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SUBCHRONIC STUDIES OF CHLOROTRIFLUOROETHYLENE

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A single application of CTFE (3.1 oil) to intact rabbit skin produced no signs of irritation, however, continued skin contact did produce a mild allergic response in guinea pigs. The fluid produced mild conjunctival redness in rabbit eyes one hour following application but this was resolved by 24 hours. It can be assumed that the fluid would not be irritating to human skin but would be irritating upon accidental eye contact. Washing the eyes immediately after contact does not preclude the transient irritating effects.

Repeated inhalation of CTFE vapors and aerosol, 6 hrs/d, 5 d/wk, 90 d resulted in treatment-related depression of body weight gains throughout the study, particularly in the male rat groups. The depression in body weight gain of the treated male rats was greatest during the 22 - 71 day period. Alkaline phosphatase, AST, and ALT values examined at the conclusion of the study indicated a treatment-response effect in the male test rats but not in the female test rats. Relative kidney and liver weights of test male and female rats were (over)

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19. significantly elevated when compared to the respective control groups. The primary target organ of CTFE was the liver. Gross liver hypertrophy and microscopic hepatocytomegaly were the principal manifestations of CTFE-induced hepatotoxicity. Dose-response data from these studies indicated the need for additional exposures at lower concentrations to identify a no-effect level.

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted in the Toxic Hazards Research Unit, NSI Technology Services Corporation – Environmental Sciences. This document serves as a final report on selected toxicity studies of chlorotrifluoroethylene (CTFE). The research described in this report began in September 1987 and was completed in September 1988. It was performed under U.S. Air Force Contract No. F33615-85-C-0532. Melvin E. Andersen, Ph.D., served as a Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory.

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

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ABBREVIATIONS

ACA	Automated Chemistry Analyzer
CTFE	Chlorotrifluoroethylene
dL	Deciliter
ECD	Electron capture detector
F-344	Fischer 344 (rats)
fL	Femtoliter
g	Gram
GC	Gas chromatography
h	Hour
IR	Infrared
kg	Kilogram
L	Liter
µg	Microgram
µL	Microliter
MCV	Mean corpuscular volume
mg	Milligram
mL	Milliliter
mm	Millimeter
MMAD	Mass median aerodynamic diameter
N	Number
ng	Nanogram
NZW	New Zealand White (rabbits)
p	Probability
PB-PK	Physiologically based pharmacokinetic
PFDA	Perfluorodecanoic acid
RER	Rough endoplasmic reticulum
SEM	Standard error mean
SER	Smooth endoplasmic reticulum
TCD	Thermal conductivity detector
TEM	Transmission electron microscopy
USA	United States Army
U/L	International units / liter

SECTION 1

INTRODUCTION

Chlorotrifluoroethylene (CTFE; 3.1 oil) oligomer is a nonflammable, saturated, and hydrogen-free chlorofluorocarbon oil. It is noncorrosive, has high thermal stability, good lubricity and high dielectic strength. These properties make CTFE promising for potential use in hydraulic fluid systems. Recent dermal and inhalation studies indicated that CTFE has a low degree of toxicity. There were no deaths among rabbits dermally exposed to 2 g CTFE/kg body weight (Gargus, 1983), and there were no deaths among rats exposed for a 4-h period to atmospheres containing saturated-vapor concentrations of CTFE (Coate, 1984; Kinkead et al., 1987).

Following oral and inhalation exposures, CTFE was readily absorbed and free fluoride liberated. Plasma and urine fluoride levels remained elevated for more than one week following oral treatment and for at least 24 h following inhalation exposure. However, analysis of blood and urine following 24-h dermal application on rabbits detected only minimal amounts of CTFE (Kinkead et al., 1987). Histopathologic examination of nerve tissue from hens dosed with CTFE showed no lesions consistent with organophosphate toxicity. The acute toxicity evaluation of CTFE compared favorably with that of other hydraulic fluids tested in this laboratory (Kinkead et al., 1987).

The subchronic inhalation test reported here was conducted to provide information on health hazards likely to arise from repeated inhalation exposures over a limited time. Data were also collected to provide information on acute cutaneous effects of CTFE, on target organs from repeated inhalation exposures, and for use in selecting dose levels for future chronic studies. Blood and tissues were taken to obtain pharmacokinetic data which were used to support the development of a physiologically based pharmacokinetic (PB-PK) model. The PB-PK model for CTFE allows prediction of distribution of CTFE in body tissues at different exposure concentrations and, once fully validated, prediction of the pharmacokinetic behavior in man. The model will then be used for risk assessment and thereby play an important role in cost-benefit discussions on the use of CTFE.

SECTION 2

MATERIALS AND METHODS

ANIMALS

Upon receipt from Charles Rivers Breeding Labs (Kingston, NY), 40 male and 40 female Fischer 344 (F-344) rats, 7 weeks of age, were quality control tested and found to be in acceptable health. The animals were randomized using a standard random number system which assigned animals to groups. They were group housed (2 to 3 per cage) in clear plastic cages with wood chip bedding prior to the study. The rats (10 weeks of age at initial exposure) were individually housed and assigned to specific exposure cage locations during the study. The exposure cages were rotated in a clockwise manner (moving one position) within the 690 L inhalation chambers each exposure day. Water and feed (Purina Formulab #5008) were available *ad libitum* except during the inhalation exposure period when food was removed and when the rats were fasted for 10 h prior to sacrifice. Ambient temperatures were maintained at 21 to 25°C and the light/dark cycle was set at 12-h intervals (light cycle starting at 0700 h).

Male and female New Zealand White (NZW) rabbits weighing between 2 and 3 kg were obtained from Clerco Research Farms (Cincinnati, OH) for use in the dermal ano eye irritation studies. Quality control assessment confirmed the acceptable health of the proposed study animals. The rabbits were housed individually in wire-bottom stainless-steel cages. Water and food (Purina Rabbit Chow #5320 and Carnation Rabbit Chow) were available *ad libitum* and the rabbits were maintained on a 12-h light/dark cycle.

Male Hartley albino guinea pigs weighing between 300 and 350 g were purchased from Murphy Breeding Labs (Indianapolis, IN) for use in the sensitization study. The animals were shcwn to be in acceptable health during the quality control evaluations. The guinea pigs were housed individually in clear plastic shoebox cages with wood chip bedding. Water and food (Purina Formulab #5015) were available *ad libitum* and the guinea pigs were maintained on a 12-h light/dark cycle.

TEST AGENT

The CTFE sample used in this study was supplied by the U.S. Air Force. Three plastic cans and two gallon bottles containing approximately 14 gallons of hydraulic fluid were received. The labels on the containers identified the sample as shown below:

MLO-87-124

Safetol® 3.1

Hydraulic Fluid

Batch #86-134

10-24-86

P O F3360186M0335

This CTFE oligomer mixture contained 1 0% (vol/vol) of a rust inhibitor additive, neutral barium dinonylnaphthalene sulfonate A second additive was 0 05% (vol/vol) of a proprietary anti-wear compound (composition unknown).

Chemically, halocarbon oils like CTFE are saturated, low molecular weight polymers of CTFE having the general formula $(CF_2CFCl)_n$. They are made using a controlled polymerization technique and are stable; the terminal groups being completely halogenated The products are then separated by vacuum distillation into various fractions, from light oils to waxes

TEST AGENT QUALITY CONTROL

Samples were taken for analyses from each of the five supply containers. An infrared (IR) spectrum of CTFE was generated using a Beckman Acculab 4 (Beckman Instruments, Inc., Fullerton, CA) infrared spectrophotometer In addition, a gas chromatogram (GC) of CTFE in hexane (1 µg/mL), using a Varian 3500 GC (Varian Associates, Palo Alto, CA) equipped with an electron capture detector (ECD), was obtained on each of the five samples No differences were observed in the five samples by either method of analysis **Figure 1** is a typical IR spectrum of the supplied hydraulic fluid

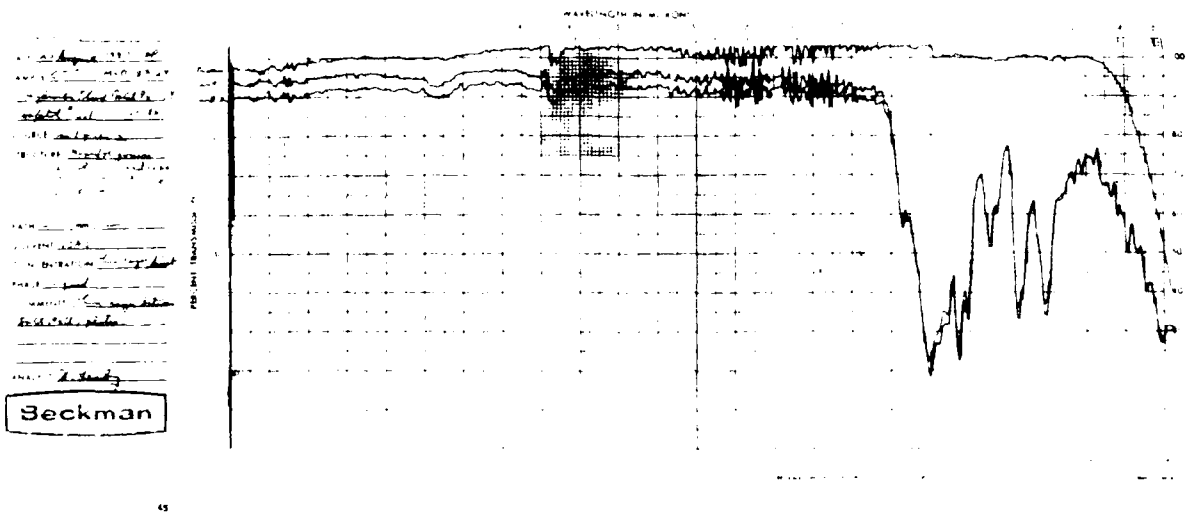

Figure 1. Infrared spectrum of Supply CTFE (MLO 87-124 batch #86-134).

The composition of the hydraulic fluid was verified by mass spectrometry. Electron bombardment mass spectral data were obtained using a Hewlett-Packard 5880 Gas Chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a 12-meter HP-1 column with 0.20 i.d. The detector was a Hewlett-Packard Model 5970 Mass Selective Detector (MSD). Chemical ionization mass spectra were obtained using a Hewlett-Packard 5986 Gas Chromatograph-Mass Spectrometer equipped with a 30-meter HP-1 column. Molecular ions were not obtained from either set of data. Molecular weights were obtained from the interpretation of ion fragment data. The number of chlorines was determined using the naturally occurring isotopic abundances of Cl_{37} to calculate the number of chlorine atoms present.

EYE IRRITATION ASSESSMENT

Nine female NZW rabbits, weighing 2 to 3 kg, were examined with fluorescein stain prior to use to ensure the absence of lesions or injury. A topical anesthetic (Ophthetic, Allergan Pharmaceuticals, Inc., Irvine, CA; Proparacaine HCl 0.5%) was instilled in the eyes, treated and control, of all rabbits approximately 2 min prior to application of the test material. One tenth of a mL of the test material was applied to one eye of each of the nine albino rabbits. The opposite eye was left untreated and served as the control. The treated eye of three rabbits was flushed with lukewarm deionized water for 1 min starting 30 sec after instillation. The treated eyes of the remaining six rabbits were not flushed. Examination for gross signs of eye irritation were made at 1, 24, 48, and 72 h following treatment. Irritation was scored according to the method of Draize (1944) (**Appendix A**), in which the total score for the eye was the sum of the cornea, iris, and conjunctiva scores.

SKIN IRRITATION ASSESSMENT

Six female NZW rabbits were clipped on the back and sides 24 h prior to treatment to allow for recovery of the skin from any abrasion resulting from the clipping. The test agent was applied in the amount of 0.5 mL to a designated patch area and was covered by a 2.5 cm square of surgical gauze two single layers thick. Strips of Elastoplast tape held the gauze patch in place and the entire area was covered with dental dam and secured with Vetrap (3M Corp., Minneapolis, MN) and Elastoplast tape. Patches remained in place for 4 h, then all wrappings were removed and residual test agent wiped from the animals. Test areas were evaluated for irritation using the Draize Scoring System (1944) (**Appendix B**) as a reference standard at 4, 24, 48, and 72 h. Total scores of the four observations for all rabbits were divided by 24 to yield a primary irritation rating which was interpreted using the National Institute for Occupational Safety and Health (NIOSH) skin test rating (**Appendix C**).

SENSITIZATION ASSESSMENT

Ten male guinea pigs were treated with 0.1 mL of the test material on the clipped left flank. The site of the sensitization test was an area just behind the shoulder girdle. The site was clipped with an Oster® animal clipper and depilated with a commercial depilatory (Surgex Hair Remover Cream, Sparta Instrument Corp., Hayward, CA) 4 h prior to treatment. A Vetrap frame with a 1.5 x 1.5 cm opening at the site of the depilated area was affixed to the guinea pig. The test material was topically applied to the test area, covered with gauze, dental dam, and adhesive tape. The animals were treated on a Monday, Wednesday, Friday, and Monday until a total of four sensitizing treatments were applied. In addition to the third sensitizing treatment, 0.2 mL of a 50% aqueous dilution of Freund's adjuvant (Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, MI) was injected intradermally using two or three sites next to the test site on each animal. Following the fourth sensitizing treatment, the animals were rested for two weeks. Both flanks were again clipped and each animal was challenged on one flank with 0.1 mL of the test material. The challenge application was not occluded. The skin response at these sites was recorded at 24 and 48 h after application (scoring method in **Appendix D**). Any animal eliciting a score of two or more at the test solution challenge site for the 48-h scoring was rated a positive responder. If the results of the challenge application were not conclusive, a second challenge application was done 14 days later. The frequency of the reaction is the important statistic in determining sensitization potential (**Appendix E**).

INHALATION TOXICITY

Generation and Analysis

Exposure atmospheres were generated by aerosolization using Collison (BGI, Inc., Waltham, MA) compressed air nebulizers. A single jet nebulizer was used to generate the 0.25 and 0.50 mg CTFE/L concentrations, whereas a three-jet nebulizer was required to generate the 1.0 mg CTFE/L atmosphere. **Figure 2** is a schematic of the generation and introduction system used. Chamber atmospheres produced by this generation system contained both vapor and aerosol fractions at a 90:10 proportion. Aerosol concentrations within the exposure chambers were determined by gravimetric analysis of aerosol collected on glass-fiber filter media. The size distribution of the aerosols was determined using a Lovelace Multijet Cascade Impactor (Intox Products, Albuquerque, NM). The chamber vapor concentrations were monitored by Miran Infrared analyzers (Foxboro, S. Norwalk, CT) at the absorption band at 1200 cm^{-1} (wave number). **Figure 3** is a schematic of the analytical system, including the data acquisition system.

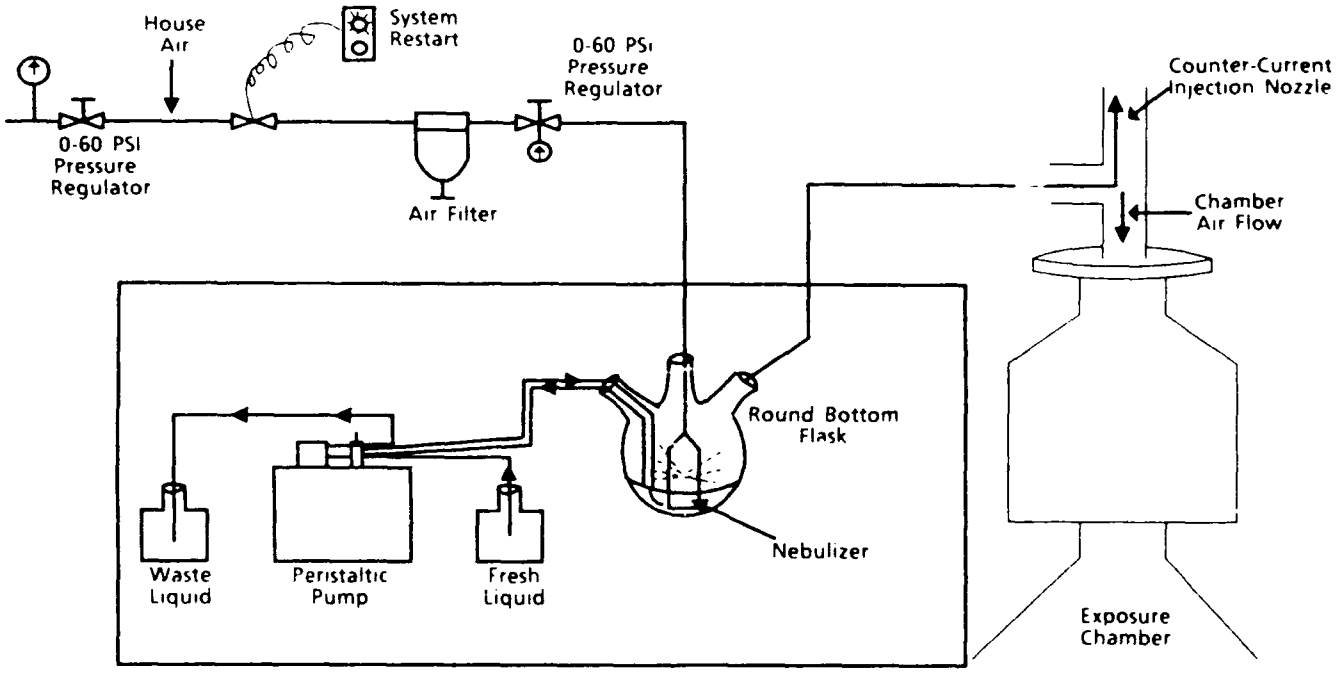

Figure 2. CTFE Vapor/Aerosol Generation System.

