National Toxicology Program
Toxicity Report Series
Number 45

NTP Technical Report
on Renal Toxicity Studies of Selected

Halogenated Ethanes

Administered by Gavage
to F344/N Rats

John R. Bucher, Ph.D., Study Scientist
National Toxicology Program
Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication 96-3935
February 1996

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Note to the Reader

The National Toxicology Program (NTP) is made up of four charter agencies of the United States Department of Health and Human Services (DHHS):

- the National Cancer Institute (NCI) of the National Institutes of Health;
- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

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The studies described in this toxicity study report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

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Post Office Box 12233
Research Triangle Park, NC 27709

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CONTRIBUTORS

This NTP report on the toxicity studies of halogenated ethanes is based primarily on studies that took place from January through March 1992.

National Toxicology Program
Evaluated experiment, interpreted results, and reported findings

John R. Bucher, Ph.D., Study Scientist
Leo T. Burka, Ph.D.
Rajendra S. Chhabra, Ph.D.
Michael R. Elwell, D.V.M., Ph.D.
Joel Mahler, D.V.M.
Bernard A. Schwetz, D.V.M., Ph.D.
Gregory S. Travlos, D.V.M.
Kristine L. Witt, M.S.
Oak Ridge Associated Universities

Microbiological Associates, Inc.
Principal contributors

Martin L. Wenk, Ph.D., Principal Investigator
J. Das, Ph.D.
Walter F. Loeb, V.M.D., Ph.D.
Lynda L. Pippin, D.V.M.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assessment

Jerry F. Hardisty, D.V.M.

Pathology Associates, Inc.
Stained and evaluated slides

Sandra Eldridge, Ph.D.

NTP Pathology Working Group
Evaluated slides and prepared pathology report

John C. Seely, D.V.M., Chair
PATHCO, Inc.
Michael R. Elwell, D.V.M., Ph.D.
National Toxicology Program
Akiko Enomoto, D.V.M.
NIEHS
Ronald A. Herbert, D.V.M., Ph.D.
National Toxicology Program
Joel Mahler, D.V.M.
National Toxicology Program
Ann Radovsky, D.V.M., Ph.D.
National Toxicology Program

Analytical Sciences, Inc.
Provided statistical analyses

Sarah Rosenblum, M.S.
Richard W. Morris, M.S.
Sarah Rosenblum, M.S.

Biotechnical Services, Inc.
Provided toxicity report preparation

Daphne D. Lambright, Ph.D.,
Principal Investigator
C. Michael Bailey, B.S. Pharm.
Waynette D. Sharp, B.A., B.S.

PEER REVIEW

The draft report on the toxicity study of halogenated ethanes was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly. While the reviewers' comments receive careful consideration, the final interpretation of these studies represents the position of the NTP.

Thomas L. Goldsworthy, Ph.D.
Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Brian G. Short, D.V.M., Ph.D.
SmithKline Beecham Pharmaceuticals
King of Prussia, PA

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ABSTRACT

Halogenated Ethanes

The National Cancer Institute and National Toxicology Program have performed 2-year toxicology and carcinogenesis studies with a number of ethanes substituted with chlorine or bromine. A review of the results of studies with these halogenated ethanes has revealed several consistencies between the pattern of halogen substitution and neoplastic responses in some affected organs. One of these consistencies was the finding of a modest increase in the incidence of renal tubule cell neoplasms in male rats administered penta- or hexachloroethane. Certain aspects of the nephropathy also noted in these studies resembled what is now recognized as a distinct hyaline droplet nephropathy typically associated with the accumulation of α_{2u}-globulin in renal tubule cells. In an attempt to determine some of the structure activity relationships involved in the induction of hyaline droplet nephropathy by halogenated ethanes, a series of commercially available ethanes substituted with three or more chlorines, four or more bromines, or a combination of chlorines and fluorines was studied in a short-term renal toxicity assessment in male F344/N rats.

All chemicals were administered by gavage in corn oil to groups of five male rats once daily for 21 days. The doses selected for study, 0.62 and 1.24 mmol/kg per day, were based on those used in the 2-year pentachloroethane studies. The following chemicals were evaluated: 1,1,1,2- and 1,1,2,2-tetrachloroethane; pentachloroethane; 1,1,2,2-tetrachloro-1,2-difluoroethane; 1,1,1-trichloro-2,2,2-trifluoroethane; 1,2-dichloro-1,1-difluoroethane; 1,1,1-trichloroethane; hexachloroethane; 1,1,1,2- and 1,1,2,2-tetrabromoethane; and pentabromoethane. Evaluations included survival, mean body weight gains, clinical signs, organ weights, urinalysis, and histopathologic examination of the right kidney and liver. The kidneys of rats that showed a difference in renal protein droplet accumulation compared to the controls were evaluated for replicative DNA synthesis by staining for proliferating cell nuclear antigen.

