000 ppm ETBE had fewer spermatocytes within tubule cross sections compared with control animals. The range of severity of the degeneration within affected tubules was greater in the treated animals compared with the controls. There were also greater numbers of affected tubules in ETBE treated animals compared to controls. ETBE did not have an effect on the frequency of stage of sperm development. No treatment effect was seen in testes from rats exposed to 500 ppm ETBE. The impact of the response of the testes to ETBE would best be measured by sperm count, motility assessment, and breeding studies.

No other treatment related effects were seen in any other tissue. Incidence summaries for all tissues and observations for individual animals are presented in the Histopathology Appendix in Appendix 12.

All organs taken at necropsy from high dose and control female rats were examined microscopically. Only the target tissues of femoral bone marrow, kidney, liver and lung were examined from animals exposed to the intermediate concentrations. A summary of the microscopic findings for these tissues is presented in Table 28. The bone marrow had an increased incidence of congestion in the 1750 and 5000 ppm exposure groups (Table 28). Although the bone marrow congestion was associated with treatment, the hematopoietic cell population appeared unaffected. The change identified in peripheral hematology (increased MCV) was not considered clinically significant and it is unlikely that congestion of the bone marrow has any clinical relevance. No other treatment related effects were seen in any other tissue. Histopathology observations for individual animals are presented in the Histopathology Appendix in Appendix 12.

Table 25
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Histopathology Incidence Summary for Selected Tissues in Male Rats[a]

Tissue	Exposure Group (ppm) Control	500	1750	5000
Epididymis				
No. Examined	11	11	11	11
Diagnosis				
None[b]	11	11	10	11
Granuloma	0	0	1	0
Liver				
No. Examined	11	11	11	11
Diagnosis				
None	11	11	11	11
Lungs				
No. Examined	11	11	11	11
Diagnosis				
None	11	11	11	11
Kidneys				
No. Examined	11	11	11	11
Diagnosis				
None	7	1	0	0
Regenerative foci	4	10	11	11
Testes				
No. Examined	11	11	11	11
Diagnosis				
None	0	0	0	1
Degeneration	11	11	11	10
Sloughed epithelium	7	3	3	7

[a]Number depicts number examined or number having lesion
[b]None - indicates no diagnosis upon examination

Table 26
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Percent of Seminiferous Tubules with Spermatocyte Degeneration and Lumenal Debris[a]

Group	Degeneration	Lumenal Debris
Control	2 ± 1	2 ± 2
	(1-4)[b]	
500 ppm	2 ± 2	1 ± 2
	(1-7)	
1750 ppm	8 ± 4	3 ± 8
	(3-16)	
5000 ppm	13 ± 11	1 ± 1
	(0-39)	

[a]Data are mean percent ± standard deviation
[b]range

Table 27
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Summary of Percent of Seminiferous Tubules in Specified Developmental Stages[a]

Group	Stages I–V	Stages VI–VIII	Stages IX–XIII	Stage XIV
Control	36 ± 5	30 ± 6	29 ± 7	5 ± 2
500 ppm	32 ± 5	27 ± 4	37 ± 3	5 ± 2
1750 ppm	34 ± 5	31 ± 3	30 ± 5	5 ± 2
5000 ppm	36 ± 6	29 ± 6	31 ± 6	5 ± 2

[a]Data are mean percent ± standard deviation

Table 28
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Histopathology Incidence Summary for Selected Tissues in Female Rats[a]

	Exposure Group (ppm)			
Tissue	Control	500	1750	5000
Femur				
No. Examined	10	11	11	11
Diagnosis				
None	5	8	4	0
Bone marrow congestion	0	0	5	11
Bone marrow necrosis	5	3	3	2
Kidneys				
No. Examined	10	11	11	11
Diagnosis				
None	10	11	11	11
Liver				
No. Examined	10	11	11	11
Diagnosis				
None	9	11	11	10
Inflammation	1	0	0	1
Lungs				
No. Examined	10	11	11	11
Diagnosis				
None	10	11	11	11

[a]Number depicts number examined or number having lesion
[b]None - indicates no diagnosis upon examination

Renal Cell Proliferation and Special Stains of Kidneys

The labeling index (LI) of the proximal tubules of the kidney was increased in male rats after one week and 4 weeks of treatment in the 5000 ppm exposure group only. In contrast, the labeling index was increased in the proximal tubules of all ETBE exposed male rats at 13 weeks (Table 29). The variation in LI values of control rats at the 1, 4, and 13 week time points is associated with growth and maturation. At the early time points the kidneys are still growing. The LI at the end of the study (13 weeks) is typical of a mature

kidney. Data for individual animals and group statistics (means and 95% confidence intervals) are in Appendix 13.