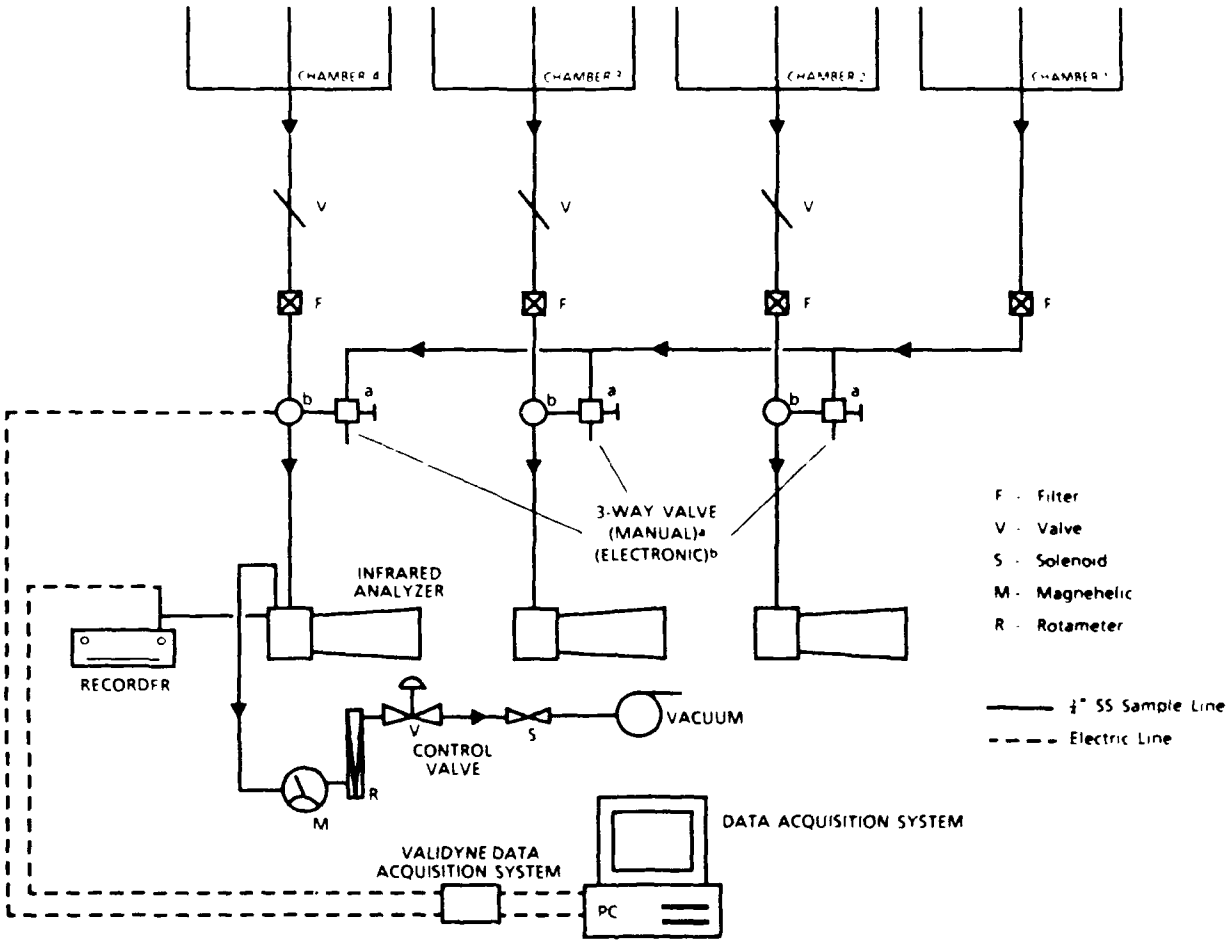

Figure 3. Infrared Analyzer System for CTFE vapor concentrations. Analyzers for chambers 2 and 3 were connected in the same manners as that shown for chamber 4.

Exposure Regimen

Ten male and ten female F-344 rats, age 9 to 11 weeks, were placed in each of four 690 L inhalation chambers and exposed for 6 h/day, 5 days/week, for 13 weeks (65 exposures over a 90-day test period) to either air only, 0.25 mg CTFE/L, 0.50 mg CTFE/L, or 1.00 mg CTFE/L.

Animal Response Assessment

Records were maintained for body weights (one day pre-exposure, weekly during the first four weeks, then biweekly thereafter), signs of toxicity, and mortality. All rats were sacrificed on the day following the final exposure. Euthanasia was accomplished via halothane inhalation overdose. At sacrifice, gross pathology was performed and tissues (**Table 1**) harvested for histopathologic

examination. Wet tissue weights were determined on adrenals, brain, heart, kidneys, liver, lungs, ovaries (females), spleen, testes (males), and thymus. Tissues for histopathologic examination were fixed in 10% neutral buffered formalin, trimmed, and further processed via routine methods for HE-stained paraffin-embedded sections (Luna, 1968).

TABLE 1. TISSUES HARVESTED FROM CONTROL AND CTFE EXPOSED F-344 RATS FOR HISTOPATHOLOGIC EXAMINATION

Gross lesions	Thymus
Brain	Kidneys
Lungs	Adrenals
Trachea	Pancreas
Heart	Ovaries/testes
Liver	Nasal turbinates
Spleen	Uterus (females)
Duodenum	Esophagus
Jejunum	Stomach
Ileum	Colon
Urinary bladder	Rectum
Mandibular lymph nodes	Sternum
Mesenteric lymph nodes	Sciatic nerve
Teeth (incisors)	Skeletal muscle
Bone (femurs, including stifle)	

Additionally, blood was drawn for hematology (**Table 2**) and clinical chemistry (**Table 3**) assays. Erythrocytes were enumerated on a Coulter counter (Coulter Electronics, Hialeah, FL), and sera for clinical chemistry evaluation were assayed on an Automated Chemistry Analyzer (ACA) (DuPont Company, Wilmington, DE). Selected hematological parameters and absolute leukocyte differentials were determined according to established procedures. Sera were processed according to the procedures in the ACA Operations Manual.

TABLE 2. ASSAYS PERFORMED ON WHOLE BLOOD FROM CONTROL AND CTFE EXPOSED F-344 RATS

Hematocrit
Hemoglobin
Red blood cell count
Total and differential leukocyte count

TABLE 3. SERUM CHEMISTRY ASSESSMENTS OF CONTROL AND CTFE EXPOSED F-344 RATS

Creatinine	Alkaline phosphatase
Chloride	Blood urea nitrogen
Calcium	Serum glutamic-pyruvic transaminase
Phosphorus	Serum glutamic-oxalacetic transaminase
Total protein	

Tissue Morphometric Analysis

At sacrifice, following the 90-day inhalation exposure, a 1 mm slice of left liver lobe of the three rats per sex per exposure group was collected for transmission electron microscopy (TEM) examination. The liver slices were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 and minced into 1 mm^3 sections. The minced tissue was postfixed with 2% osmium and then processed into plastic capsules. Sections, one micron in thickness, were cut to identify centrolobular zones. Thin sections were cut from the centrolobular and intermediate zones of liver lobules. These sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100B Transmission Electron Microscope (USA Inc., Peabody, MA) at 60 kV. For each animal, 15 to 30 photographs were taken of three or more hepatocytes, representative of the liver for that animal. For mitochondria, smooth endoplasmic reticulum, and peroxisomes, photographs were scored to derive a semi-quantitative interpretation of the changes between treatment and sexes.

PHARMACOKINETICS AND PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PB-PK) MODELING

Exposure Regimen

Two groups of twelve male F-344 rats, age 9 to 11 weeks, were placed in each of two 690 L inhalation chambers and exposed 6 h, 5 days/week for 13 weeks (65 exposures over a 90-day period) to either air alone or 0.5 mg CTFE/L. A group of four male F-344 rats of the same age group were maintained in an animal facility as an unexposed control group. The animals maintained in the exposure chambers were subdivided into three groups of four rats each, resulting in a total of seven groups of four rats.

Blood, urine, and selected tissue samples were obtained from the control and the CTFE-exposed rats for use in developing a PB-PK model. One group of CTFE-exposed rats was removed after 84 days and sacrificed 48 h later. The other two groups of four CTFE-exposed rats were removed after 90 days; one group was sacrificed immediately, while the second group was held for one-year postexposure. Two groups of rats (four each, previously in control chamber) were exposed for a single 6-h period to 0.5 mg CTFE/L. One group was sacrificed 48 h after exposure, the other group was sacrificed 14 days following exposure. The final group of four rats from the control chamber was not exposed to CTFE.

To evaluate the possible progression or resolution of the liver lesion, each of the four control and four test rats being maintained for one-year postexposure was subjected to a median hepatic lobe hepatectomy 105 and 236 days postexposure. Approximately 1.5 to 2.5 g of liver were aseptically removed and prepared for histopathologic examination by light microscopy and TEM using methods as previously described for animals necropsied immediately postexposure.

Blood samples were taken via lateral tail vein from all rats at intervals following exposure for CTFE analysis. Urine and feces were collected daily from one exposed and one control group for a

period of two weeks following conclusion of the 90-day study for CTFE analysis. Urine collected daily during the two-week period, then once per week for a total of 90 days postexposure, was also analyzed for fluoride concentration. Kidney, lung, liver, testes, brain, and fat were collected from all rats at sacrifice for CTFE analysis.

Analysis of Biologic Samples

Blood for CTFE analysis was collected from the lateral tail vein in a 79.8 µL capillary tube. The blood sample was transferred to a 20 mL scintillation vial containing 5.0 mL hexane. To reduce CTFE vaporization, the transfer occurred below the surface. CTFE was extracted for 2 h using an Evapotec® mixer (Haak Buchler Instruments, Inc., Saddlebrook, NJ).

To avoid analysis interference, no refrigerant or preservative was used during the collection of 24-h urine samples for either CTFE or fluoride analysis. The samples were transferred into hexane following the same procedure as described for blood. CTFE was extracted using an Evapotec® mixer for one h.

Fluoride concentration of the urine was determined by ion specific electrodes using the method of Neefus (1970). Urine weight was converted to volume using a value of 1.06 g/mL for urine density. Fluoride concentration of the urine was determined by diluting the urine by 50% with a total ionic strength buffer (TISUB) (Neefus et al., 1970), and measuring the resulting cell potential with an Orion 701A analyzer (Orion Research, Inc., Cambridge, MA). The instrument was calibrated by measuring the cell potential of standard solutions using the same buffer and dilution conditions.

A weighed sample of feces was collected daily over a two-week postexposure period. The CTFE was extracted overnight in hexane using an Evapotec® mixer. After centrifugation the hexane layer was removed and stored at -70°C until analyzed.

Bone and tooth fluoride concentrations were determined using the method of Singer and Armstrong (1968). The sample (bone or tooth) was cleaned by immersion in a 10% solution of papain until void of extraneous tissue, then rinsed in deionized water and dried overnight at 100°C in a vacuum oven. A mortar and pestle was used to break the bones/teeth into small pieces, which were then ground to a fine powder in a ball mill. The powdered sample was then weighed into a nickel crucible and ashed at 550°C. The ash was dissolved in dilute hydrochloric acid, buffered, brought up to volume, and then assayed by fluoride electrode. The instrument was calibrated using standard fluoride solutions made in the same buffer.

Tissues collected for CTFE analysis were weighed and then maintained in hexane on ice until homogenization. The tissues were homogenized using a Tissue-mizer® (Tekmar, Cincinnati, OH), then mixed overnight on an Evapotec® mixer. Following centrifugation, the hexane layer was stored at -70°C, but allowed to return to room temperature before analysis. Tissue extracts were diluted with hexane to reduce the CTFE in the samples to less than 500 ng/mL for calibration purposes.

Gas Chromatographic Analysis

A Varian® 3700 GC equipped with an ECD was used to analyze the extracts of biologic samples. A Nelson integration system was programmed to handle the GC output. CTFE-hexane solution standards were used to quantitate the ECD signal. The usable standard range was between 1 and 500 ng/mL.

STATISTICAL ANALYSIS

Comparisons of mean body weights were performed using the Multivariate Analysis of Covariance for Repeated Measures Test (Barcikowski, 1983; Dixon, 1985). A two-factorial analysis of variance with multivariate comparisons was used to analyze the hematology, clinical chemistry, and organ weight data. The histopathology data were analyzed using one of the following nonparametric tests: Fisher's Exact Test or, if not valid, Yates' Corrected Chi-square (Zar, 1974). A probability of 0.05 inferred a significant change from controls.

MODEL DEVELOPMENT

A PB-PK model was written in Simusolv (Mitchell and Gauthier Associates, Concord, MA), a Fortran-based continuous simulation language with optimization capabilities and run on a VAX 8530 (Digital Equipment Corp., Maynard, MA). The general form of the model follows that of Ramsey and Andersen (1984). Fat, lung, liver, kidney, brain, testis, rapidly perfused and slowly perfused organ groups were described. The CTFE oligomer separated into two distinctive groups of chromatographic peaks, a lower and a higher molecular weight grouping referred to as Group I and Group II, respectively. The modeling effort to date has focused on Group I, which existed in the chambers only as a vapor. Group II, which existed as a vapor and aerosol, has not yet been modeled. Partition coefficients were estimated from tissue:blood concentration ratios immediately after the 90-day exposure.

All data used in the development of the PB-PK model were derived from animal exposures described under the heading "Pharmacokinetics and Physiologically Based Pharmacokinetic (PB-PK) Modeling." Study animals and exposure conditions are detailed in depth.

SECTION 3

RESULTS

EYE IRRITATION

Nine animals were used in this study. In prescreening the rabbit eyes with fluorescein stain prior to treatment, several showed mild to intense staining of the corneal epithelium. The eyes showing epithelial staining were not used in the study.

Mild conjunctival redness was present in the eyes of six rabbits 1 h after treatment with CTFE. Redness was present in one rabbit at the 48-h evaluation, but no rabbits showed conjunctival redness at the 24- or 72-h evaluation. One rabbit exhibited mild chemosis and another exhibited mild discharge 1 h after treatment. However, neither chemosis nor discharge were present at the 24-, 48-, or 72-h evaluations (**Table 4**).

TABLE 4. PRIMARY EYE IRRITATION RESULTS FOLLOWING CONTACT WITH CTFE[a]

		Examination Time (h Post-treatment)			
Rabbit No.	Washed	1	24	48	72
Y21	No	0	0	0	0
Y23	No	2	0	0	0
Y27	No	2	0	2	0
Y29	No	0	0	0	0
Y33	No	2	0	0	0
Y35	No	4	0	0	0
Y37	Yes	2	0	0	0
Y39	Yes	2	0	0	0
Y67	Yes	2	0	0	0

[a] Draize scale for interpreting the scores can be located in Appendix A.

SKIN IRRITATION

Following 4 h of skin contact with the test compound, the rabbits were unwrapped and the residual test agent was wiped off. At 4 h postexposure no animal exhibited any sign of erythema, edema, or necrosis. All animals received scores of zero for the remaining 24-, 48-, and 72-h evaluation periods. The test compound was classified as nonirritating.

SENSITIZATION

Ten guinea pigs were treated with CTFE during the challege applications. Six animals exhibited very slight erythema (score of 1), and one exhibited slight erythema (score of 2) at the initial

challenge (**Table 5**). A score of 2 or above denotes a positive responder. In questionably sensitized guinea pigs, rechallenge has been shown to be an effective confirmatory step in determining sensitization (Gad et al., 1986), therefore, a rechallenge was performed two weeks following the initial challenge application. The 48-h rechallenge score revealed three animals to be positive responders. Based on the scale for determining sensitization potential (**Appendix E**), the 30% (3 of 10 animals) sensitization rate would classify CTFE a mild sensitizing agent.

TABLE 5. RESPONSE OF GUINEA PIGS TO CHALLENGE DOSE OF CTFE

Guinea Pig No.	48-h Reaction Score	
	Initial Challenge	Rechallenge
170	2	2
171	1[a]	1
173	1	0
175	1	1
176	1	1
177	0	0
178	1	2
179	0	0
182	0	1
183	1	2

[a] A score of less than two is not rated a positive responder.

INHALATION TOXICITY

The quality control procedures for the test material included comparisons between the CTFE sample as received and CTFE vapor-aerosol atmospheres within the exposure chambers. These chromatographic comparisons are provided for the 0.25, 0.50, and 1.0 mg CTFE/L chambers (**Figures 4, 5,** and **6**, respectively). In addition, samples of chamber atmospheres were analyzed by GC to compare the distribution of oligomers in these test atmospheres to the distribution of oligomers in the test agent. These comparisions are provided in **Figures 7** and **8**. These data indicate that the composition of the test material within the exposure chambers did not differ from the CTFE sample as received.

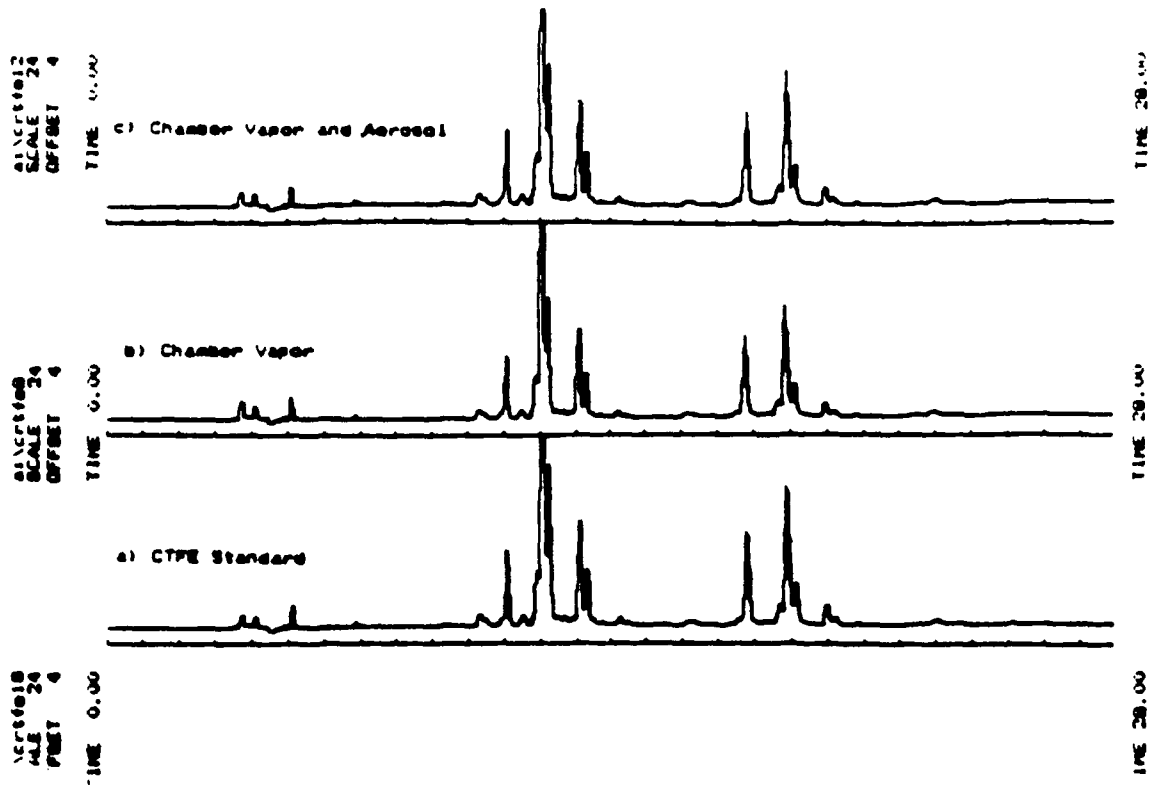

Figure 4. Comparison of GC chromatograms of CTFE a) Stock Supply, b) Chamber Vapor, and c) Chamber Vapor and Aerosol from the 0.25 mg/L Chamber (all samples at 1 µL injection volume of a 1 µg/mL solution in hexane).