For most groups, survival was not affected by chemical treatment; however, all rats administered either dose of 1,1,2,2-tetrabromoethane died by Day 11, and all rats administered 1.24 mmol/kg pentabromoethane, 1,1,1,2-tetrabromoethane, or 1,1,2,2-tetrachloroethane died before the end of the study. Rats receiving 0.62 mmol/kg pentabromoethane gained less weight than the controls, and rats in the 0.62 mmol/kg 1,1,1,2-tetrabromoethane group lost weight during the study. Increased kidney weights and signs of renal toxicity, indicated by urinalysis results, were noted in rats in many of the groups administered halogenated ethanes, but these observations were not always coincident with a diagnosis of hyaline droplet nephropathy. Hyaline droplet nephropathy was observed only in rats receiving penta-, hexa-, or 1,1,1,2-tetrachloroethane. The renal tubule cell labeling index was increased, indicating replicative DNA synthesis, in male rats receiving chemicals that induced hyaline droplet nephropathy as well as in males receiving pentabromoethane or 1,1,2,2-tetrachloroethane and in female negative control rats administered pentachloroethane; thus some of the halogenated ethanes appeared to cause significant renal toxicity not associated with hyaline droplet nephropathy.

In summary, of the halogenated ethanes studied, the capacity to induce hyaline droplet nephropathy in male rats was restricted to ethanes containing four or more halogens, and only the chlorinated ethanes were active. If the ability to induce hyaline droplet nephropathy is the determining factor in the induction of renal tubule cell neoplasms by halogenated ethanes, then an absence of kidney neoplasms in male rats would be predicted in the event that 2-year studies were performed with the bromo- or chlorofluoroethanes.

INTRODUCTION

The National Cancer Institute (NCI) and the National Toxicology Program (NTP) have performed chronic toxicology and carcinogenesis studies in rats and mice on 11 members of the halogenated ethane chemical class. These range from the singly halogenated chemicals chloroethane (NTP, 1989a) and bromoethane (NTP, 1989b) through 1,1- and 1,2-dichloroethane (NCI, 1978a,b); 1,2-dibromoethane (NCI, 1978c; NTP, 1982); 1,1,1- and 1,1,2-trichloroethane (NCI, 1977, 1978d); 1,1,1,2- and 1,1,2,2-tetrachloroethane (NCI, 1978e; NTP, 1983a); and pentachloroethane (NTP, 1983b); to the fully halogenated hexachloroethane (NCI, 1978f; NTP, 1989c). The findings of these studies reveal some general neoplasm patterns in relation to chemical structure.

Among these findings were a marked uterine neoplasm response in mice exposed to bromoethane (NTP, 1989b) or chloroethane (NTP, 1989a) and a weaker response to 1,2-dichloroethane (NCI, 1978b). No clearly increased incidences of uterine neoplasms were observed in animals receiving the more highly halogenated ethanes. Recent studies have failed to show changes in the concentrations of circulating sex hormones in response to exposure to chloro- or bromoethane (Bucher *et al.*, 1995), and the mechanism by which chloro- and bromoethane induce these neoplasms remains unknown.

In another finding, male and female mice administered any of the ethanes with three or more halogens had greater incidences of liver neoplasms than the controls. An exception to this was the study of 1,1,1-trichloroethane (NCI, 1977), in which there were no increased incidences of liver neoplasms in dosed rats or mice; however, this study was considered an inadequate assessment of carcinogenicity due to poor survival and because the duration of dosing was less than 2 years. Apparently, none of the halogenated ethanes induce neoplasms in the rat liver.

A third finding was that site-of-contact neoplasms (forestomach neoplasms in gavage studies and nasal cavity neoplasms in inhalation studies) occurred in studies of ethanes with one halogen on each carbon. The neoplasms developed in exposed rats and mice and were more likely to occur with brominated rather than chlorinated ethanes.

The fourth finding was that renal tubule cell neoplasms occurred primarily in male rats that received pentachloroethane (NTP, 1983b) or hexachloroethane (NCI, 1978f; NTP, 1989c). These neoplasm incidences were dose related and were associated with evidence of α_{2u}-globulin accumulation in the renal tubular epithelium. A single neoplasm (an incidence of 2%) also occurred in each of the high-

dose groups in the 1,1,1,2- and 1,1,2,2-tetrachloroethane studies (NCI, 1978e; NTP, 1983a) and in each of the two dosed groups in the 1,1,2-trichloroethane study (NCI, 1978d); none were observed in any of the control groups of these three studies.