There were time and ETBE concentration-dependent increases in nephropathy in the male rats as determined by the increase in mean number of regenerative foci (Table 30). The Mallory-Heidenhain stain showed increased protein droplet accumulation in the renal proximal tubule for ETBE exposed male rats (Table 31). There was α2u-globulin immunoreactivity present in the protein droplets of the renal proximal tubule epithelium of male rat kidney from all exposure groups. All parameters, nephropathy, regenerative foci, protein droplet accumulation, and α2u-globulin immunoreactivity, correlate well with the proposed mechanism of α2u-globulin mediated nephrotoxicity. There is a concentration-dependent increase in protein droplet accumulation and these protein droplets are immunoreactive for α2u-globulin. The α2u-globulin containing droplet accumulation is directly related to the treatment-dependent increases in nephropathy and LI.

Table 29
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Mean Labeling Indices in Kidneys from Male Rats

Exposure Group	1 Week	4 Weeks	13 Weeks
Control	3.52	3.27	0.91
500 ppm	4.90	4.04	2.16[a]
1750 ppm	4.34	2.80	3.40[b]
5000 ppm	7.12[a]	8.98[b]	2.47[b]

[a] Different from control at $p<.01$
b Different from control at $p<.001$

Table 30
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Mean Number of Regenerative Foci in Kidneys from Male Rats

Exposure Group	1 Week	4 Weeks	13 Weeks
Control	0	0	2
500 ppm	0	0	11
1750 ppm	0	2	17
5000 ppm	0	4	34

Table 31
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Mean Droplet Severity Grade (Mallorys Heidenhain) in Kidneys from Male Rats[a]

Exposure Group	1 Week	4 Weeks	13 Weeks
Control	1.2	1.8	1.8
500 ppm	3.4	2.6	3.0
1750 ppm	4.0	3.4	3.2
5000 ppm	4.6	3.8	3.8

[a]Mallory Heidenhain scoring grades - 1 = minimal; 2 = <10% cortex involved; 3 = 10–25% cortex involved; 4 = 25–50% cortex involved; 5 = >50% cortex involved

The kidneys had a treatment- but not concentration-dependent increase in cell proliferation of the proximal tubules in female rats exposed for one week (Table 32). This increased cell proliferation was present only in the 5000 ppm group after 4 weeks of exposure. By 13 weeks of exposure the labeling index was not different among control and treatment groups. There was no microscopic evidence of treatment related increases in nephropathy in the female rats. The Mallory-Heidenhain stain was negative for renal proximal tubule protein droplet accumulation for all exposure groups. There was no α2u-globulin immunoreactivity in the female rat kidney from any exposure group.

Table 32
Ethyl Tertiary Butyl Ether (ETBE): Ninety-Day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluations in Fischer 344 Rats
Mean Labeling Indices in Kidneys from Female Rats

Exposure Group	1 Week	4 Weeks	13 Weeks
Control	2.65	1.38	0.59
500 ppm	4.25[a]	1.42	1.02
1750 ppm	4.97[a]	1.59	0.97
5000 ppm	3.96[a]	1.81[a]	0.87

[a] Different from control at $p<.05$

DISCUSSION

The objective of this study was to determine the toxic effects, if any, in rats resulting from repeated exposure to ETBE vapor. The study consisted of 3 exposure groups and an air-exposed control group. Within each exposure group was a core group (11 rats/sex/group), a neurotoxicology subgroup (12 rats/sex/group), an interim clinical pathology subgroup (10 rats/sex/group) and a cell replication subgroup (15 rats/sex/group). Rats in the core group and the neurotoxicology subgroup were exposed by inhalation for 6 hours/day, 5 days/week, over a 90 day period to target concentrations 0, 500, 1750, or 5000 ppm of ethyl tertiary butyl ether (ETBE) vapor. Body weights and clinical signs were assessed at intervals throughout the study. A functional observational battery (FOB) was performed on rats in the neurotoxicology subgroup prior to exposure, after one exposure, and after 6, 10, 20, 42 and 65 exposures. Motor activity was assessed prior to exposure, and after 20, 42, and 65 exposures. Cell replication in kidneys was evaluated on 5 rats/sex/group from the cell replication subgroup during 1, 4, and 13 weeks of exposure. A subset of 6 rats assigned to the neurotoxicology subgroup were evaluated for evidence of neuropathology at the end of the exposures. Hematology and clinical chemistry were evaluated after at least 30 exposures in the interim clinical pathology subgroup of rats and at termination of the exposure period in the core group. Ophthalmologic evaluations were performed pretest and after the sixty-fifth ETBE exposure.