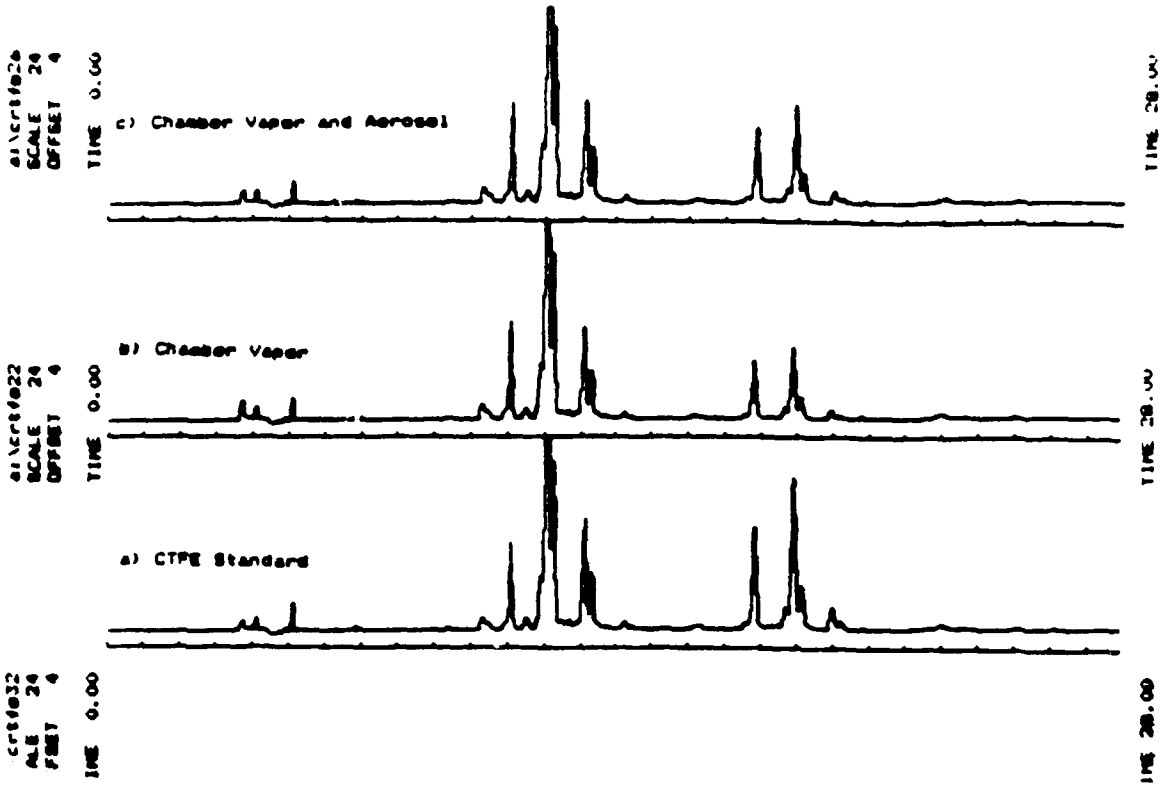

Figure 5. Comparison of GC chromatograms of CTFE a) Stock Supply, b) Chamber Vapor, and c) Chamber Vapor and Aerosol from the 0.50 mg/L Chamber (all samples at 1 µL injection volume of a 1 µg/mL solution in hexane)

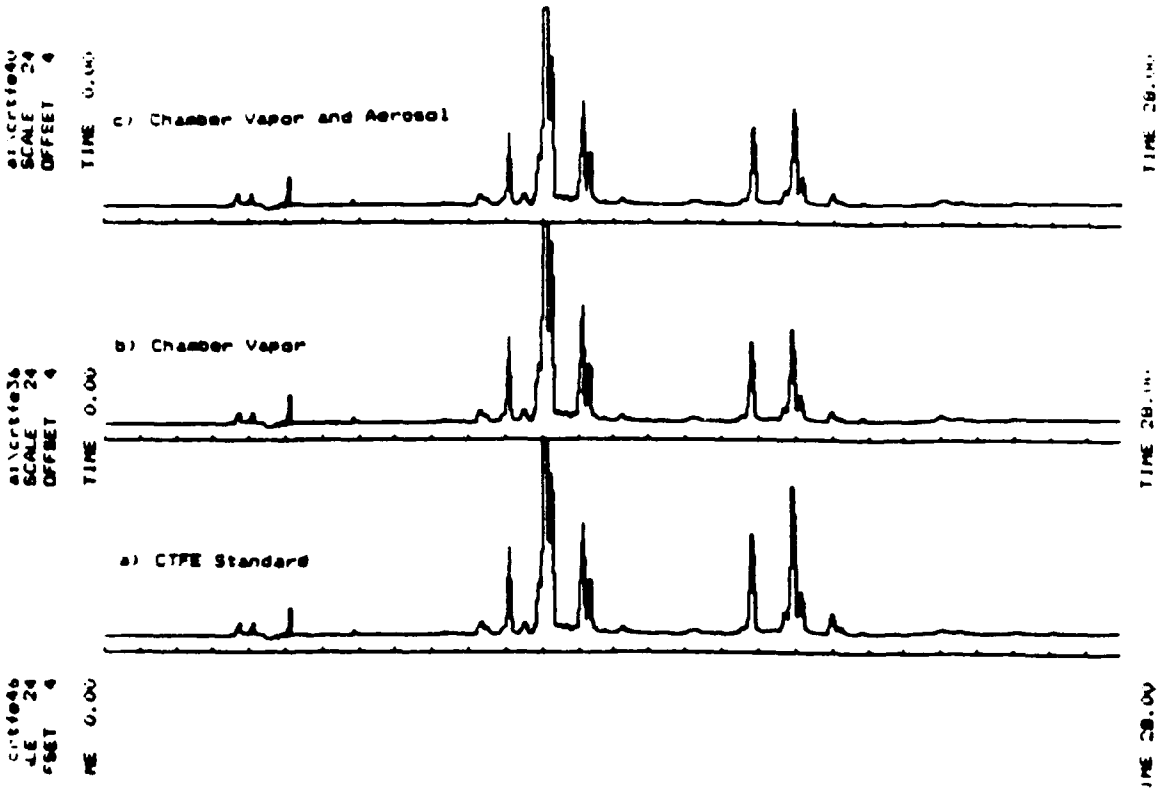

Figure 6. Comparison of GC chromatograms of CTFE a) Stock Supply, b) Chamber Vapor, and c) Chamber Vapor and Aerosol from the 1.0 mg/L Chamber (all samples at 1 µL injection volume of a 1 µg/mL solution in hexane).

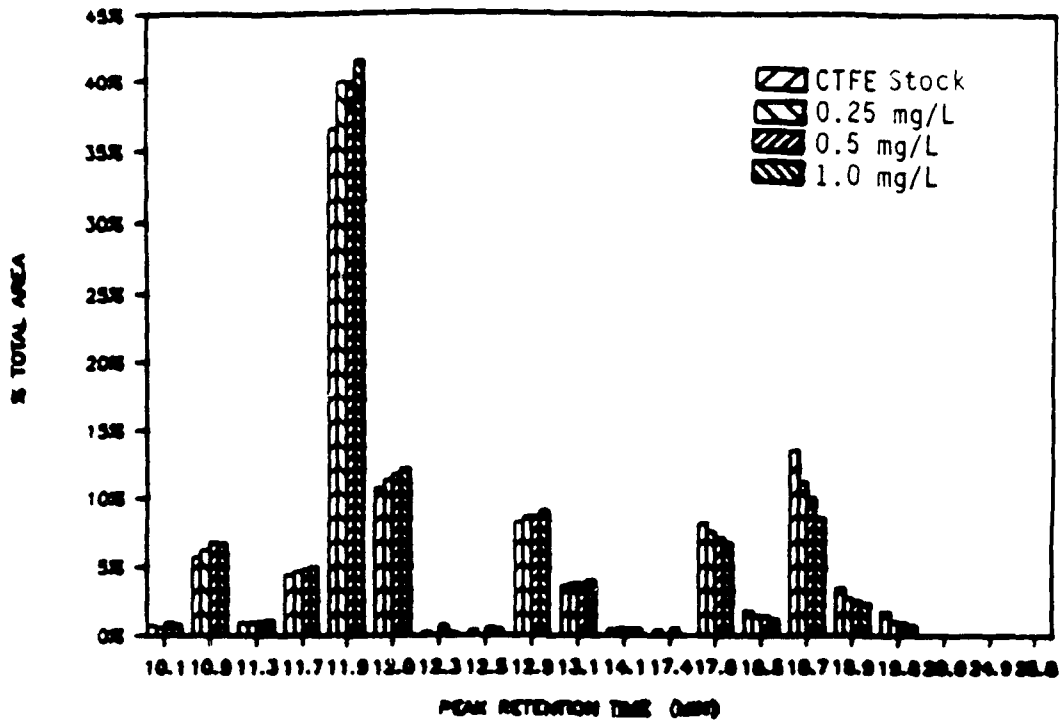

Figure 7. Comparison of the CTFE oligomers in the vapor phase of the three exposure chambers to the distribution of the CTFE stock.

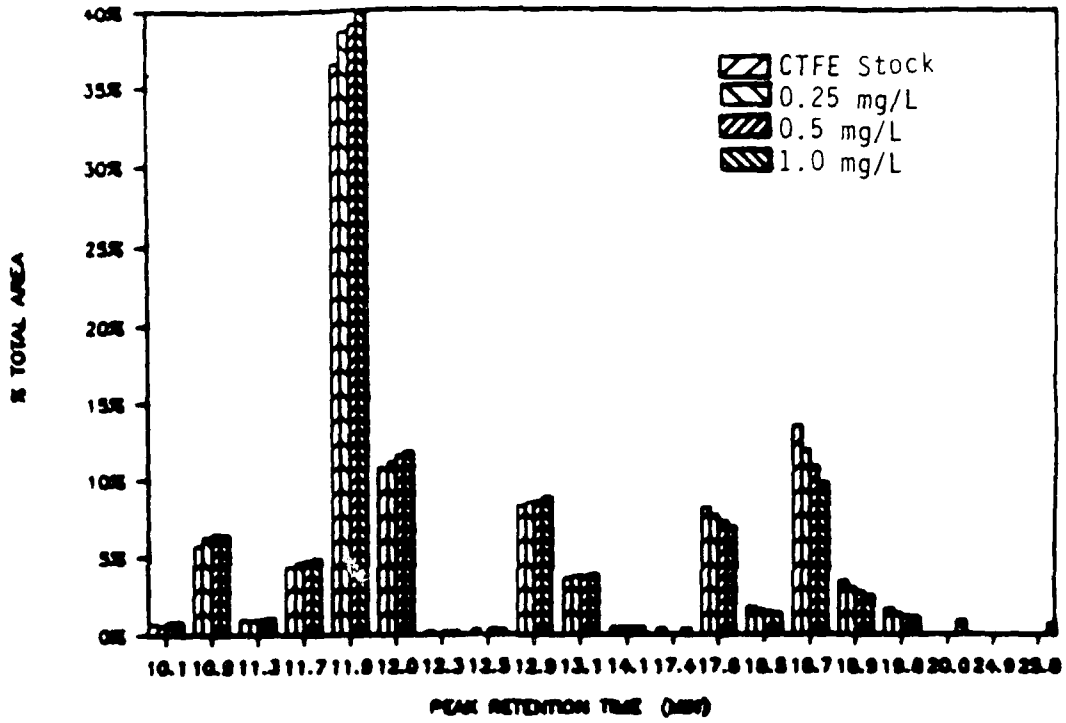

Figure 8. Comparison of the CTFE oligomers in the combined vapor and aerosol phases of the three exposure chambers to the distribution of the CTFE stock.

Analyzed vapor-aerosol mixture concentrations were within four percent of the selected target values (**Table 6**). The aerosol fraction of the total chamber atmospheres averaged less than 10% at each concentration level. No outward signs of toxic stress were observed and no exposure-related deaths occurred during the 90-day exposure period. A male rat from the 0.50 mg/L group was euthanatized following accidental injury during the study. To assess the general health of the rats, a standard battery of serology assays was performed on selected animals at the conclusion of the 90-day study; all were negative.

TABLE 6. ANALYSIS OF CTFE CONCENTRATIONS INHALED BY MALE AND FEMALE F-344 RATS FOR 90 DAYS

Target concentration, mg/L	0.25	0.50	1.00
Mean Concentration, mg/L (N = 66)	0.25	0.48	0.98
Standard error	<0.01	<0.01	<0.01
Lowest daily average, mg/L	0.21	0.43	0.93
Highest daily average, mg/L	0.28	0.53	1.04
Mean percent aerosol	5.30	6.80	8.90
Mean MMAD	1.20	0.97	1.14
Geometric standard deviation	2.59	2.15	2.17

A significant difference ($p<0.05$) in group mean body weights was observed at Day 0 of the study, which required that statistical analysis of all mean body weight data be performed on group mean body weight gains. With the exception of the 14- and 21-day weighings, the male control rats consistently gained body weight at a rate greater than any treated male rat group (**Figure 9, Appendix F**). The difference in body weight gains was greatest during the mid-portion of the study, Days 28 through 70, where a definite treatment-related effect occurred. Body weight gains among the 1.0 mg/L exposed female rats were slightly less than controls during the first three weeks of the study, but closely paralleled the other groups thereafter.

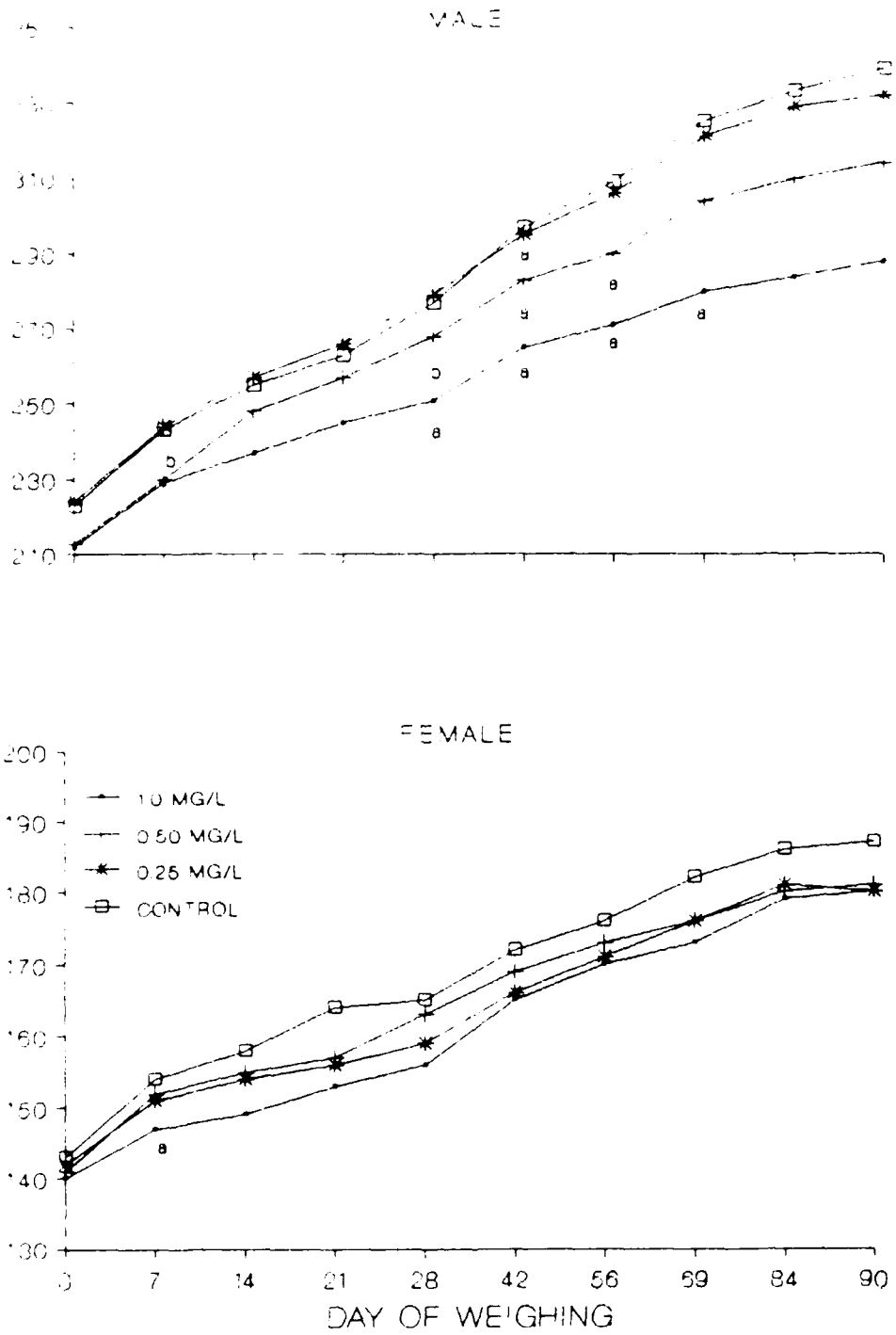

Figure 9. Effect of a 90-day CTFE inhalation exposure on body weight gain ($n \geq 9$). a = different from control, $p < 0.01$; b = compared with control, $p < 0.05$ as determined by the Multivariate Analysis of Covariance for Repeated Measures Test.

Blood chemistry data collected at sacrifice are listed in **Tables 7** and **8**. The mean alkaline phosphatase value for the male rats was significantly different ($p<0.01$) from controls for all treatment groups with the high concentration group exhibiting a threefold increase over the control group. Increased ($p<0.01$) BUN values were observed in the intermediate and high concentration male groups, and increased SGOT and SGPT were observed only in the high concentration male group. The treated female rats did not demonstrate differences from the controls.

The hematology data from the male and female rats are shown in **Tables 9** and **10**. Mean corpuscular volume (MCV) was increased ($p<0.01$) in all male treatment groups. Only in the female high concentration group (1.00 mg CTFE/L) was there a statistical ($p<0.05$) difference from controls for this parameter. All other hematology values did not differ from control values.

TABLE 7. BLOOD CHEMISTRY DATA[a] FROM MALE F-344 RATS FOLLOWING 90-DAY INHALATION EXPOSURE TO CTFE

Parameters	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
BUN (mg/dL)	11.7 ± 0.3 (3)	14.1 ± 1.3 (7)	17.4 ± 0.8 (4)[b]	19.3 ± 1 2 (6)[b]
CREA (mg/dL)	0.6 ± 0.1 (5)	0.5 ± 0.1 (5)	0.6 ± 0.1 (4)	0.5 ± 0.1 (6)
ALK PHOS (U/L)	99.7 ± 3.2 (10)	148.1 ± 4.8 (9)[b]	197.0 ± 6.6 (6)[b]	345.1 ± 18.3 (7)[b]
SGOT (U/L)	73.7 ± 9.0 (10)	82.3 ± 8.2 (9)	77.2 ± 6.9 (6)	117.8 ± 6.8 (6)[b]
SGPT (U/L)	62.5 ± 4.4 (10)	78.4 ± 8.9 (9)	68.7 ± 2.3 (6)	112.7 ± 12.7 (7)[b]

[a] Mean ± S.E.M.

[b] Significantly different than control, $p<0.01$, as determined by a two-factorial analysis of variance with multivariate comparisons.

TABLE 8. BLOOD CHEMISTRY DATA[a] FROM FEMALE F-344 RATS FOLLOWING 90-DAY INHALATION EXPOSURE TO CTFE

Parameters	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
BUN (mg/dL)	14.3 ± 0.7 (7)	14.6 ± 0.4 (5)	13.0 ± 0.6 (7)	14.5 ± 0.8 (6)
CREA (mg/dL)	0.5 ± 0.1 (7)	0.5 ± 0.1 (3)	0.4 ± 0.1 (3)	0.5 ± 0.0 (6)
ALK PHOS (U/L)	73.3 ± 2.2 (9)	91.0 ± 6.4 (8)	89.4 ± 8.5 (9)	89.1 ± 5.8 (9)
SGOT (U/L)	61.3 ± 8.1 (5)	68.8 ± 10.2 (5)	68.7 ± 7.3 (7)	52.5 ± 9.5 (4)
SGPT (U/L)	42.8 ± 6.3 (9)	53.7 ± 2.6 (7)	43.6 ± 2.9 (9)	47.6 ± 3.1 (7)

[a] Mean ± S.E.M.