These relationships between chemical structure and neoplastic response are relatively clear because, in most instances, the neoplasm incidences in dosed groups represent marked increases over the control incidences, and there is little doubt that similar diagnostic criteria were used between the various studies. However, several factors complicate more detailed analyses. The studies were performed from the mid 1970's to the present. Study dosing durations ranged from 78 weeks to 104 weeks (although some groups were killed earlier due to poor survival), and the Osborne-Mendel rat strain, rather than the Fischer 344, was used in some of the studies. In addition, descriptions of observed specific organ toxicity are limited for some of the earlier prechronic studies that were used for dose setting for the chronic studies. Thus, more subtle relationships, particularly between toxic and carcinogenic effects, cannot be determined.

This lack of comparative information on prechronic study lesions makes it particularly difficult to understand the patterns of toxic effects produced in the rat kidney. Certain of these chemicals have caused "toxic nephropathy" (1,2-dibromoethane); others have caused "toxic tubular nephropathy" with lesions consistent with α_{2u}-globulin or hyaline droplet nephropathy (penta- and hexachloroethane); and 1,1,1,2-tetrachloroethane caused papillary mineralization and an accumulation of crystals in the tubule lumen, lesions that are suggestive of the α_{2u}-globulin syndrome.

The importance of closely examining the spectrum of toxic renal effects produced with this class of chemicals lies in the decision of the United States Environmental Protection Agency (USEPA, 1991) calling for a distinction between renal tubule neoplasms produced in male rats in "association with CIGA [Chemicals Inducing alpha$_{2u}$-Globulin Accumulation]-induced alpha$_{2u}$-nephropathy" and "other renal tubule tumors in terms of use in human risk assessment." This is based on the view that the occurrence of the α_{2u}-globulin is specific to sexually mature male rats, and the hyaline droplet syndrome described in male rats exposed to chemicals is a result of a tight association of the parent chemical or a metabolite with the globulin, thus rendering the protein-chemical complex resistant to normal lysosome degradation in renal tubule epithelial cells. According to the USEPA (1991):

> "Excessive accumulation of hyaline droplets in proximal tubules, representing lysosomal overload, leads to tubule cell degeneration, cell loss, and regenerative cellular proliferation. Cell debris in the form of granular casts accumulates at the corticomedullary junction with associated dilation of the affected tubule segment and more distally, mineralization of tubules within the renal medulla. Single cell necrosis accompanied by compensatory cell proliferation and exacerbation of the chronic progressive nephropathy (CPN) characteristically found in aging rats occurs. Renal tubule hyperplasia and neoplasia develop subsequently."

Study Rationale and Design

As part of a larger effort to understand the structure activity relationships and putative mechanisms of carcinogenesis of halogenated ethanes, the study presented in this report was designed specifically to examine the influence of the number, type, and distribution of halogens over the ethane molecule on hyaline droplet nephropathy in the proximal and distal tubule epithelium of the male rat kidney. To evaluate this relationship further, sexually mature male Fischer 344/N rats received equimolar doses of the chemicals listed in Table 1 by gavage once daily for 21 days without interruption. The liver and kidney of these rats were evaluated histopathologically, and selected samples were stained for proliferating cell nuclear antigen to allow an estimate of the fraction of cells undergoing replicative DNA synthesis. Urinalysis was also performed as an additional measure of renal injury. Groups of female rats used as controls received either pentachloroethane in corn oil or the corn oil vehicle only.

The chemicals selected for study (Table 1) were drawn from those commercially available and containing three or more halogens or combinations of halogens (chlorine, bromine, and fluorine); iodinated compounds were not studied. Each of the chemicals tested for mutagenicity in *Salmonella typhimurium* was either not mutagenic or only weakly positive (Table 2). The doses used in this study were equivalent to those used in the 2-year gavage studies of pentachloroethane (NTP, 1983b). In some instances, these doses proved excessive and mortality occurred.