Average ETBE exposure concentrations were: 505, 1748, and 4971 ppm of ETBE. Two female rats in the control group died during the study. The only noteworthy clinical

finding was ataxia which was sometimes observed in male rats in the 5000 ppm exposure group during mortality checks made following the daily exposure during the duration of the study. Subtle and inconsistent changes in FOB and motor activity were noted throughout the study. However, none demonstrated a dose-response relationship or a consistent pattern of neurologic dysfunction. Male and female rats in the 1750 and 5000 ppm group had a 25% decrease in body weight gain compared with controls during the first week of the study suggesting a transitory adjustment to ETBE exposure. By study termination, the final body weights were either similar to controls (males) or significantly increased (females in the 5000 ppm group) relative to controls. The only significant differences in hematology parameters for both the 6 and 13 week time points were an increase in platelets and MCHC in male rats and an increased MCV in females exposed to 5000 ppm. The relationship of these small changes to ETBE is unclear. ETBE-associated changes in clinical chemistry parameters in male rats consisted of changes in chloride and total protein at the interim and final time points. No consistent alterations were seen in females at these two time points. No changes were considered to be indicative of a disease process. Statistically significant increases in organ weights for liver, kidney, adrenal gland and heart (female only) were noted in male and female rats compared with controls. An increased number of regenerative foci were noted in the kidneys of male rats exposed to all 3 concentrations of ETBE compared with controls. An increased incidence of degenerative tubules was observed in the testicles of male rats exposed to 1750 and 5000 ppm ETBE. No treatment effect was seen at 500 ppm ETBE. Results of the cell proliferation evaluations indicated that kidneys of male rats exhibited time and concentration dependent increases in the labeling index of the proximal tubule cells. An increase in protein droplet accumulation and evidence of α2u-globulin immunoreactivity was noted in these animals. No evidence of treatment related nephropathy, protein droplet accumulation or α2u-globulin immunoreactivity was observed in female rats. An increased incidence of congestion was noted in bone marrow of female rats exposed to 1750 and 5000 ppm ETBE.

These findings suggest a mild toxicity in rats in the 1750 and 5000 ppm exposure groups. ETBE does not appear to be a neurotoxicant apart from the solvent related ataxia observed immediately after exposure. Renal lesions, increased cell proliferation, and presence of α2u-globulin in kidney cells of male rats only is consistent with the male rat

specific syndrome of α2u nephropathy. The lesions noted in testes of male rats suggest a no-observable-adverse-effect-level of 500 ppm for repeated exposure of ETBE in rats.

Detailed examination of the kidneys was based on potential structure-activity relationships of ETBE with methyl tertiary butyl ether (MTBE). It was hypothesized that like MTBE, ETBE might result in male rat kidney nephropathy. Since the α2u-globulin syndrome is a likely cause of this nephropathy, the hallmarks of this syndrome, protein droplet accumulation, increased cell replication, and immunoreactivity with α2u-globulin were evaluated in parallel with the toxicity and histopathology evaluations. As indicated above, the results of this study implicate α2u-globulin in the development of protein droplet accumulation, increased cell replication, and presence of regenerative foci in the kidneys of male rats exposed to ETBE. The absence of these same lesions in kidneys of female rats similarly exposed to ETBE further implicates this syndrome in the development of kidney toxicity and suggests that this endpoint may be of no relevance for humans potentially exposed to ETBE vapors.

Structure activity relationships also hold for other endpoints examined. ETBE, like MTBE, does not appear to be a neurotoxicant under the exposure conditions of this study. Ataxia was sometimes noted in male rats exposed to the high concentration immediately after exposure but this effect was transient, most likely a generalized solvent effect. Like MTBE, a mild transient depression in body weight gain was noted in the high and mid exposure groups and slight hematologic effects were observed at both the interim and final time points. Clinical chemistry parameters, similar to MTBE, were unremarkable. Like MTBE, increases in liver and adrenal weights were noted at the final necropsy. There were no exposure-related microscopic changes in adrenal weight that could account for the observed change in weight as was the case with MTBE.

In contrast to MTBE, bone marrow congestion was observed for female rats exposed to 5000 ppm. This lesion was not noted in male rats exposed to ETBE and thus its relevance to MTBE is unclear, especially since it did not correlate with any change in peripheral hematology. An increased incidence in the percent of degenerated tubules was noted in the testes of male rats. These lesions occurred in the 1750 and 5000 ppm exposure groups and suggest a no-observable-adverse-effect level of 500 ppm for repeated exposure of ETBE in rats.

REFERENCES

Hess RA (1990) Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. Biology of Reproduction 43:525-542

Institute of Laboratory Animal Resources Commission on Life Sciences (1996) Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, DC

RETENTION OF RECORDS

The final report, tissue specimens, nonperishable slides and raw data will be retained in the archives of the Chemical Industry Institute of Toxicology.

REVIEW AND APPROVAL

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Data Tables are excluded
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