TABLE 9. HEMATOLOGY DATA[a] FROM MALE F-344 RATS FOLLOWING 90-DAY INHALATION EXPOSURE TO CTFE

Parameters	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
WBC (x 10^3 cells/mm^3)	8.7 ± 0.6 (10)	9.1 ± 0.5 (10)	8.6 ± 0.9 (9)	10.7 ± 2.3 (10)
RBC (x 10^3 cells/mm^3)	8.8 ± 0.1 (10)	8.3 ± 0.1 (10)	8.2 ± 0.2 (9)	8.3 ± 0.1 (10)
HGB (g/dL)	16.5 ± 0.2 (10)	16.2 ± 0.2 (10)	16.2 ± 0.3 (9)	15.5 ± 0.3 (10)
HCT (%)	44.1 ± 0.5 (10)	43.3 ± 0.5 (10)	43.5 ± 1.0 (9)	42.5 ± 1.0 (10)
MCV (fl)	50.2 ± 0.3 (10)	53.0 ± 0.3 (10)[b]	52.5 ± 0.2 (9)[b]	50.6 ± 0.3 (10)
MCHC (g/dL)	36.2 ± 0.5 (10)	36.4 ± 0.6 (10)	36.6 ± 0.6 (9)	37.0 ± 0.5 (10)
NEUTRO (%)	25.5 ± 1.5 (10)	24.1 ± 1.4 (10)	24.3 ± 2.2 (9)	20.0 ± 1.5 (10)
LYMPH (%)	68.1 ± 1.5 (10)	70.3 ± 1.2 (10)	69.2 ± 2.0 (9)	73.0 ± 1.8 (10)
MONO (%)	2.1 ± 0.5 (7)	2.5 ± 0.4 (8)	1.7 ± 0.2 (7)	2.3 ± 0.4 (9)
ATYP (%)	3.0 ± 0.6 (9)	3.6 ± 0.7 (7)	3.8 ± 0.7 (8)	3.0 ± 0.6 (9)

[a] Mean ± S.E.M.

[b] Significantly different than control, $p<0.01$, as determined by a two-factorial analysis of variance with multivariate comparisons.

TABLE 10. HEMATOLOGY DATA[a] FROM FEMALE F-344 RATS FOLLOWING 90-DAY INHALATION EXPOSURE TO CTFE

Parameters	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
WBC (x 10^3 cells/mm^3)	4.8 ± 0.3 (10)	5.6 ± 0.3 (10)	5.4 ± 0.3 (10)	6.1 ± 0.5 (10)
RBC (x 10^3 cells/mm^3)	8.1 ± 0.2 (10)	7.8 ± 0.1 (10)	7.7 ± 0.1 (10)	7.7 ± 0.2 (10)
HGB (g/dL)	17.5 ± 0.3 (10)	16.7 ± 0.3 (10)	16.8 ± 0.1 (10)	16.8 ± 0.2 (10)
HCT (%)	42.7 ± 0.8 (10)	41.9 ± 0.4 (10)	41.5 ± 0.4 (10)	41.6 ± 0.8 (10)
MCV (fl)	53.1 ± 0.3 (10)	53.6 ± 0.3 (10)	53.7 ± 0.2 (10)	54.3 ± 0.4 (10)[b]
MCHC (g/dL)	41.0 ± 0.7 (10)	40.7 ± 0.3 (10)	40.4 ± 0.4 (10)	40.3 ± 0.4 (10)
NEUTRO (%)	22.7 ± 2.0 (10)	26.0 ± 2.1 (10)	23.1 ± 1.6 (10)	23.3 ± 1.5 (10)
LYMPH (%)	70.8 ± 2.4 (10)	69.0 ± 2.2 (10)	70.1 ± 1.9 (10)	70.6 ± 2.1 (10)
MONO (%)	1.8 ± 0.2 (9)	2.0 ± 0.5 (6)	2.3 ± 0.4 (8)	2.0 ± 0.4 (7)
EOSIN (%)	1.7 ± 0.5 (7)	1.6 ± 0.6 (7)	2.1 ± 0.4 (9)	1.6 ± 0.4 (8)
NRBC (%)	1.7 ± 0.3 (3)	1.0 ± <0.1 (2)	1.5 ± 0.5 (2)	1.0 ± <0.1 (1)
ATYP (%)	3.3 ± 0.6 (10)	3.0 ± 0.6 (8)	3.1 ± 0.4 (8)	3.7 ± 0.3 (9)

[a] Mean ± S.E.M.

[b] Significantly different than control, $p<0.05$, as determined by a two-factorial analysis of variance with multivariate comparisons.

Significant concentration-related increases ($p<0.01$) in relative kidney weights occurred in both sexes of rats (**Tables 11** and **12**). Relative kidney weights of the treated male rats were increased over controls by 30, 32, and 47% in the 0.25, 0.5, and 1.0 mg/L groups, respectively. The female rats had relative kidney weight increases of 8, 11, and 19% in the respective treatment groups. Similarly, a concentration-related increase in relative liver weights occurred in both sexes of rats. Relative liver weights of the treated male rats were increased over controls by 84, 133, and 213% in the 0.25, 0.5, and 1.0 mg/L groups, respectively; those of the treated female rats were increased over controls by 16, 34, and 77% at the respective exposure concentrations. An increase in the relative testes weights of the treated male rats was noted; however, the absolute weights of the testes were comparable to those of controls.

Gross pathologic findings at the conclusion of the 90-day exposures consisted of gross liver enlargement in all CTFE-exposed rats which was subsequently determined to be statistically significant when compared to controls ($p<0.01$). Among female rats, the incidences of grossly detected paraovarian cysts were 40, 10, 10, and 0% in the control, 0.25, 0.5, and 1.0 mg CTFE/L concentration groups, respectively.

TABLE 11. ORGAN WEIGHTS[a] AND ORGAN TO BODY WEIGHT RATIOS (%) OF MALE F-344 RATS FOLLOWING 90-DAY INHALATION EXPOSURE TO CTFE

Organs	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
Kidney	2.48 ± .06	3.14 ± .09[c]	3.01 ± .04[c]	3.08 ± .04[c]
Ratio[b]	0.77 ± .01	1.00 ± .03[c]	1.01 ± .01[c]	1.13 ± .01[c]
Heart	1.12 ± .03	1.07 ± .02	1.02 ± .03[d]	0.98 ± .02[d]
Ratio	0.35 ± <0.01	0.34 ± .01	0.34 ± .01	0.36 ± .01
Brain	1.91 ± .01	2.00 ± .01	1.95 ± .02	1.92 ± .02
Ratio	0.60 ± .01	0.62 ± .01	0.66 ± <0.01	0.70 ± .01
Liver	9.12 ± .22	16.46 ± .26[c]	19.65 ± .40[c]	24.27 ± .43[c]
Ratio	2.83 ± .04	5.22 ± .07[c]	6.61 ± .11[c]	8.87 ± .11[c]
Spleen	0.67 ± .01	0.67 ± .01	0.67 ± .02	0.62 ± .02[d]
Ratio	0.21 ± <0.01	0.21 ± <0.01	0.23 ± <0.01	0.23 ± .01
Thymus	0.39 ± .15	0.24 ± .01	0.24 ± .01	0.22 ± .02
Ratio	0.12 ± .05	0.08 ± <0.01	0.08 ± <0.01	0.08 ± .01
Lungs	2.04 ± .06	2.00 ± .04	2.21 ± .35	1.89 ± .03
Ratio	0.64 ± .02	0.63 ± .01	0.74 ± .01	0.69 ± .01
Adrenal	0.07 ± <0.01	0.08 ± .01	0.08 ± <0.01	0.09 ± <0.01
Ratio	0.02 ± <0.01	0.02 ± <0.01	0.03 ± <0.01	0.03 ± <0.01
Testes	3.24 ± .03	3.31 ± .04	3.28 ± .04	3.21 ± .04
Ratio	1.01 ± .02	1.05 ± .01[d]	1.10 ± .01[c]	1.18 ± .02[c]
Whole Body	322.2 ± 6.0	315.5 ± 3.5	297.4 ± 3.7[c]	273.7 ± 4.5[c]

[a] Mean ± S.E.M., N = 10 for all groups except the 0.50 mg/L group where N = 9.

[b] Organ weight/body weight × 100.

[c] Significantly different than control, $p<0.01$, as determined by a two-factorial analysis of variance with multivariate comparisons.

[d] Significantly different than control, $p<0.05$, as determined by a two-factorial analysis of variance with multivariate comparisons.

TABLE 12. ORGAN WEIGHTS[a] AND ORGAN TO BODY WEIGHT RATIOS (%) OF FEMALE F-344 RATS FOLLOWING 90-DAY INHALATION EXPOSURE TO CTFE

Organs	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
Kidney	1.45 ± .02	1.52 ± .03	1.57 ± .02	1.66 ± .02[d]
Ratio[b]	0.81 ± .01	0.89 ± .01[c]	0.90 ± .01[c]	0.97 ± .01[c]
Heart	0.70 ± .02	0.71 ± .02	0.71 ± .02	0.73 ± .02
Ratio	0.39 ± .01	0.41 ± .01	0.41 ± .01	0.42 ± .01
Brain	1.78 ± .02	1.79 ± .02	1.77 ± .03	1.81 ± .01
Ratio	1.00 ± .02	1.03 ± .02	1.02 ± .01	1.05 ± .01
Liver	4.71 ± .07	5.30 ± .08	6.12 ± .07[c]	8.01 ± .07[c]
Ratio	2.64 ± .04	3.06 ± .05[c]	3.52 ± .04[c]	4.67 ± .06[c]
Spleen	0.44 ± .01	0.47 ± .01	0.48 ± .01	0.49 ± .01
Ratio	0.25 ± .01	0.27 ± .01	0.28 ± .01	0.29 ± .01
Thymus	0.20 ± .01	0.18 ± .01	0.20 ± .01	0.21 ± .01
Ratio	0.11 ± .01	0.11 ± .01	0.12 ± .01	0.12 ± .01
Lungs	1.43 ± .04	1.49 ± .09	1.34 ± .05	1.58 ± .06
Ratio	0.80 ± .02	0.84 ± .05	0.77 ± .02	0.92 ± .03
Adrenal	0.08 ± <0.01	0.09 ± <0.01	0.09 ± <0.01	0.09 ± <0.01
Ratio	0.04 ± <0.01	0.05 ± <0.01	0.05 ± <0.01	0.05 ± <0.01
Ovary	0.14 ± .01	0.15 ± .01	0.15 ± .01	0.14 ± .01
Ratio	0.08 ± <0.01	0.09 ± .01	0.09 ± .01	0.08 ± <0.01
Whole Body	178.8 ± 2.5	173.4 ± 2.9	173.7 ± 1.9	171.9 ± 2.2

[a] Mean ± S.E.M., N = 10.

[b] Organ weight/body weight × 100.

[c] Significantly different than control, $p<0.01$, as determined by a two-factorial analysis of variance with multivariate comparisons.

[d] Significantly different than control, $p<0.05$, as determined by a two-factorial analysis of variance with multivariate comparisons.

Histologically, the prevalence of hepatocytomegaly was 100% in CTFE-exposed male and female rats at each test level with no occurrence of this lesion noted in control rats of either sex (**Table 13**). Among male rats exposed to 1.0 and 0.5 mg CTFE/L, 9 of 10 and 8 of 10 rats, respectively, had hyaline droplet accumulation in the renal proximal tubules. Compared to the control male rats, the incidence of hyaline droplet accumulation for each test group was significantly increased at the $p<0.01$ level. The histopathologic examination also confirmed each paraovarian cyst in female rats and also revealed paraovarian cysts that were not grossly detected in other female rats. The microscopic incidences of paraovarian cysts were 6 of 10, 7 of 10, 9 of 10, and 9 of 10 among the

control, 0.25, 0.5, and 1.0 mg CTFE/L groups. No statistical differences between groups were noted for the incidence of this lesion.

TABLE 13. INCIDENCE (%) SUMMARY OF SELECTED MICROSCOPIC LESIONS OF RATS FOLLOWING 90-DAY INHALATION EXPOSURE TO CTFE

	Male				Female			
Organ	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
Liver								
Hepatocellular cytomegaly	0	100[a]	100[a]	100[a]	0	100[a]	100[a]	100[a]
Kidneys								
Laminated concretions	100	100	100	90	100	90	90	100
Hyaline droplet formation	0	0	80[a]	90[a]	0	0	0	0
Ovaries								
Paraovarian cyst	—	—	—	—	60	70	90	90

[a] Significantly different than control, $p<0.01$, as determined by Chi-squared test.

Additional histopathologic findings, with treatment/sex group incidences of two or less rats affected, included: colonic nematodiasis, focal myocarditis, renal retention cysts, pulmonary subpleural histiocytosis, focal dacryoadenitis, rhinitis, and multifocal chronic hepatitis. One male rat in the high concentration group had nephroblastoma in one kidney. Ninety percent or greater of the 10 rats in each of the male and female control and CTFE-exposed groups had a few foci of laminar concretions within renal tubules.

Descriptively, the light microscopic liver lesions consisted of multifocal to diffuse enlargement of individual hepatocytes with a massive increase in the cytoplasm and slightly increased nuclear size (**Figure 10**). Compared to unaffected cells in controls, individual hepatocytes in CTFE-exposed rats had at least a twofold increase in size. The cytoplasm exhibited a loss of the normal basophilic stippling and was replaced by eosinophilic granularity. The engorged cells distorted the hepatic cords and in some areas, obliterated sinusoids. The liver lesions in male rats were more severe than in female rats at each CTFE exposure concentration. The liver lesions in male rats were more diffusely distributed than in female rats where the lesions were multifocal and centrolobular oriented. Attempts to grade the lesions according to CTFE exposure level proved unsuccessful as differences in the severity of lesions between the 1.0 and 0.5 mg CTFE/L groups were morphologically indistinguishable.

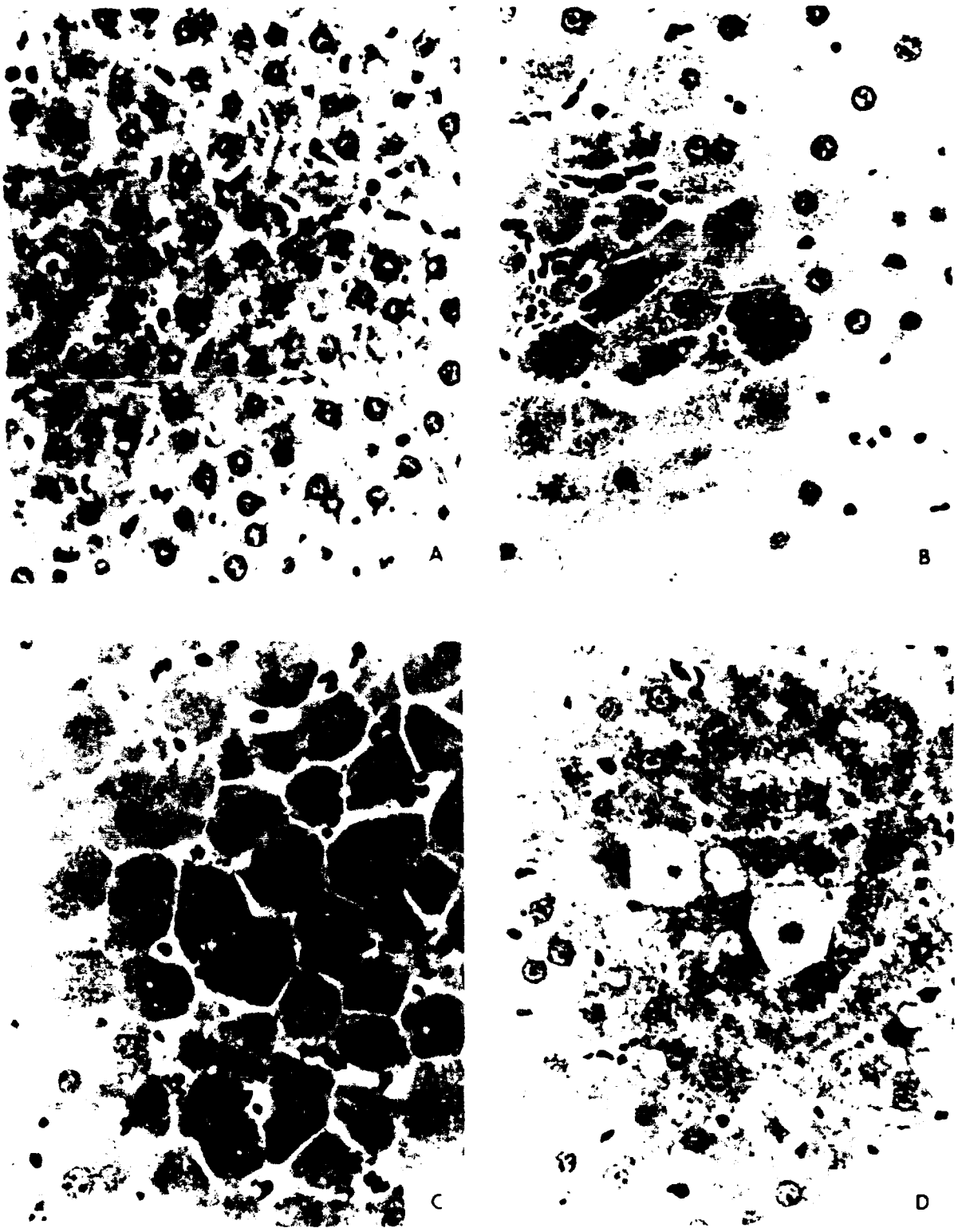

Figure 10. Photomicrographs of liver sections taken from male F-344 rats demonstrating diffuse enlargement of hepatocytes in the exposed rats. (A) Control (B) 0.5 mg/L (C) 1.0 mg/L after 90 days and (D) 0.5 mg/L at 105 days postexposure.

Table 14 presents data from the morphometric examination of hepatocytic ultrastructure in livers from control and CTFE-exposed rats. The ultrastructural examination revealed mild to moderate mitochondrial swelling in the hepatocytes of rats of both sexes exposed to CTFE. In CTFE-exposed rats hypertrophied hepatocytes had increased amounts of smooth endoplasmic reticulum (SER) and numbers of peroxisomes. The relative amounts of SER indicated a treatment dependent increase of SER in hepatocytes of both sexes with no difference between sexes. The number of peroxisomes per visual field was increased as the CTFE concentration was increased (**Figures 11** and **12**). The number of peroxisomes was increased ($p<0.05$) in male rat hepatocytes as compared to hepatocytes from female rats exposed to the same concentrations of CTFE. Rough endoplasmic reticulum (RER) was unaffected in female CTFE-exposed rats, whereas male CTFE-exposed rats had less RER as the amount of SER increased. The number of lipid vacuoles was increased in male rats after CTFE exposure, but decreased in female rats after CTFE exposure. In neither sex of rat did the number of lipid vacuoles present appear to be treatment dependent. In CTFE-exposed rats, membranous inclusions were seen in hepatocytic cytoplasm of male rat livers at each concentration level, but were only seen in hepatocytes of female rats exposed to 0.5 and 1.0 mg CTFE/L.

TABLE 14. MORPHOMETRIC EVALUATION OF HEPATOCYTE ULTRASTRUCTURE FOLLOWING 90-DAY INHALATION EXPOSURE OF F-344 RATS TO CTFE

		Exposure Groups (N = 3)			
Sex	Organelle	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
Males	Mitochondria[a]	0.0 ± <0.1	1.3 ± 0.6	1.2 ± 0.7	2.1 ± 0.6
	SER[a]	1.0 ± 0.6	1.7 ± 0.3	2.0 ± <0.1	3.3 ± 0.3
	Peroxisomes[b]	3.2 ± 0.6	7.7 ± 2.0	16.3 ± 3.1[c]	15.2 ± 0.0[c]
Females	Mitochondria[a]	1.2 ± 0.2	1.7 ± 0.3	1.8 ± 0.3	2.3 ± 0.4
	SER[a]	1.0 ± <0.1	1.3 ± 0.3	2.4 ± 0.3	3.0 ± <0.1
	Peroxisomes[b]	4.4 ± 0.4	8.1 ± 0.6	7.2 ± 0.6[d]	8.2 ± 0.9[d]

[a] Mean ± S.E.M. (N = 3) for grades of severity of mitochondrial swelling, and relative amounts of smooth endoplasmic reticulum based on 0 = normal; 1 = minimal; 2 = mild; 3 = moderate; 4 = severe; and 5 = necrotic.