TABLE 1 Physical and Chemical Characteristics of Halogenated Ethanes

Compound	CAS Number	Chemical Formula	Molecular Weight	Physical Description	Specific Gravity
PART A					
1,1,2,2-Tetrachloroethane	79-34-5	$C_2H_2Cl_4$	167.85	colorless liquid	1.5866 at 25° C[1]
1,1,1,2-Tetrachloroethane	630-20-6	$C_2H_2Cl_4$	167.85	colorless liquid	1.56 at 20° C[2]
Pentachloroethane	76-01-7	C_2HCl_5	202.8	colorless liquid	1.6712 at 25° C[1]
PART B					
1,1,2,2-Tetrachloro-1,2-difluoroethane	76-12-0	$C_2Cl_4F_2$	203.8	colorless liquid	1.6447 at 25° C[3]
1,1,1-Trichloro-2,2,2-trifluoroethane	354-58-5	$C_2Cl_3F_3$	187.5	colorless liquid	1.579[2]
1,2-Dichloro-1,1-difluoroethane	1649-08-7	$C_2H_2Cl_2F_2$	134.9	colorless liquid	1.4163 at 20° C[3]
1,1,1-Trichloroethane	71-55-6	$C_2H_3Cl_3$	133.41	colorless liquid	1.3376 at 20° C[1]
PART C					
Hexachloroethane	67-72-1	C_2Cl_6	236.74	white crystalline powder	2.09[1]
1,1,2,2-Tetrabromoethane	79-27-6	$C_2H_2Br_4$	345.67	colorless liquid	2.964[1]
Pentabromoethane	75-95-6	C_2HBr_5	424.55	pale yellow crystalline solid	not available
1,1,1,2-Tetrabromoethane	630-16-0	$C_2H_2Br_4$	345.67	yellow liquid	2.885[2]

[1] *Merck Index*, 1989.
[2] Provided by the supplier.
[3] *Hawley's Condensed Chemical Dictionary*, 1987.

TABLE 2 Results of NTP *Salmonella typhimurium* Mutagenicity Tests with a Series of Halogenated Ethane Compounds

Compound	CAS Number	*Salmonella typhimurium* Mutagenicity Test Results
1,1,1,2-Tetrachloroethane	630-20-6	negative[1]
1,1,2,2-Tetrachloroethane	79-34-5	negative[1]
Pentachloroethane	76-01-7	negative[1]
1,1,2,2-Tetrachloro-1,2-difluoroethane	76-12-0	weakly positive[2]
1,1,1-Trichloro-2,2,2-trifluoroethane	354-58-5	not tested
1,2-Dichloro-1,1-difluoroethane	1649-08-7	not tested
1,1,1-Trichloroethane	71-55-6	negative[3]
Hexachloroethane	67-72-1	negative[1]
1,1,2,2-Tetrabromoethane	79-27-6	negative[2]
Pentabromoethane	75-95-6	negative[2]
1,1,1,2-Tetrabromoethane	630-16-0	weakly positive with rat S9[2]

[1] Haworth *et al.*, 1983.
[2] NTP, unpublished data.
[3] Zeiger *et al.*, 1987.

The collected results of this study are presented in the text for each chemical individually. Tabular information is presented for each endpoint, in most cases retaining the groupings of chemicals studied in each segment of the three-part study (Parts A, B, and C; Table 1). These groupings allow comparisons to be made with the appropriate control group. Mortality, weight gains, clinical signs, liver and kidney weights and histopathology, urinalysis, and renal tubule cell labeling indexes are presented. Information on potential hepatotoxicity is presented because marked hepatotoxicity can significantly impair synthesis of serum proteins, including α_{2u}-globulin, possibly leading to an underexpression of chemically induced hyaline droplet nephropathy.

MATERIALS AND METHODS

Procurement and Characterization of Halogenated Ethanes

The lot numbers and suppliers of the 11 halogenated ethanes are listed in Table 3. Information on identity, purity, and stability was provided by the suppliers.

1,1,2,2-TCE was identified by infrared and nuclear magnetic resonance spectroscopy. PCE, 1,1,1-TriC, 1,1,2,2-TBE, and HCE were identified by infrared spectroscopy. The purity of all chemicals except PBE was determined by gas chromatography and ranged from approximately 98% to 100% (Table 3). Because PBE is unstable thermally and in polar solvents, purity was determined based on a bromine content of 94.1%; a purity of greater than 99% was calculated. The following impurities were also identified with gas chromatography: 0.2% tri-, 0.2% tetra-, and 1.6% hexachloroethane in PCE; 0.8% 1,1,2-trichloro-1,2,2-trifluoroethane in 1,1,1-TriC-2,2,2-TFE; and 1.6% 1,1,2,2-tetrabromoethane and 0.6% 1,1,2-tribromoethane in 1,1,1,2-TBE.

Because material safety data sheets from the suppliers and data provided by the NTP (Tracor Jitco, Inc., 1980; Arthur D. Little, Inc., 1984, 1989; MRI, 1988) indicate that the bulk chemicals are stable under normal laboratory conditions, no stability studies were performed by the study laboratory. Throughout the 3-week studies, all bulk chemicals except HCE were stored in the dark at room temperature; HCE was stored refrigerated. 1,1,2,2-TCE and 1,1,1-TriC were stored under a nitrogen headspace.

The study laboratory reanalyzed the bulk chemicals at receipt with infrared spectroscopy; the spectra of all chemicals except 1,1,1,2-TBE were identical to reference spectra provided by chemical suppliers or Midwest Research Institute (MRI, 1984) or taken from the literature (Bucker and Nielsen, 1963; Grasselli and Ritchey, 1975). The identity of 1,1,1,2-TBE was confirmed by comparing its spectrum to the reference spectrum of 1,1,1,2-TCE, which has a similar structure.

TABLE 3 Purity and Supplier Information for Halogenated Ethanes

Chemical	CAS Number	Lot Number	Purity[1] (%)	Supplier
PART A				
1,1,2,2-Tetrachloroethane (1,1,2,2-TCE)	79-34-5	06226JW	99%	Aldrich Chemical Company, Milwaukee, WI
1,1,1,2-Tetrachloroethane (1,1,1,2-TCE)	630-20-6	AX01	99%	TCI America, Portland, OR
Pentachloroethane (PCE)	76-01-7	AY01	98%	TCI America, Portland, OR
PART B				
1,1,2,2-Tetrachloro-1,2-difluoroethane (1,1,2,2-TC-1,2-DFE)	76-12-0	11162	100%	PCR, Inc., Gainesville, FL
1,1,1-Trichloro-2,2,2-trifluoroethane (1,1,1-TriC-2,2,2-TFE)	354-58-5	0730191-2	99%	Columbia Organic Chemical Company, Inc., Camden, SC
1,2-Dichloro-1,1-difluoroethane (1,2-DC-1,1-DFE)	1649-08-7	9658	98%	PCR, Inc., Gainesville, FL
1,1,1-Trichloroethane (1,1,1-TriC)	71-55-6	01802CX	100%	Aldrich Chemical Company, Milwaukee, WI
PART C				
Hexachloroethane (HCE)	67-72-1	02624BW	100%	Aldrich Chemical Company, Milwaukee, WI
1,1,2,2-Tetrabromoethane (1,1,2,2-TBE)	79-27-6	02816TT	98%	Aldrich Chemical Company, Milwaukee, WI
Pentabromoethane (PBE)	75-95-6	AP01	>99%	TCI America, Portland, OR
1,1,1,2-Tetrabromoethane (1,1,1,2-TBE)	630-16-0	0730091	98%	Columbia Organic Chemical Company, Inc., Camden, SC

[1] Purity documented by supplier.

Dose Formulations

Dose formulations were prepared by mixing each chemical with corn oil and magnetically stirring the mixtures.

Stability studies of the dose formulations under storage conditions and under use conditions (under a nitrogen head space; simulated dosing procedure was performed after a storage interval) were performed by the study laboratory with gas chromatography with flame ionization detection (FID). The results indicated that the chemical/corn oil solutions were stable for 28 days when stored at 5° C. 1,1,1-TriC-2,2,2-TFE showed losses in concentration when stored at room temperature. 1,1,1-TriC-2,2,2-TFE and 1,2-DC-1,1-DFE also showed losses in concentration under use conditions after storage at room temperature or at 5° C. These results were attributed to the volatility of these compounds. All other dose formulations were stable when stored at room temperature or under use conditions.

One set of dose formulations for each chemical except 1,1,1,2-TBE was prepared 1 week before the 3-week study began. A second set of the 1,1,1-TriC-2,2,2-TFE formulations was prepared for the last week of dosing. To prevent losses due to volatility, dose formulations of 1,1,1,2-TBE were prepared 2 days before the study began and weekly thereafter. The dose formulations were stored under a nitrogen head space at 5° C. The study laboratory analyzed the dose formulations and animal room samples once by gas chromatography with FID. All dose formulations administered to the rats were within 10% of the theoretical dose. The animal room sample of 0.62 mmol/kg 1,2-DC-1,1-DFE was slightly less than 90% of the theoretical dose; all other animal room samples were within 10% of the theoretical dose.

Toxicity Study Designs

BASE STUDY

Male and female F344/N rats were obtained from Taconic Farms (Germantown, NY) and were 12 weeks of age at receipt. The rats were quarantined 15 days and were 15 weeks old when the study began. Additional details concerning the study design are provided in Table 4.