[b] Mean ± S.E.M. (N = 3) of the number of peroxisomes in a 30,000x field using an 8 x 10 photograph (9 to 20 photographs per animal.

[c] Significantly different than control and 0.25 mg/L groups at $p<0.05$ as determined by a two-factorial analysis with multivariate comparisons.

[d] Significantly different than corresponding male rats at $p<0.05$ as determined by a two-factorial analysis of variance with multivariate comparisons.

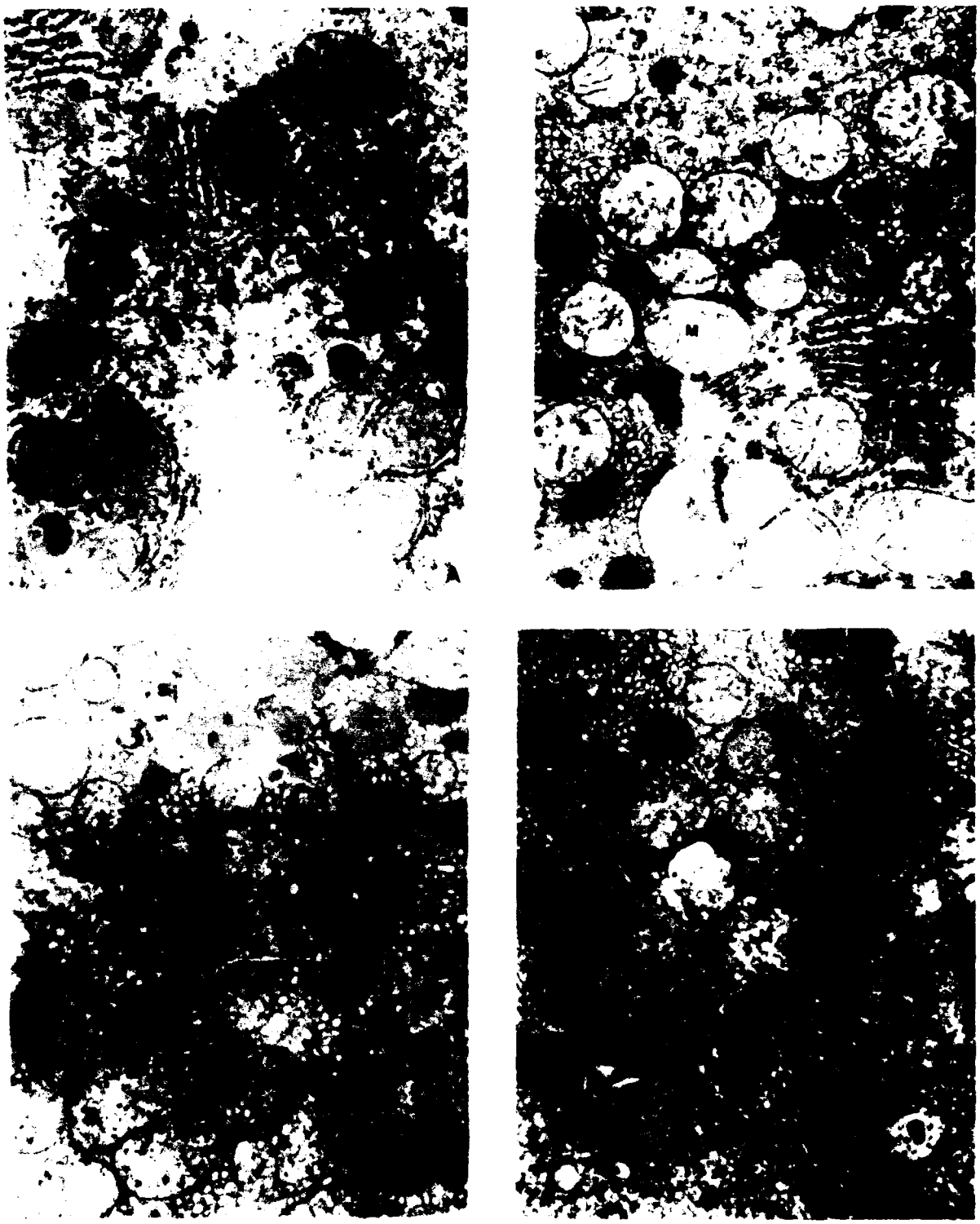

Figure 11. **Electron micrographs of hepatocytes from male F-344 rats exposed to (A) air only, (B) 0.25, (C) 0.50, and (D) 1.0 mg CTFE/L for 90 days.** M = mitochondria; S = smooth endoplasmic reticulum; P = peroxisomes; R = rough endoplasmic reticulum; MW = membranous whorls.

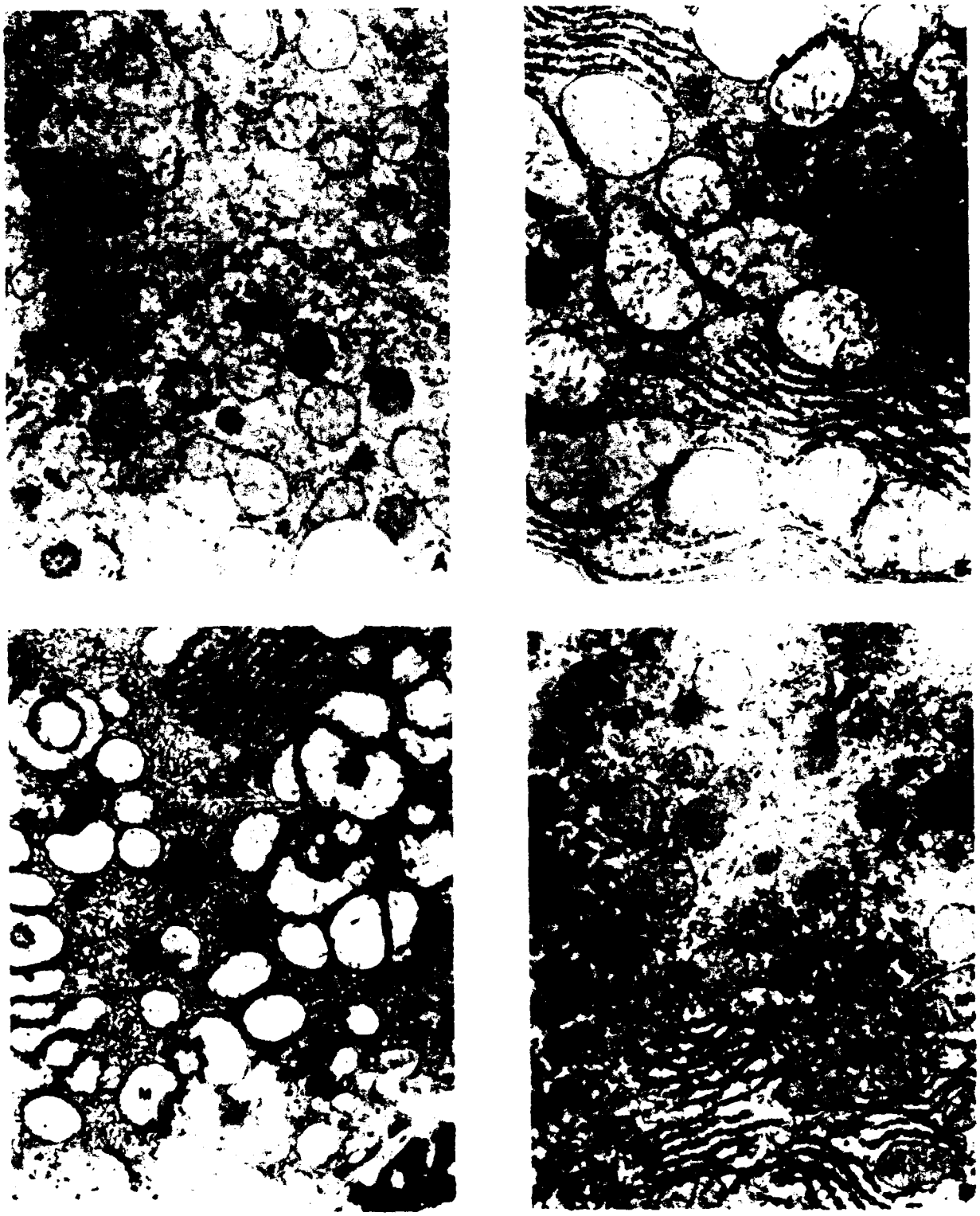

Figure 12. **Electron micrographs of hepatocytes from female F-344 rats exposed to (A) air only, (B) 0.25, (C) 0.50, and (D) 1.0 mg CTFE/L for 90 days.** M = mitochondria; S = smooth endoplasmic reticulum; P = peroxisomes; R = rough endoplasmic reticulum; MW = membranous whorls.

The kidney lesions were limited to the epithelial cells of the proximal convoluted tubules and consisted of hyaline droplet accumulation within the cytoplasm and laminar concretions that appeared to be anchored to the basement membrane. The second lesion, laminar concretions, was present in most rats of each sex among both the control and CTFE-exposed groups. The hyaline droplet formation appeared to be slightly more severe in male rats in the high concentration (1.0 mg CTFE/L) group in that the number of proximal convoluted tubule epithelial cells affected and the number and size of droplets appeared to be greater in this group.

Two of four rats included in this study for pharmacokinetic modeling purposes and exposed to 0.5 mg CTFE/L died, while anesthetized, prior to the beginning of the surgical procedure to collect a liver specimen at 105 days postexposure. Histopathologic examination of liver specimens from the two rats that died and the two CTFE-exposed rats that survived the biopsy procedure disclosed diffuse hepatocytomegaly with cytoplasmic eosinophilic granules and variable-sized clear vacuoles. Via light microscopy, the vacuolar changes were suggestive of lipid accumulation (**Figure 10D**). Multifocal aggregates or single enlarged hepatocytes, with poorly stained ground-glass cytoplasm and no apparent nuclear alteration, were also found in livers of the CTFE-exposed rats. Using periodic acid-Schiff staining, glycogen could not be detected in hepatocytes possessing ground-glass cytoplasm, but was present in other non-necrotic hepatocytes. Lipid accumulation and a lack of glycogen was confirmed by electron microscopy. Foci of hepatocytic necrosis with and without associated inflammation were seen in liver sections of CTFE-exposed rats. The foci of hepatic necrosis were usually associated with enlarged hepatocytes having extensive cytoplasmic vacuolation or poorly stained cytoplasm.

At 236 days after cessation of inhalation exposure to 0.5 mg CTFE/L, the hepatocytes of the CTFE-exposed rats still had increased eosinophilic cytoplasmic granularity, compared to controls, and occasionally contained microvacuoles suggestive of fat accumulation. The hepatocytes were essentially the same size as most hepatocytes in control rats, differing from the hepatocytomegaly seen in livers of CTFE-exposed rats immediately postexposure and at 105 days postexposure. Compared to hepatocytes in livers of CTFE-exposed rats at earlier postexposure points, the cytoplasm in hepatocytes of CTFE-exposed rats contained more basophilic material at 236 days after CTFE exposure. Both control and CTFE-exposed rats had chronic pericholangitis with biliary duplication and foci of hepatocytic necrosis.

Transmission electron microscopy of the liver hepatocytes at 236 days postexposure revealed mitochondria ranging from normal to various degrees of swelling, with some mitochondria appearing almost necrotic. The size of lipid droplets was greater in the exposed rats; however, the lipid content appeared to be the same in both control and exposed rats. RER of the test rats was dilated when compared to control animals. The exposed rats also had a greater amount of SER than

the controls, a small percentage of which was dilated. The numbers of peroxisomes of the test rats were in the same range as that of the controls. (Data not shown)

PHARMACOKINETICS

Gas chromatograms of CTFE showed two distinct groups of peaks (**Figure 13**). The initial peak in Group I had an 8.7 min retention time and comprised four area percent of the CTFE standards. This peak is not included in the total area reported for Group I oligomers because it was also determined to be a possible metabolite in the urine and kidney extracts. Group I oligomers eluded in five peaks with retention times between 10 and 14 min. Group II oligomers also had five peaks with retention times between 22 and 28 min. The average concentration of the only exposure for which pharmacokinetic data were collected was 0.48 mg/L. Apportioning the areas for the two groups resulted in Group I and Group II oligomer concentrations of 35 and 13 mg/L, respectively. Since ECD normally responds to the halides in compounds it would be expected to respond to all CTFE components in proportion to their concentration. To confirm this, CTFE was also chromatographed using a thermal conductivity detector (TCD) which normally responds to compounds in proportion to their concentration. The data using TCD analysis was similar to that obtained from ECD.

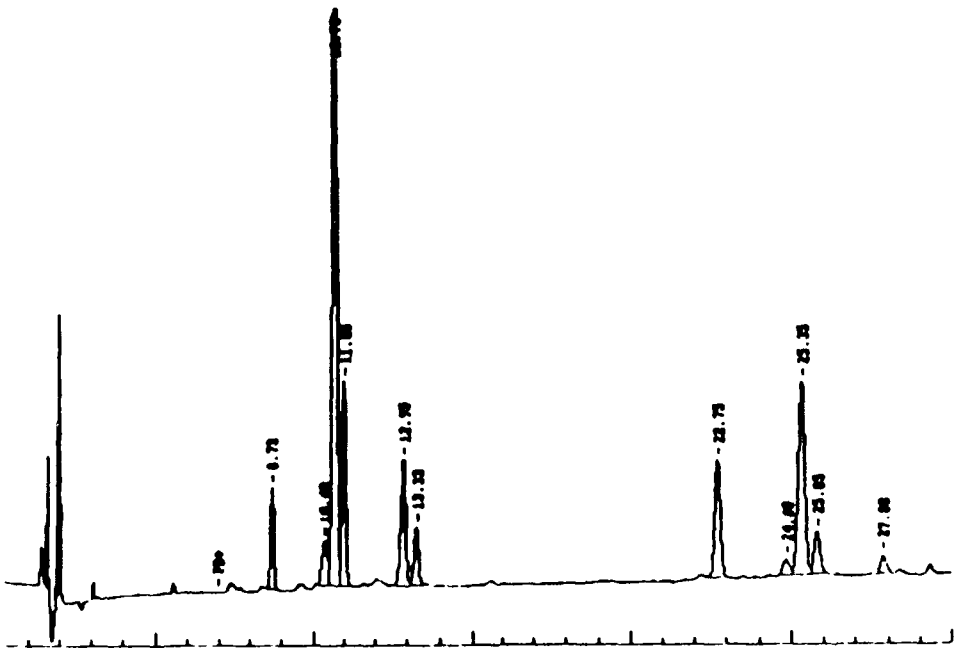

Figure 13. Typical chromatograph using ECD, showing two distinct groups of peaks.

Results of the analyses of CTFE in blood samples taken following a single, 6-h exposure to 0.5 mg CTFE/L are provided in **Table 15**. The results of the analyses following repeated exposure are provided in **Table 16**. CTFE concentration in blood samples from the singly exposed rats gradually decreased through seven days, after which the concentration was below the detection limits. CTFE concentrations found in the repeatedly exposed rat blood samples taken immediately following exposure were about twice that of the singly exposed rats. By 21 days postexposure, the CTFE concentration in the repeated exposed rat blood samples was minimal. CTFE was not detected in blood samples taken from control rats.

Only Group I oligomers were found in the urine samples of exposed rats. Urine from the repeated exposure rats initially contained approximately nine times the CTFE found in the urine of singly exposed rats (**Table 17**). CTFE was found in urine samples for three days following a single exposure and for 13 days following repeated exposures. CTFE was not detected in urine collected from control rats.

An increase ($p<0.01$) in total inorganic fluoride excreted in the urine of the repeated exposure rats was noted for eight weeks postexposure (**Table 18, Figure 14**). The inorganic fluoride concentration of the control rat urine remained relatively stable throughout the testing period. No increase in urinary fluoride excretion was seen in singly exposed rats.

TABLE 15. BLOOD CONCENTRATIONS (ng CTFE/mL) OF GROUP I, GROUP II CTFE OLIGOMERS, AND OF TOTAL CTFE FOLLOWING A SINGLE 6-h EXPOSURE TO CTFE

General Sample Time	Actual Time (h)	GC Peak Group I	GC Peak Group II	Total
Immediately	0.05	1563	1135	2698
Following	0.05	1843	899	2742
Exposure	0.07	2672	2159	4830
	0.08	1753	1114	2868
0.15 h	0.10	1768	1007	2776
Following	0.12	2033	1203	3236
Exposure	0.15	1651	1055	2706
	0.18	1964	1390	3354
0.5 h	0.47	1503	925	2428
Following	0.55	1171	876	2048
Exposure	0.55	1286	801	2087
	0.58	1484	1009	2493
1 h	0.97	418	681	1099
Following	1.00	488	714	1203
Exposure	1.02	540	792	1332
	1.08	457	697	1154
1 Day	23.98	325	171	496
Following	24.00	304	161	465
Exposure	24.00	270	132	402
	24.03	317	304	621
2 Days	47.50	149	—[a]	149
Following	47.52	152	—	152
Exposure	47.53	153	—	153
	47.57	153	—	153
7 Days	167.52	171	—	171
Following	167.58	87	—	87
Exposure	167.62	67	—	67
	167.77	76	—	76
14 Days	335.20	—	—	—
Following	335.22	—	—	—
Exposure	335.25	—	—	—
	335.33	—	—	—

[a] (—) = Concentrations below detection limit.

TABLE 16. BLOOD CONCENTRATIONS (ng CTFE/mL) OF GROUP I, GROUP II CTFE OLIGOMERS, AND OF TOTAL CTFE FOLLOWING REPEATED INHALATION EXPOSURE TO CTFE

General Sample Time	Actual Time (h)	GC Peak Group I	GC Peak Group II	Total
immediately	0.05	3313	2949	6262
Following	0.08	3103	2723	5826
Exposure	0.08	2829	2317	5145
	0.08	3460	2867	6327
	0.12	3259	2774	6033
0.15 h	0.12	3419	3439	6858
Following	0.13	2912	2448	5359
Exposure	0.13	3055	2460	5515
	0.15	3052	2592	5644
	0.17	2939	2474	5413
	0.18	2830	2543	5373
	0.18	2878	2619	5497
0.5 h	0.53	2533	2148	4681
Following	0.63	2742	2393	5135
Exposure	0.65	2148	1718	3866
	0.67	2983	2698	5680
3 h	2.88	1749	1906	3656
Following	2.90	1738	1632	3370
Exposure	2.93	2208	2446	4653
	2.95	1819	1746	3566
1 Day	23.57	1275	1126	2401
Following	23.58	1614	1692	3306
Exposure	23.63	1373	996	2369
	23.65	1186	1216	2402
2 Days	47.53	1099	962	2061
Following	47.53	1029	1054	2083
Exposure	47.58	1209	1197	2407
	47.60	1019	904	1923
7 Days	167.60	451	408	859
Following	167.63	430	590	1020
Exposure	167.68	437	522	958
	167.72	323	378	701

Continued

TABLE 16. (CONT.)

General Sample Time	Actual Time (h)	GC Peak Group I	GC Peak Group II	Total
21 Days	503.10	—[a]	—	—
Following	503.12	96	—	96
Exposure	503.13	89	—	89
	503.20	—	243	243
35 Days	839.20	—	—	—
Following	839.22	—	—	—
Exposure	839.23	—	—	—
	839.25	—	—	—
49 Days	1176.20	—	—	—
Following	1176.23	—	138	138
Exposure	1176.23	—	—	—
	1176.27	—	—	—

[a] (—) = Concentrations below detection limit.

TABLE 17. CTFE CONTENT[a] OF URINE FROM RATS EXPOSED TO 0.5 mg CTFE/L

Following Single Inhalation		Following Repeated Inhalation	
Time (Days)	Total CTFE (ng)	Time (Days)	Total CTFE (ng)
1	204 ± 30	1	1780 ± 194
2	107 ± 36	2	1966 ± 287
3	301 ± 21	3	1103 ± 176
4	88[b]	4	840 ± 58
5	—[c]	5	545 ± 62
6	—	6	602 ± 99
7	—	7	543 ± 64
8	—	8	877 ± 120
9	—	9	516 ± 69
		10	392 ± 34
		11	326 ± 29
		12	222 ± 74
		13	183 ± 119
		14	57[b]
		21	77[b]
		28	—
		35	—
		42	—
		49	—

[a] Mean ± S.E.M. (N = 4) of amount of CTFE excreted during a 24-h period.

[b] Value for one animal only, the other three had no detectable CTFE.

[c] (—) = Concentration below detection limit.

TABLE 18. TOTAL INORGANIC FLUORIDE[a] (µg FLUORIDE/24 h) IN RAT URINE FOLLOWING REPEATED INHALATION OF 0.5 mg CTFE/L

Days Postexposure	Control	Test
1	33 ± 5	131 ± 7[b]
2	43 ± 3	161 ± 4[b]
3	48 ± 4	164 ± 7[b]
4	50 ± 1	134 ± 6[b]
5	38 ± 5	105 ± 3[b]
6	46 ± 2	128 ± 7[b]
7	44 ± 1	124 ± 7[b]
8	44 ± 3	127 ± 4[b]
9	41 ± 2	129 ± 2[b]
10	43 ± 2	130 ± 2[b]
11	52 ± 1	136 ± 4[b]
12	46 ± 4	133 ± 5[b]
13	44 ± 1	124 ± 5[b]
14	45 ± 2	116 ± 5[b]
21	47 ± 10	122 ± 5[b]
28	49 ± 3	96 ± 3[b]
35	51 ± 3	87 ± 5[b]
42	51 ± 4	92 ± 4[b]
49	63 ± 6	83 ± 6
56	53 ± 3	75 ± 3[b]
63	48 ± 5	64 ± 7
70	53 ± 4	66 ± 2[c]
77	59 ± 4	68 ± 5
84	54 ± 3	69 ± 1[c]
91	64 ± 4	71 ± 2

[a] Mean ± S.E.M. (N = 4).

[b] Significantly different than control, $p<0.01$, as determined by the Multivariate Analysis of Covariance for Repeated Measures Test.

[c] Significantly different than control, $p<0.05$, as determined by the Multivariate Analysis of Covariance for Repeated Measures Test.

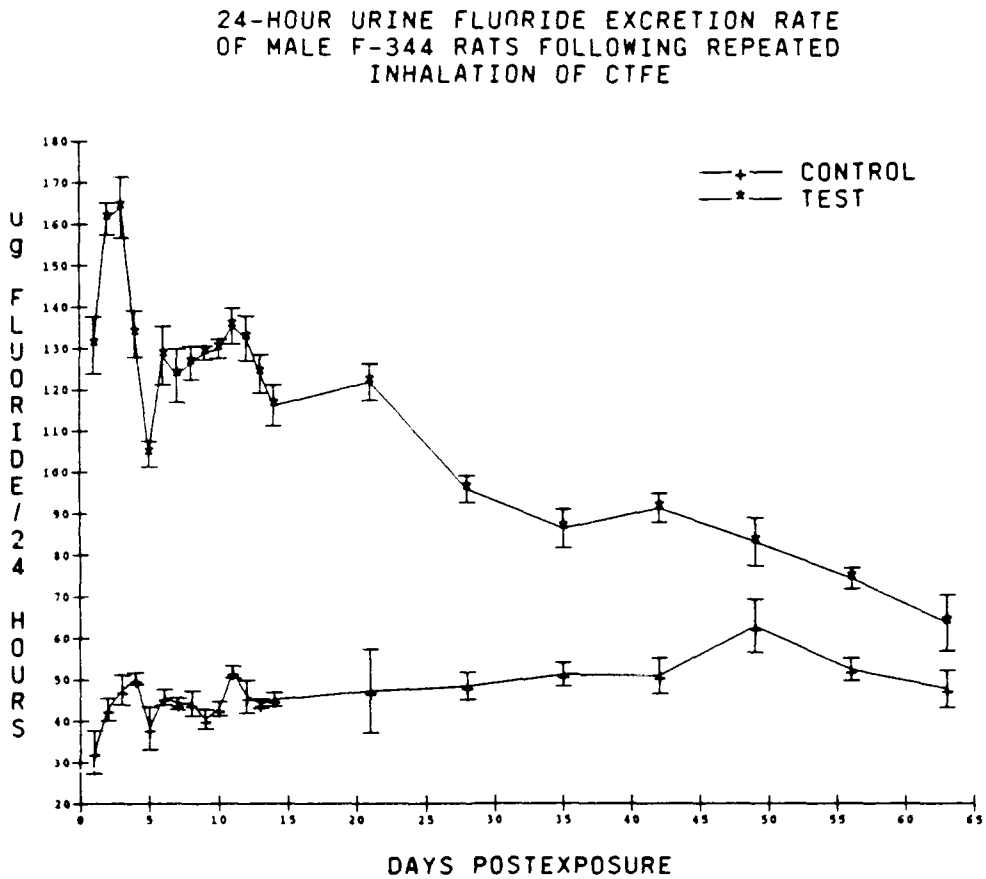

Figure 14. Total inorganic fluoride excreted in the urine of male rats (µg fluoride/24 h) following repeated exposure to 0.5 mg CTFE/L. Statistical differences are noted in Table 18.

Feces - CTFE Analysis

Gas chromatograms of CTFE extracted from feces showed poor resolution and low amplitude of peaks. No useful information was obtained.

Bone and Tooth - Fluoride Analysis

The analyses of inorganic fluoride revealed that concentrations in bones after a single 6-h exposure were not different from control (**Table 19**). However, a slight, but statistically significant increase was noted in the inorganic fluoride concentrations in bone following repeated exposure to 0.5 mg CTFE/L. The results of incisor fluoride analysis were so highly variable that meaningful statistical analysis could not be conducted.

TABLE 19. MEAN[a] BONE INORGANIC FLUORIDE CONCENTRATIONS (μgF/g BONE) OF MALE RATS EXPOSED TO 0.5 MG CTFE/L

Test Regimen	Inorganic Fluoride Concentration
Control	255 ± 43
Repeat Exposure[b] Sac. Immediately	593 ± 74[c]
Repeat Exposure[d] Sac. at 48 h	582 ± 73[c]
Single Exposure Sac. at 48 h	326 ± 55
Single Exposure Sac. at 14 days	279 ± 61

[a] Mean ± S.E.M. Measurements were made using a single femur from each rat. A minimum of five measurements per rat were made.

[b] 90 days on study.

[c] Significantly different than control, $p<0.05$, as determined by the Multivariate Analysis of Covariance for Repeated Measures Test.

[d] 84 days on study.

Tissues - CTFE Analysis

The results of the analysis of CTFE in rat tissues taken following a single, 6-h exposure and following repeated exposures are provided in **Tables 20** and **21**. Tissues taken from the repeated exposure group were probably close to CTFE concentration equilibrium while the tissues removed from the singly exposed rats probably were not. There was wide variation in tissue CTFE concentrations from the single exposure rats sacrificed at 48-h postexposure. Liver, testes, and brain tissues were lowest in CTFE concentration while kidney, lung, and fat were highest in CTFE concentration. A substantial decrease in tissue CTFE concentration occurred in the single exposure rats held for 14 days. Although Group I and Group II compounds decreased, Group I compounds decreased more rapidly. The concentration of Group I oligomers in the fat also decreased with time. The data on Group II oligomers concentration in fat suggested an increase from 2 to 14 days postexposure. This result seems unlikely and the data on Group II oligomers in fat must, therefore, be viewed with suspicion.

The CTFE tissue concentration of the repeated exposure rats decreased 50% in most tissues (except for fat) when the rats were held for 48 h postexposure. Group I oligomers decreased more rapidly than those of Group II. The more rapid Group I oligomer loss continued as rat tissue examined 105 days postexposure showed the loss of all Group I oligomers and what little CTFE remained was detected as Group II. By 105 days the total CTFE concentration in fat had decreased 94% from its highest concentration immediately following exposure. Control rat tissues did not contain CTFE. An interfering peak found among lung tissue Group II peaks was subtracted from Group II peak area and the total concentration reported.

TABLE 20. TISSUE CONCENTRATIONS[a] (ng CTFE/g), GROUP I, GROUP II OLIGOMERS, AND TOTAL CTFE[b] FROM RATS FOLLOWING A SINGLE 6-H EXPOSURE TO CTFE

		Tissue Removed Postexposure	
	Organ	48 h	14 Days
Kidney:	Group I	2585 ± 362	618 ± 106
	Group II	661 ± 326	359 ± 122
	Total	3246 ± 663	977 ± 196
Lung:	Group I	4247 ± 754	1109 ± 53
	Group II	3700 ± 629	1011 ± 84
	Total	7947 ± 1361	2120 ± 113
Liver:	Group I	604 ± 108	—[d]
	Group II	572 ± 64	—
	Total	1176 ± 170	—
Testes:	Group I	523 ± 52	181 ± 15
	Group II	571 ± 26	—
	Total	1093 ± 70	181 ± 15
Brain:	Group I	738 ± 57	—
	Group II	567 ± 96	—
	Total	1305 ± 105	—
Fat:	Group I	44718 ± 1286	25870 ± 2943
	Group II	2399[c]	18023 ± 4410
	Total	47117 ± 2573	43893 ± 7019

[a] Mean ± S.E.M., N = 4.

[b] Values assuming 100% CTFE extraction from tissue.

[c] Value for one animal only, the other three had no detectable CTFE.

[d] (—) = Concentration below detection limits.

TABLE 21. TISSUE CONCENTRATIONS[a] (ng CTFE/g) OF GROUP I, GROUP II OLIGOMERS, AND TOTAL[b] CTFE FROM RATS FOLLOWING REPEATED INHALATION EXPOSURE TO CTFE

		Tissue Removed Postexposure		
	Organ	0 Days[c]	2 Days[d]	105 Days[c]
Kidney:	Group I	40782 ± 2576	16227 ± 365	—[e]
	Group II	19660 ± 1344	16689 ± 1371	1970 ± 46
	Total	60442 ± 3404	32916 ± 1384	1970 ± 46[f]
Lung:	Group I	15975 ± 2397	8834 ± 2223	—
	Group II	35202 ± 5188	20989 ± 4981	541 ± 45
	Total	51117 ± 7479	29823 ± 7204	541 ± 45[f]
Liver:	Group I	25329 ± 1290	9356 ± 778	—
	Group II	23174 ± 2078	14784 ± 1560	495
	Total	48503 ± 2093	24139 ± 2327	495[g]
Testes:	Group I	12503 ± 1542	4740 ± 492	—
	Group II	10474 ± 1865	7124 ± 628	270
	Total	22977 ± 3401	11864 ± 1105	270[f,g]
Brain:	Group I	17562 ± 1836	6033 ± 161	—
	Group II	7871 ± 583	6453 ± 265	—
	Total	25433 ± 2404	12486 ± 250	—[f]
Fat:	Group I	734670 ± 12745	738268 ± 33689	—
	Group II	879787 ± 28415	990291 ± 43707	100475 ± 15536
	Total	1614457 ± 40448	1728559 ± 75308	100475 ± 15536[f]

[a] Mean ± S.E.M., N = 4.
[b] Values assuming 100% CTFE extraction from tissue.
[c] 90 Days on study.
[d] 84 Days on study.
[e] (—) = Concentration below detection limit.
[f] N = 2.
[g] Value represents only one animal, tissue concentrations of remaining animals below detection limit.

Partition Ratio

Blood and tissue CTFE concentrations were used to calculate partition ratios. It was assumed that the test rats were exposed to CTFE for a long enough time so that the CTFE in tissues had approached an equilibrium with the blood. The mean blood concentration at the termination of exposure to 0.5 mg/L was 3176 ng CTFE (Group I oligomers)/g and 2714 ng CTFE (Group II oligomers)/g. The partition ratios were calculated from CTFE concentrations of test rat tissues divided by the blood concentrations immediately after the exposure end (Table 22).

TABLE 22. TISSUE CONCENTRATION[a] (ng CTFE/g) AND PARTITION RATIO (TISSUE/BLOOD)[b]

	Mean Tissue Concentration[c]		Ratio	
Tissue	Group I	Group II	Group I	Group II
Kidney	40782	19660	12.8	7.2
Lung	15975[d]	35202[d]	5.0	13.0
Liver	25329	23174	8.0	8.5
Testes	12503	10474	3.9	3.9
Brain	17562	7871	5.5	2.9
Fat	734670	879787	231.3	324.2

[a] Tissue data from animals exposed 90 days and sacrificed immediately.
[b] Blood concentrations were 3176 ng/g and 2714 ng/g for Groups I and II, respectively.
[c] Values assume 100% of CTFE extracted from tissues.
[d] Lung concentrations less interferring peak.

Possible Metabolites

Analysis of urine and kidney extracts showed a significant increase in the relative area of the first peak identified as a CTFE oligomer in standards. This increase was not observed in other tissues to the same degree nor was this peak present in control samples of tissue. For these reasons, the peak was identified as a possible metabolite. Because there were eleven discrete peaks identified in the standards and this peak was obviously not totally composed of starting material, it was excluded from the total area for quantitation. Although the disproportionation occurred only in selected extracts, the peak was eliminated from interpretation of all chromatograms in the interest of consistency.

Mass spectral data from electron impact and chemical ionization mass spectrometry were used to tentatively identify the possible metabolite as $C_5F_7Cl_5$. In the absence of a molecular ion by either technique, a definitive identification was not possible. The elution of this material and the available mass spectral data were consistent with the identification.

Model Development

Preliminary examination of the data coplotted with the simulation indicated that only by having diffusion limited transport of the oligomer into fat would it be possible to simulate the measured fat concentrations. Therefore, the fat compartment was modeled with diffusion limitation which was set proportional to a weight scaled diffusion constant and to blood flow to the compartment.

Metabolism was assumed to be first order. An estimate of the rate of metabolism was made from the amount of fluoride in the urine collected 24 h after the end of the 90 day exposure. There was an average of 99 μg fluoride in the urine collected from male rats weighing about 300 g. This is equivalent to 4.125 μg fluoride excreted per h or 2.2×10^{-7} mol fluoride per h (1.9×10^{7} μg fluoride per mol). Assuming 1.5 mol fluoride produced per mol of CTFE oligomer, 1.47×10^{-7} mol CTFE were metabolized per h. On a molar basis 80% of the CTFE oligomers were contained in Group I peaks, thus 1.18×10^{-7} mol or .05 mg of Group I oligomers were metabolized per h. The first order rate constant was determined to be 0.131 hr^{-1} by iteratively running the simulation under equilibrium conditions and a metabolic rate of .05 mg hr^{-1} was predicted.

Simulations (solid line) of the acute exposure (**Figures 15-22**) and of the subchronic exposure (**Figures 23-31**) are shown coplotted with actual data (squares) collected during the postexposure period of each study. Tissue data were collected 2 days and 2 weeks postexposure for the acute study and immediately, 2 days, and 105 days postexposure for the subchronic study. Blood and urine samples were collected more frequently.

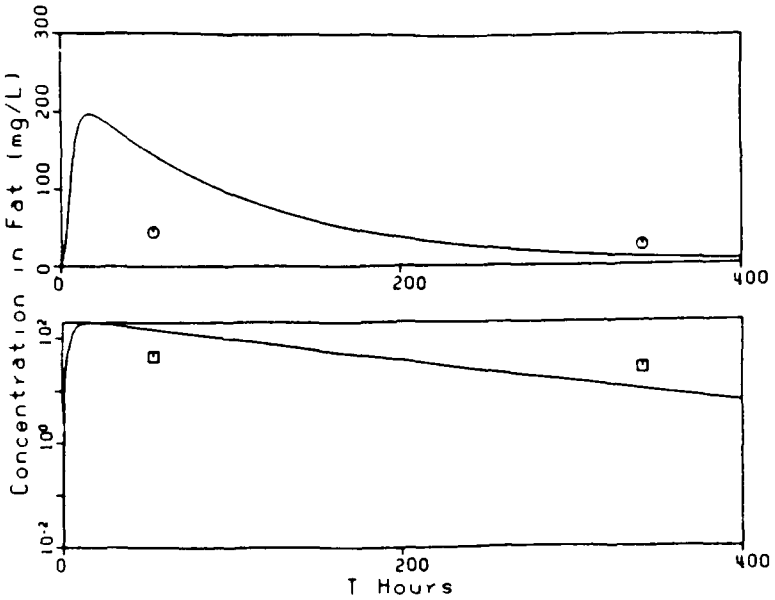

Figure 15. Concentration of CTFE (Group I Oligomers) in fat during and after single 0.5 mg/L, 6-h exposure. Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

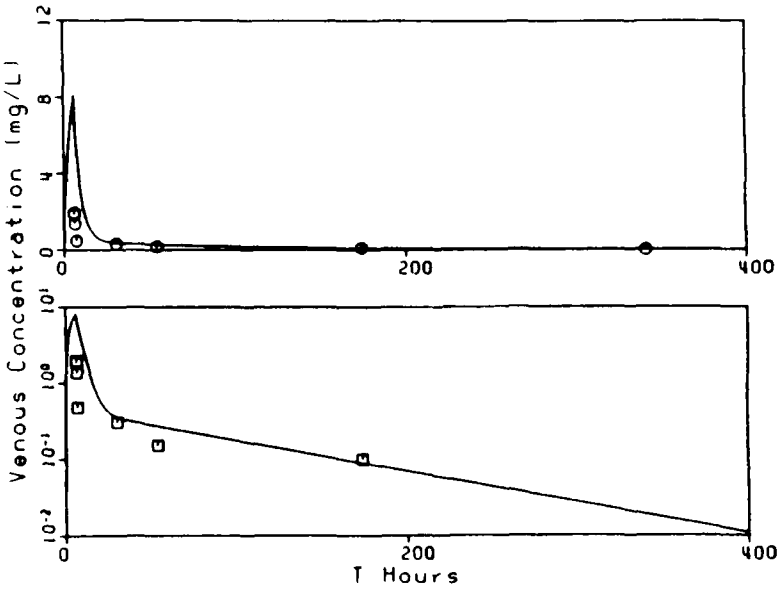

Figure 16. Concentration of CTFE (Group I Oligomers) in venous blood during and after single 0.5 mg/L, 6-h exposure. Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