The highest pentachloroethane dose used in the 3-week study was selected to be equimolar to the highest dose used in previous 2-year studies (NTP, 1983b) and was expected to produce a clear hyaline droplet accumulation; the low dose selected was one-half the high dose. The other halogenated ethanes were administered at the same equimolar doses to provide a comparison of potential effects. In Part A of the study, groups of five male rats were administered 0.62 or 1.24 mmol/kg PCE, 1,1,2,2-TCE, or 1,1,1,2-TCE by gavage in a corn oil vehicle (5 mL/kg body weight) 7 days a week for 3 weeks. Additionally, five male and five female rats received the corn oil vehicle only, and five females designated as negative controls were administered 1.24 mmol/kg PCE. In Part B, groups of five male rats were administered the vehicle or 0.62 or 1.24 mmol/kg 1,1,2,2-TC-1,2-DFE, 1,2-DC-1,1-DFE, 1,1,1-TriC-2,2,2-TFE, or 1,1,1-TriC. In Part C, groups of five male rats were administered the vehicle or 0.62 or 1.24 mmol/kg HCE, 1,1,2,2-TBE, PBE, or 1,1,1,2-TBE.

Rats were housed five per cage by sex. The animal room was maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and at least 10 room air changes per hour. Feed and drinking water were available *ad libitum*.

Necropsies were performed on all rats that survived to the end of the study and on four rats administered 0.62 mmol/kg 1,1,2,2-TBE that died early. The right kidney, liver, and right testis were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A Mallory-Heidenhain stain was applied to sections of kidney to allow for a more sensitive evaluation of protein droplets. Histopathologic examinations were performed on the four necropsied rats from the 0.62 mmol/kg 1,1,2,2-TBE group and on all rats that survived to the end of the study. The right kidney, the left lobe of the liver, and any lesions observed grossly at necropsy were examined microscopically.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

SUPPLEMENTAL EVALUATIONS

Urinalysis

Urinalysis was performed on all rats at the end of the 3-week study. Urinalysis samples were collected from all rats during an overnight period that began 4 days before the end of the study. Urine samples were collected over a 16-hour period from fasted rats individually housed in metabolism cages. The foil-wrapped urine collection container was surrounded by wet ice during the collection period. After volume and specific gravity were measured, creatinine, glucose, total protein, aspartate aminotransferase, γ-glutamyl transpeptidase, and *N*-acetyl-β-D-glucosaminidase were measured with a chemistry analyzer.

Cell Proliferation Analyses

Cell proliferation analyses were performed on kidney sections from rats in Parts A and C that survived to the end of the 3-week study; rats from Part B were not evaluated because no evidence of hyaline droplet nephropathy was observed in routine histopathology or with Mallory-Heidenhain stain. Sections were stained with proliferating cell nuclear antigen stain. At least 4,000 proximal and distal tubule epithelial cells were scored per animal. The labeling index, equal to the percentage of proximal and distal tubule epithelial cells in S-phase, was determined for each animal, and group means were calculated. Cells in S-phase were identified by uniformly dark-stained nuclei. Details of these methods have been reported by Eldridge *et al.* (1993).

TABLE 4 Experimental Design and Materials and Methods in the 3-Week Gavage Study of Halogenated Ethanes

Part A	Part B	Part C
EXPERIMENTAL DESIGN		
Study Laboratory Microbiological Associates, Inc. (Bethesda, MD)	Same as Part A	Same as Part A
Strain and Species F344/N rats	Same as Part A	Same as Part A
Animal Source Taconic Farms (Germantown, NY)	Same as Part A	Same as Part A
Size of Study Groups Five males per group plus five vehicle control and five negative control females	Five males	Five males
Doses/Duration of Dosing Male: Corn oil vehicle or 0.62 or 1.24 mmol/kg PCE, 1,1,2,2-TCE, or 1,1,1,2-TCE by gavage for 3 weeks Female: Corn oil vehicle or 1.24 mmol/kg PCE by gavage for 3 weeks	Corn oil vehicle or 0.62 or 1.24 mmol/kg 1,1,2,2-TC-2,2,2-DFE, 1,1,1-TriC-2,2,2-TFE, 1,2-DC-1,1-DFE, or 1,1,1-TriC in corn oil by gavage for 3 weeks	Corn oil vehicle or 0.62 or 1.24 mmol/kg HCE, 1,1,2,2-TBE, PBE, or 1,1,1,2-TBE in corn oil by gavage for 3 weeks
Date of First Dose 31 January 1992	7 February 1992	14 February 1992
Date of Last Dose 20 February 1992	27 February 1992	5 March 1992
Date of Necropsy 21 February 1992	28 February 1992	6 March 1992
Type and Frequency of Observation Animals were observed twice daily. Animals were weighed and clinical observations were recorded at the beginning of the study, weekly thereafter, and at necropsy.	Same as Part A	Same as Part A
Necropsy and Histologic Examinations Necropsies and histopathologic evaluations were performed on all rats surviving at the end of the study. The following organs were weighed at the end of the study: right kidney, liver, and right testis. The right kidney, left liver lobe, and gross lesions were histopathologically examined.	Same as Part A	Necropsies and histopathologic examinations were performed on four rats administered 0.62 mmol/kg 1,1,2,2-TBE that died early and on all rats surviving at the end of the study. Organs weighed and organs microscopically examined were the same as in Part A.