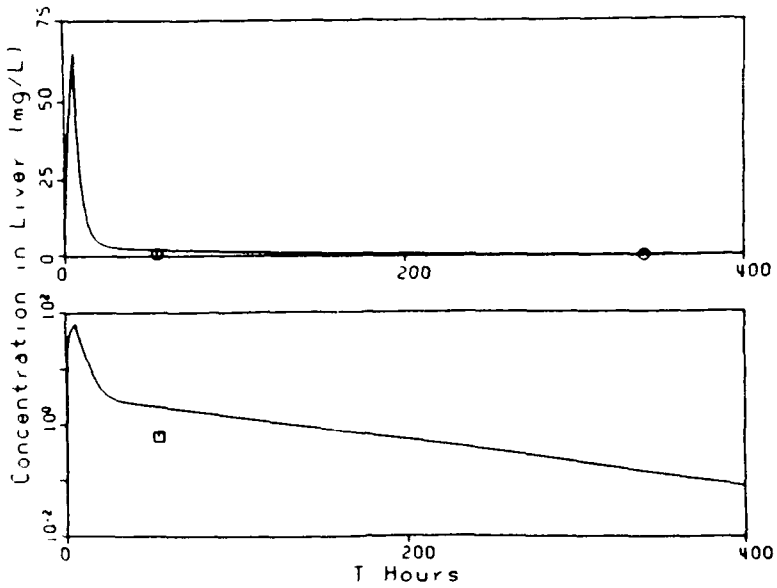

Figure 17. **Concentration of CTFE (Group I Oligomers) in liver during and after single 0.5 mg/L, 6-h exposure**. Solid lines represent simulations, points represent data Linear plot, upper curve and hexagonal points Log plot, lower curve and square points

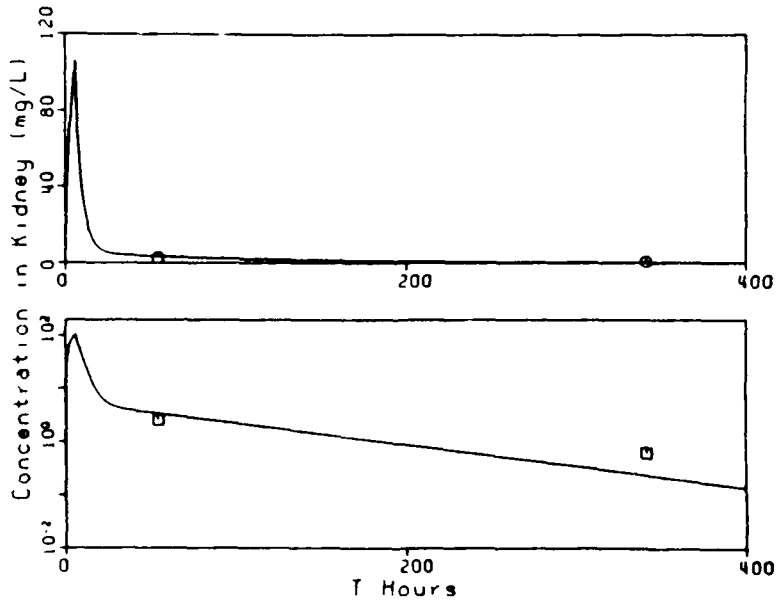

Figure 18. **Concentration of CTFE (Group I Oligomers) in kidney during and after single 0.5 mg/L, 6-h exposure**. Solid lines represent simulations, points represent data Linear plot, upper curve and hexagonal points Log plot, lower curve and square points

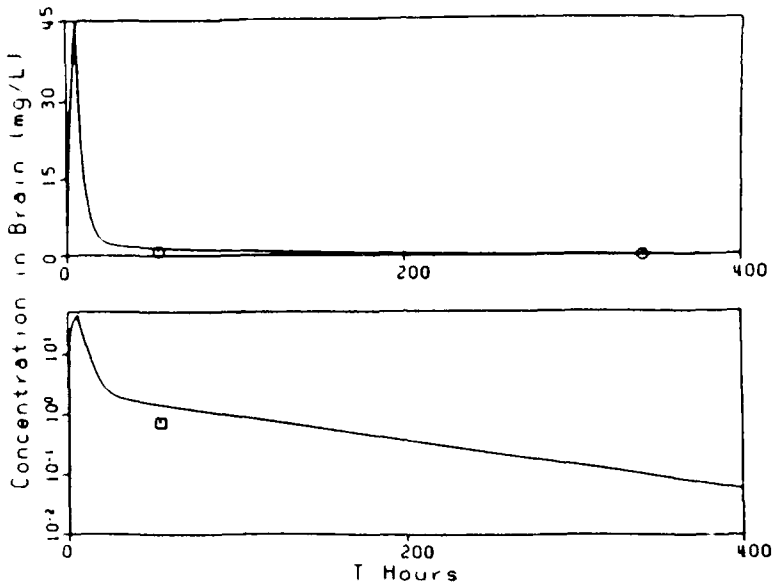

Figure 19. **Concentration of CTFE (Group I Oligomers) in brain during and after single 0.5 mg/L, 6-h exposure.** Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

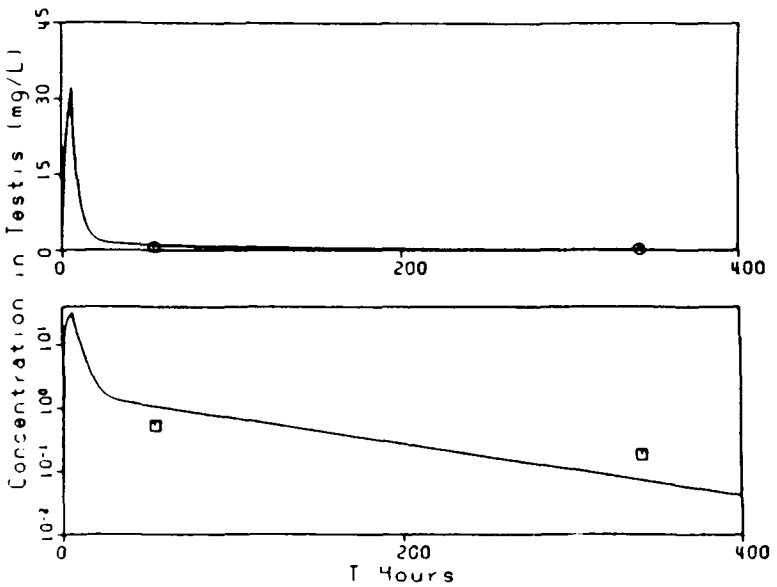

Figure 20. **Concentration of CTFE (Group I Oligomers) in testes during and after single 0.5 mg/L, 6-h exposure.** Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

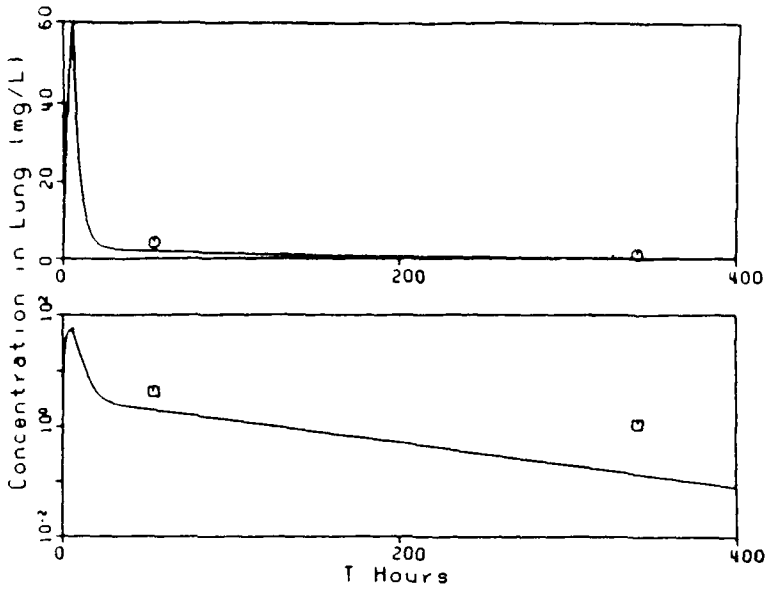

Figure 21. **Concentration of CTFE (Group I Oligomers) in lung during and after single 0.5 mg/L, 6-h exposure.** Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

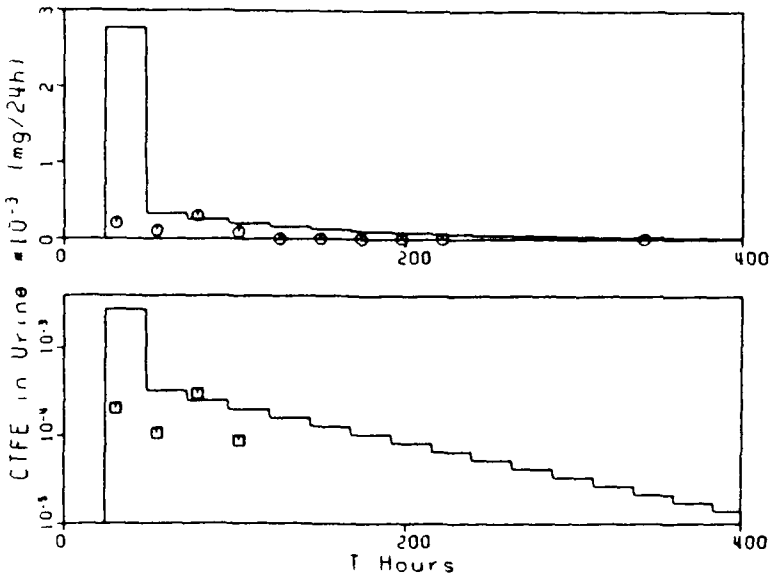

Figure 22. **Amount of CTFE (Group I Oligomers) excreted in urine every 24 h after single 0.5 mg/L, 6-h exposure.** Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

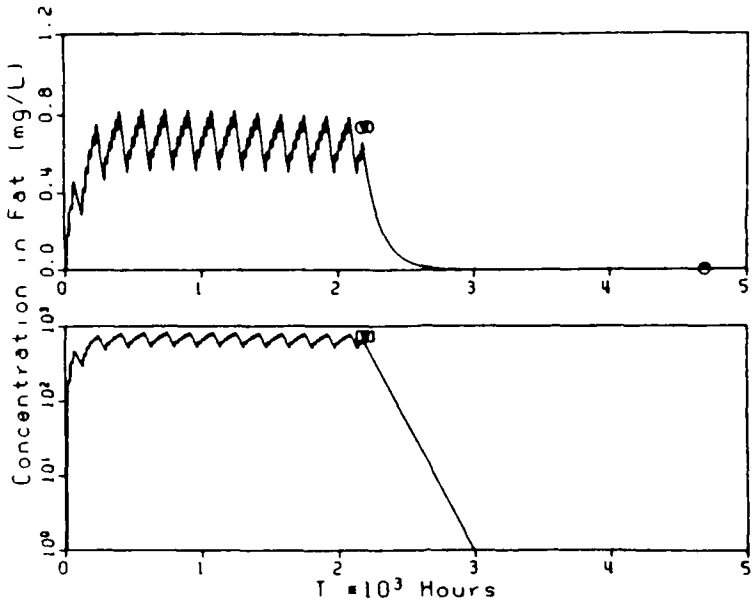

Figure 23. Concentration of CTFE (Group I Oligomers) in fat during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

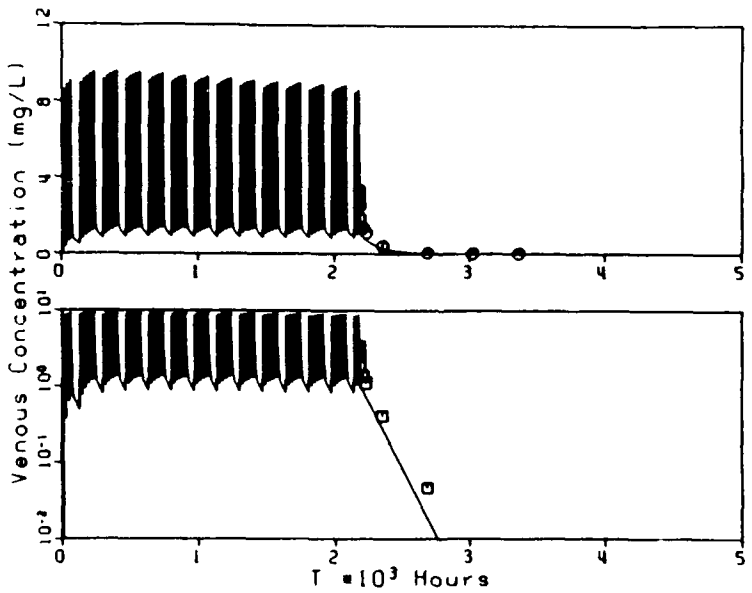

Figure 24. Concentration of CTFE (Group I Oligomers) in venous blood during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

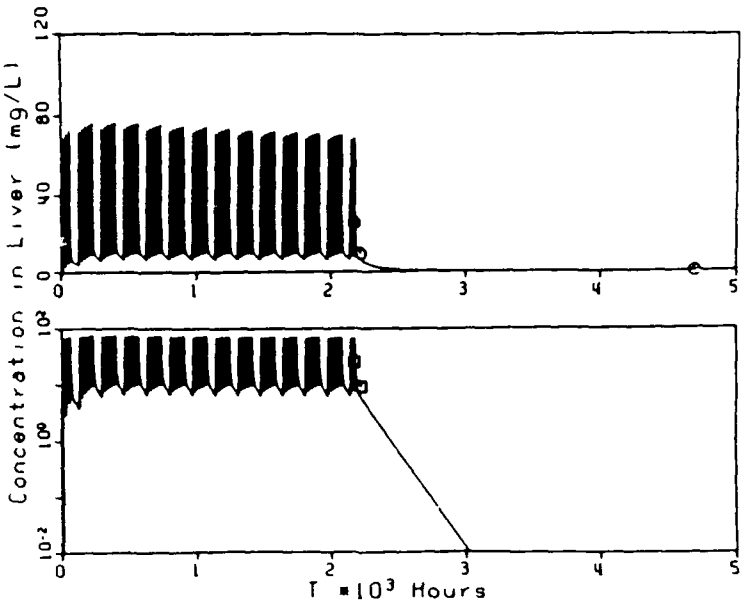

Figure 25. Concentration of CTFE (Group I Oligomers) in liver during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data Linear plot, upper curve and hexagonal points Log plot, lower curve and square points

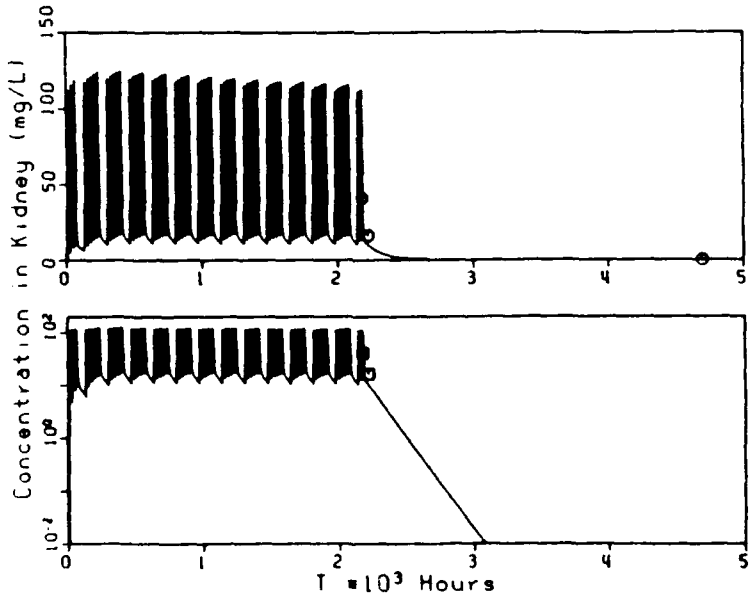

Figure 26. Concentration of CTFE (Group I Oligomers) in kidney during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data Linear plot, upper curve and hexagonal points Log plot, lower curve and square points

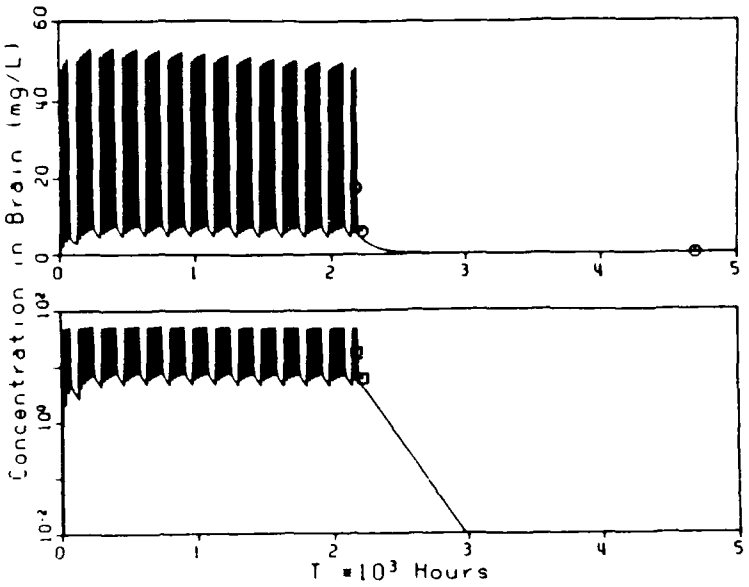

Figure 27. Concentration of CTFE (Group I Oligomers) in brain during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data Linear plot, upper curve and hexagonal points Log plot, lower curve and square points

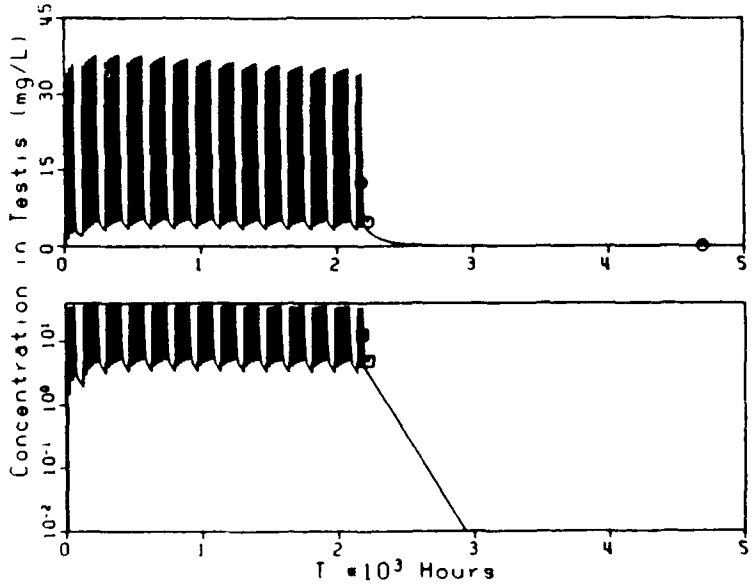

Figure 28. Concentration of CTFE (Group I Oligomers) in testes during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data Linear plot, upper curve and hexagonal points Log plot, lower curve and square points

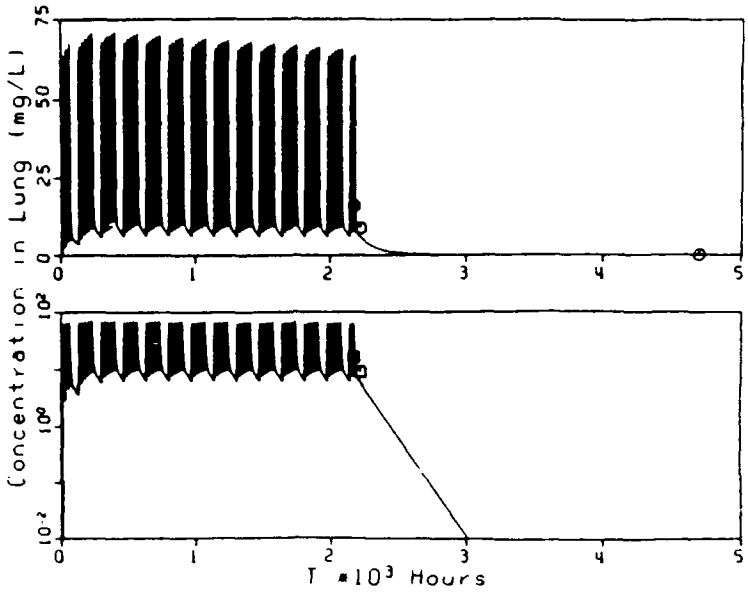

Figure 29. Concentration of CTFE (Group I Oligomers) in lung during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data Linear plot, upper curve and hexagonal points Log plot, lower curve and square points

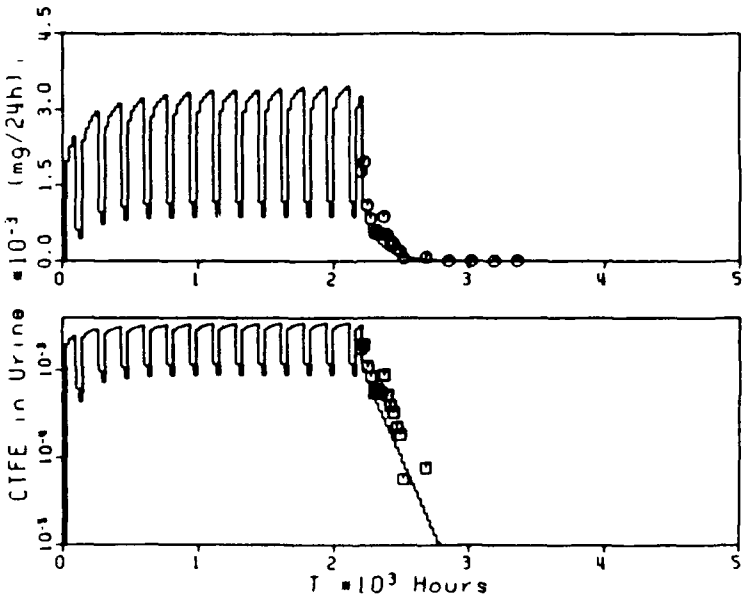

Figure 30. Amount of CTFE (Group I Oligomers) excreted in urine every 24 h during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data Linear plot, upper curve and hexagonal points Log plot, lower curve and square points

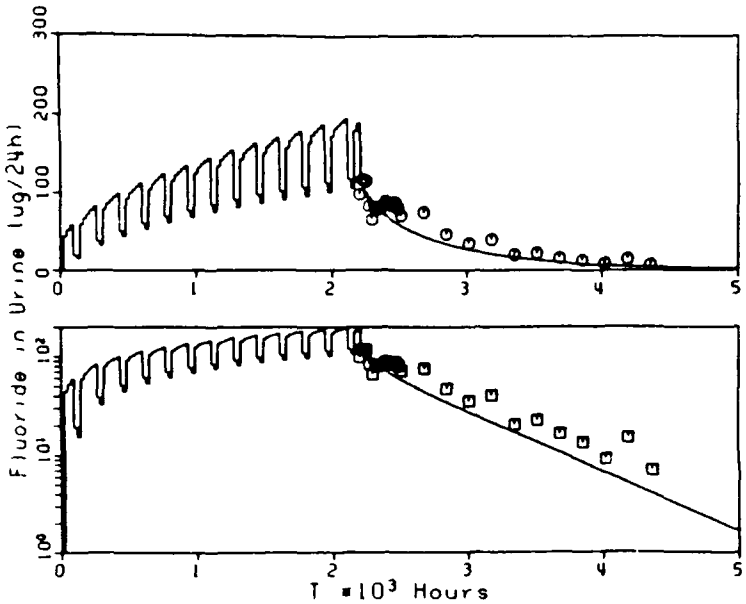

Figure 31. Amount of Inorganic Fluoride excreted in urine every 24 h during and after 0.5 mg/L, 6-h/day, 5 days/week, 90-day exposure. Solid lines represent simulations, points represent data. Linear plot, upper curve and hexagonal points. Log plot, lower curve and square points.

SECTION 4

DISCUSSION

The application of CTFE to intact rabbit skin produced no signs of irritation, however, it appears that continued skin contact could produce an allergic response in sensitive individuals. CTFE produced mild conjunctival redness in rabbit eyes one h following application but this was resolved by 24 h. It can be assumed that the fluid would not be irritating to human skin but would cause minimal irritation upon accidental eye contact. Washing the eyes immediately after contact did not preclude the transient irritating effects.

The changes observed in serum alkaline phosphatase, SGOT, and SGPT activities were considered directly related to CTFE exposure since there was significant morphologic alteration in hepatocytes of all CTFE-exposed rats. The hypertrophy of hepatocytes with resultant compression of sinusoids suggested that there was concurrent compression of the biliary duct system. These effects can contribute to intrahepatic biliary obstruction and subsequent induction of alkaline phosphatase synthesis. SGPT and SGOT elevations were probably a consequence of hepatocytic necrosis, a feature seen most frequently in the high concentration male rats. BUN mean values, though statistically different from the control group mean, were not considered to have pathophysiologic significance because they were within the 12.6 - 35.8 mg/dL range reported for historical controls within this laboratory. Although hyaline droplet accumulation in proximal tubules was prevalent in the median and high dose male rat kidneys, renal epithelial necrosis or other morphologic indicators of renal disease were not present. The MCV values were also considered to have no pathophysiologic significance, since values for all male and female rat dose groups were within the 44.6 - 54.5 μm^3 and 52.4 - 72.6 μm^3 historical control ranges for male and female F-344 rats, respectively.

Of prime importance was CTFE's toxic effect on the liver. Many of the hepatotoxic effects of CTFE appeared to be treatment- and sex-dependent. The morphologic results documented gross liver hypertrophy and microscopic hepatocytomegaly as the principal manifestations of CFTE-induced hepatotoxicity. Electron microscopic examination demonstrated peroxisomal proliferation and increased smooth endoplasmic reticulum as the primary structural factors responsible for the hepatocytomegaly. Treatment-dependent morphologic changes in CTFE-exposed rats included increased SER in hepatocytes of both sexes of rats, increased cytoplasmic membranous profiles in hepatocytes of female rats exposed to median and high concentrations of CTFE, and progressive decreases in the amount of RER in hepatocytes of male rats as the CTFE concentration increased. The sex-dependent hepatotoxic effects demonstrated via light microscopy were diffuse

hepatocytomegaly in male CTFE-exposed rats and multifocal centrolobular-oriented hepatocytomegaly in female rats.

Increased hyaline droplet formation, usually limited to male rats, tends to spontaneously increase in severity with age. Several hydrocarbon fuels are known to induce hyaline droplet formation (Bruner, 1984). The organophosphorus chemical dimethylmethylphosphonate also induces renal tubular hyaline droplet formation (Mattie and Hixson, 1987). Hyaline droplet formation is considered to have pathophysiologic significance when associated with resultant renal tubular necrosis. Such necrosis was not a feature in CTFE-exposed rats, therefore, hyaline droplet formation in male rat kidneys after CTFE exposure may not be a significant or toxic effect.

The finding of a nephroblastoma was probably unrelated to CTFE toxicity despite its occurrence in a rat that received a 1.0 mg CTFE/L exposure. The tumor has occasionally been found in rats, however, most commonly in younger animals less than one year old (Altman and Goodman, 1979). Other gross and histologic findings reported in the Results section are also considered to be background lesions.

Following a 90-day inhalation exposure to CTFE, the hepatocytic injury apparently becomes more severe as evidenced by multifocal enlargement of cells that became necrotic by 105 days postexposure. Although abnormal hepatocytic eosinophilic granularity persisted, hepatocytes of CTFE-exposed rats appeared to partially recover at 236 days postexposure, as indicated by reduced cytoplasmic volume and reduction in the frequency and severity of cytoplasmic vacuolar degeneration.

A retrospective histopathologic review of archived rat liver histology slides from a subchronic study of the effects of orally administered Halocarbon 27-S (unpublished data), another CTFE polymer, revealed slightly increased hepatocytic volume, accumulation of eosinophilic cytoplasmic granules, and reduced cytoplasmic basophilia. Perfluorodecanoic acid (PFDA), a chemical structurally related to CTFE, also causes hepatocytic enlargement that was less severe than that caused by CTFE in this study (Van Rafelghem et al., 1987). Light microscopically, the enlarged hepatocytes from PFDA-treated rats had increased cytoplasmic eosinophilic granularity with reduced cytoplasmic basophilia. Electron microscopic studies of hepatocytic effects caused by PFDA exposure demonstrated peroxisome proliferation and cytoplasmic accumulation of membranous profiles (Van Rafelghem et al., 1987). Several hypolipidemic pharmaceuticals and industrial plasticizers are known to cause peroxisome proliferation in hepatocytes (Reddy and Lalwani, 1983).

It has been suggested that CTFE oligomers may be metabolized to the corresponding halo-fatty acids. Such metabolism could provide a mechanistic explanation for the similar hepatotoxicities seen with CTFE, Halocarbon 27-S, and PFDA. The exact mechanism by which perhalogenated fatty acids

might disrupt normal cellular processes is unknown. However, it is possible that these acids act as substrates in normal biosynthetic pathways, leading to the formation of, for example, halogenated acetyl-CoA esters. It is presumed that these and other related species would interfere with normal metabolic processes.

The model developed performed well in fitting both the single and 90-day exposures. However, the goodness of fit could be improved if certain additional data were available. Since rapid decreases in levels of CTFE occurred in the first two days (see blood concentrations, Figs. 16 and 24), tissue concentration data obtained immediately after the acute exposure would allow a better fit of the model. Fitting the subchronic data would be aided with additional data points taken at 14 days exposure. A second source of data uncertainty relates to the partition coefficients. These were obtained from the ratios of measured tissue and blood concentrations immediately after the subchronic exposure. Because rapid declines occurred in CTFE tissue concentration immediately post-exposure, any lags in sampling between tissues and blood would produce an error in the partition coef'icient calculations. Furthermore, the significance of the lag would be exaggerated in any tissues which presented a diffusional limitation for the passage of CTFE. The simulation indicated that such a limitation occurred in fat. The lung:air partition coefficient was determined empirically by iteration to find a best fit of the simulation to the data. Direct determination of partition coefficients should be made for all tissues described in the model.

Results were not presented for the second group of higher molecular weight oligomers because part of the exposure to Group II oligomers was in aerosol form. It was uncertain how much aerosol actually entered and was absorbed by the rats, because CTFE exposure presumably occurred both by inhalation and by oral ingestion from grooming of droplets deposited on the animals' pelage. The model has not yet been modified to account for the aerosol exposure. Subsequent experiments will be done at lower concentrations where aerosol generation will be avoided entirely.

Postexposure inorganic fluoride levels in urine of animals that were repeatedly exposed to CTFE were elevated compared to urine of control animals (**Table 18**). The levels diminished to control levels over a 91-day period. The excess fluoride is presumed to be the result of CTFE biotransformation. Because the ends of the oligomers were capped with chlorine, there were either one or two fluorides available for release after the initial oxidation of the oligomer. There would thus be an average of 1.5 mol of fluoride released for every mol of CTFE metabolized. About 80% of CTFE was of the Group I oligomers. Approximately half of the fluoride released into the circulation would have been excreted in the urine with the remainder being stored in calcified tissue (Van Gelder, 1976). **Figure 31** shows the levels of fluoride (corrected for control values) in the urine up to 91 days postexposure. These are coplotted with the simulation.

The model indicated that CTFE pharmacokinetics are sensitive to the fat:blood and blood:air partition coefficients and to diffusional restriction on CTFE movement in fat. Moreover, the model showed that although the 90–day exposure was intermittent, fat concentrations of CTFE increased over time to the point where fat storage of CTFE drove continuous blood and tissue exposure vhich was modulated upward during the daily inhalation exposure. This latter observation is probably relevant to hepatic lesions which developed during this study and which are described elsewhere. In addition to providing insights to the pharmacokinetic behavior of CTFE, this study also illustrates PB-PK modeling of mixtures of structurally similar materials.

Results from this experiment are being used to design the sampling strategy for the next set of experiments to be done with CTFE. Additional sampling points will be selected to give a better experimental description of the change in concentration of CTFE in tissues postexposure. Partition coefficients will be determined using an *in vitro* method.

SECTION 5

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APPENDIX A

DRAIZE[a] SCALE FOR SCORING OCULAR LESIONS

Parameter	Score
1. CORNEA	
A. Opacity-degree of density (area most dense taken for reading)	
No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
B. Area of cornea involved	
One-quarter (or less), but not zero	1
Greater than one-quarter, but less than one-half	2
Greater than one-half, but less than three-quarters	3
Greater than three quarters, up to whole area	4
Score = A × B × 5 Total Maximum =	80
2. IRIS	
A. Values	
Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
Score = A × 5 Total Maximum =	10
3. CONJUNCTIVAE	
A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

continued

APPENDIX A (continued)

Parameter	Score
B. Chemosis	
No swelling	0
Any swelling above normal (including nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids above half closed to completely closed	4
C. Discharge	
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3
Score = (A + B + C) x 2 Total Maximum =	20

The TOTAL MAXIMUM SCORE is the sum of all scores obtained for the cornea, iris, and conjunctivae.

Total Maximum Score Possible =	110

[a] Draize, J.H., G. Woodard, H.O. Calvery. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharm. Exp. Therap.* 32:377-390.

APPENDIX B

DRAIZE[a] SCALE FOR EVALUATION AND SCORING OF SKIN REACTIONS

Parameter	Score
1. ERYTHEMA	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness)	4
2. EDEMA	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raising approx. 1 mm)	3
Severe edema (raising more than 1 mm and extending beyond area of exposure)	4
3. NECROSIS[b]	
No necrosis	0
Slight necrosis (less than one-fourth exposed area)	5
Moderate necrosis (one-fourth to one-half exposed area)	10
Severe necrosis (more than one-half exposed area)	15

[a] Draize, J.H., G. Woodard, and H.O. Calvery. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharm. Exp. Therap.* 32:377-390.

[b] Necrosis, for the purpose of this scoring system, is defined as a chemical denaturation of tissue sufficiently severe to result in fibrotic replacement (scar tissue). Superficial eschar which heals without scar is not classified as necrosis.

APPENDIX C

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH INTERPRETATION OF SKIN TEST RATINGS[a]

	Rating	Interpretation
Intact Skin	0 – 0.9	Nonirritant; probably safe for human skin contact
	1 – 1.9	Mild irritant; may be safe for use, but appropriate protective measures are recommended during contact
	2 – 4	Too irritating for human skin contact; avoid contact

[a] Campbell, K.I., E.L. George, L.L. Hale, and J.F. Stara. 1975. Dermal Irritancy of Metal Compounds. *Arch. Environ. Health.* 30:168-170.

APPENDIX D

GRADING SYSTEM[a] FOR SENSITIZATION TEST

Erythema			Edema		
0	–	None	0	–	None
1	–	Very Slight Pink	1	–	Very Slight
2	–	Slight Pink	2	–	Slight
3	–	Moderate Red	3	–	Moderate
4	–	Very Red	4	–	Marked

[a] Toxic Hazards Research Unit grading system for sensitization test.

APPENDIX E

SCALE[a] FOR DETERMINING SENSITIZATION POTENTIAL

Sensitization Rate (%)	Grade
10	Weak
20 – 30	Mild
40 – 60	Moderate
70 – 80	Strong
90 – 100	Extreme

[a] Toxic Hazards Research Unit grading system for sensitization potential.

APPENDIX F

MEAN[a] BODY WEIGHT GAINS (g) OF F-344 RATS DURING 90-DAY INHALATION EXPOSURE TO CTFE

	MALE			
Day	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
8	20.2 ± 0.9 (10)	19.3 ± 0.7 (10)	16.2 ± 7.0 (10)[c]	17.0 ± 2.6 (10)
15	11.2 ± 1.0 (10)	12.3 ± 0.5 (10)	8.8 ± 0.9 (10)	8.0 ± 0.7 (10)
22	8.7 ± 0.8 (10)	9.4 ± 0.9 (10)	9.8 ± 0.6 (9)	7.6 ± 0.9 (10)
29	14.1 ± 0.5 (10)	13.5 ± 0.8 (10)	10.1 ± 1.1 (9)[c]	6.7 ± 1.3 (10)[b]
43	20.1 ± 1.4 (10)	15.3 ± 1.4 (10)[b]	15.4 ± 1.3 (9)[b]	13.0 ± 1.3 (10)[b]
57	12.1 ± 1.5 (10)	11.7 ± 0.9 (10)	6.6 ± 0.9 (9)[b]	6.9 ± 1.9 (10)[b]
71	15.5 ± 1.9 (10)	15.1 ± 1.2 (10)	14.3 ± 1.1 (9)	8.5 ± 1.9 (10)[b]
85	8.0 ± 1.7 (10)	7.3 ± 0.9 (10)	6.2 ± 1.2 (9)	4.2 ± 2.5 (10)
91	6.4 ± 0.8 (10)	2.9 ± 0.9 (10)	4.1 ± 1.0 (9)	3.8 ± 1.2 (10)

	FEMALE			
Day	Control	0.25 mg/L	0.50 mg/L	1.00 mg/L
8	10.8 ± 0.8 (10)	8.8 ± 0.9 (10)	11.1 ± 0.9 (10)	7.2 ± 0.9 (10)[c]
15	3.5 ± 0.8 (10)	2.4 ± 0.6 (10)	2.9 ± 1.2 (10)	1.4 ± 0.7 (10)
22	5.7 ± 0.9 (10)	2.6 ± 0.5 (10)	2.3 ± 0.6 (10)	4.3 ± 0.9 (10)
29	1.6 ± 0.4 (10)	2.9 ± 0.4 (10)	5.5 ± 0.6 (10)	3.6 ± 0.6 (10)
43	6.8 ± 0.7 (10)	7.1 ± 0.5 (10)	6.3 ± 1.3 (10)	8.7 ± 1.0 (10)
57	4.2 ± 0.8 (10)	5.3 ± 0.8 (10)	3.6 ± 0.5 (10)	4.7 ± 0.9 (10)
71	5.7 ± 1.1 (10)	4.2 ± 0.7 (10)	3.0 ± 0.6 (10)	3.4 ± 1.0 (10)
85	4.3 ± 0.8 (10)	5.2 ± 0.5 (10)	4.2 ± 0.5 (10)	6.2 ± 1.0 (10)
91	0.9 ± 0.6 (10)	-0.4 ± 0.4 (10)	1.0 ± 0.4 (10)	0.1 ± 0.6 (10)

[a] Mean ± S.E.M. (N).

[b] Statistically different from controls $p<0.01$.

[c] Statistically different from controls $p<0.05$.

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QUALITY ASSURANCE

The study, "Subchronic Studies of Chlorotrifluoroethylene," was conducted by the NSI Technology Services Corporation, Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. The various phases of this study were inspected by members of the Quality Assurance Unit. Results of these inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION:	ITEM INSPECTED:
June 15, 1987	Study Protocol.
September 21, 1987 to November 12, 1987	Skin Sensitization.
October 5-8, 1987	Skin Irritation.
October 9-23, 1987	Eye Irritation.
September 8, 1987 to December 17, 1987	90-Day Inhalation exposure.
December 8, 1987 to January 27, 1988	Modeling samples.
September 19, 1988 to January 26, 1989	Final report and data audit.

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

M. G. Schneider

M. G. Schneider
QA Coordinator
Toxic Hazards Research Unit

Date 12 June 1989