TABLE 4 Experimental Design and Materials and Methods in the 3-Week Gavage Study of Halogenated Ethanes (continued)

Part A	Part B	Part C
EXPERIMENTAL DESIGN (continued)		
Supplemental Evaluations Urinalysis Urine was collected overnight 4 days before the end of the study for urinalysis. Urinalysis parameters included creatinine, glucose, total protein, aspartate aminotransferase, γ-glutamyl transpeptidase, *N*-acetyl-β-D-glucosaminidase, volume, and specific gravity.	Same as Part A	Same as Part A
ANIMAL MAINTENANCE		
Time Held Before Study 15 days	15 days	15 days
Age When Study Began 15 weeks	15 weeks	15 weeks
Age When Killed 18 weeks	18 weeks	18 weeks
Method of Animal Distribution Animals were distributed randomly into groups of approximately equal initial mean body weight.	Same as Part A	Same as Part A
Diet NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form and water (Washington Suburban Sanitary Commission Potomac Plant) were available *ad libitum*.	Same as Part A	Same as Part A
Animal Room Environment Rats were housed five animals per cage by sex. The temperature was maintained at 69° to 75° F and relative humidity at 35% to 65%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.	Rats were housed five animals per cage. The temperature was maintained at 69° to 75° F and relative humidity at 35% to 65%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.	Same as Part B

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed with the parametric multiple comparisons procedures of Dunnett (1955). Urinalysis data, which typically have skewed distributions, were analyzed with the nonparametric multiple comparisons methods of Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF PROLIFERATING CELL NUCLEAR ANTIGEN LABELING INDEXES

Student's *t*-test for the equality of two means was used to analyze pairwise comparisons between dosed and control groups and to assess the significance of dose-response trends.

Quality Assurance

The animal studies of halogenated ethanes were performed in compliance with United States Food and Drug Administration Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance Unit of Microbiological Associates, Inc. performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

Tables of selected data for each of the halogenated ethanes are presented at the end of the Results. In these tables, survival and mean body weight data and mean proliferating cell nuclear antigen (PCNA) labeling indexes are presented by group: Group A contains 1,1,1,2- and 1,1,2,2-TCE and PCE; Group B contains 1,1,2,2-TC-1,2-DFE, 1,1,1-TriC-2,2,2-TFE, 1,2-DC-1,1-DFE, and 1,1,1-TriC; and Group C contains HCE, 1,1,1,2- and 1,1,2,2-TBE, and PBE.

Survival and body weight data are provided in Table 5. Kidney effects are summarized in Table 6. Mean PCNA labeling indexes are given in Table 7. Selected values for organ weights and urinalysis parameters of each of the halogenated ethanes are graphed as percent differences from the controls in Figures 1 through 7 at the end of the Results; complete organ weight and urinalysis data are presented by group in Appendixes A and B, respectively. Individual PCNA labeling indexes are provided in Appendix C. Data for liver lesions are not shown.

PCE

All male and female rats administered PCE survived until the end of the study. The final mean body weights and mean body weight gains of dosed males and females were similar to those of the controls. Clinical signs of toxicity in rats administered PCE included lethargy in all males and females and thinness in two males in the 1.24 mmol/kg group.

In male rats, absolute and relative right kidney and liver weights increased with increasing dose. Right kidney and liver weights of females administered 1.24 mmol/kg were also greater than those of the controls.

The urine glucose output by males in the 0.62 and 1.24 mmol/kg groups was significantly greater than that by the controls. For males in the 1.24 mmol/kg group, the *N*-acetyl-β-D-glucosaminidase (NAG) activity was higher than that of the controls. Females in the PCE control group had a slightly higher protein output than the vehicle controls.

Microscopically, kidney changes related to PCE treatment included accumulation of hyaline droplets and increased incidences of tubule regeneration and intratubular granular casts. There was marked accumulation of hyaline droplets in the renal tubules of males in each dosed group. Best seen with Mallory-Heidenhain-stained sections, the droplets were variably sized, bright red inclusions within

the cytoplasm of proximal convoluted tubule cells. In comparison with droplets observed in the controls, the droplets in dosed rats were abnormal, being more numerous, larger, or more crystalline in shape (Plates 1 through 4). Affected tubule cells were enlarged due to the presence of these abnormal droplets. Rats administered PCE had greater incidences of tubule regeneration in the kidneys than the controls. Tubule regeneration was a multifocal change characterized by clusters of immature tubule epithelial cells with more basophilic cytoplasm and a greater nuclear/cytoplasmic ratio than that observed in control rats (Plate 5). Another change in the kidneys that was associated with hyaline droplet accumulation was the presence of granular casts in the outer medullary tubules. Casts were eosinophilic, granular deposits within, and occasionally distending, the tubule lumen (Plate 6). Increased incidences of tubule regeneration and tubule casts occurred in all rats administered PCE; the severity of these changes was not dose related. The mean PCNA labeling index of cortical tubule cells was significantly higher in dosed males than in the controls. No detectable hyaline droplets or other evidence of renal histopathology was observed in female rats in the vehicle control or PCE control group; however, the mean PCNA labeling index was higher in females administered PCE than in the vehicle controls. Mineralization of the corticomedullary tubules occurred in both groups of females, with no appreciable difference in incidence between vehicle control and PCE control rats.

Minimal to mild vacuolization of hepatocytes was another microscopic effect of PCE treatment. This change occurred in four of five males and four of five females administered 1.24 mmol/kg and consisted of multiple small, clear spaces within affected cells. No zonal distribution of this change was noted. Minimal hepatocellular necrosis was observed in one male rat in the 1.24 mmol/kg group, but this lesion was of questionable toxicologic significance.

HCE

All male rats administered HCE survived until the end of the study. The final mean body weights and mean body weight gains of control and dosed males were similar. There were no clinical signs of toxicity.

Absolute and relative right kidney weights of male rats in the 0.62 and 1.24 mmol/kg groups and the relative liver weight of males in the 1.24 mmol/kg group were significantly greater than those of the controls.

For males in the 1.24 mmol/kg group, urinary creatinine and specific gravity were significantly lower and glucose output and urine volume were significantly greater than in the controls; aspartate aminotransferase (AST) and NAG activities of males in the 0.62 and 1.24 mmol/kg groups were greater than those of the controls.

Similar to PCE, HCE administration induced male rat nephropathy consisting of hyaline droplet accumulation and increased incidences of tubule regeneration and granular casts. The severity of hyaline droplets in males administered HCE was similar to that in males administered PCE; tubule regeneration and casts were less severe in HCE-treated males. The mean PCNA labeling index increased with dose and was significantly greater in both dosed groups than in the controls.

No microscopic effects were detected in the liver of males administered HCE.

1,1,1,2-TCE

All male rats administered 1,1,1,2-TCE survived until the end of the study. The final mean body weights and mean body weight gains of control and dosed males were similar. There were no clinical signs of toxicity.

Absolute and relative right kidney weights of males that received 1.24 mmol/kg were greater than those of the controls. Males that received 1.24 mmol/kg had a higher urine protein output and NAG activity and a lower γ-glutamyltransferase activity than the controls.

1,1,1,2-TCE induced nephropathy in male rats; this effect, similar to the nephropathy induced by PCE and HCE, consisted of hyaline droplet accumulation and increased incidences of tubule regeneration and granular casts. In rats administered 1,1,1,2-TCE, hyaline droplet accumulation was less severe and the number of foci of tubule regeneration per rat was lower in comparison to rats receiving the corresponding doses of PCE or HCE. Tubule casts were observed in the 1.24 mmol/kg 1,1,1,2-TCE group, but not in the 0.62 mmol/kg group. The mean renal PCNA labeling index in the 1.24 mmol/kg group was significantly higher than that in the controls.

No microscopic effects were detected in the liver of rats administered either dose of 1,1,1,2-TCE.

1,1,2,2-TCE

All male rats administered 1.24 mmol/kg 1,1,2,2-TCE died or were killed moribund before the end of the study. The final mean body weights and mean body weight gains of males in the 0.62 mmol/kg group were similar to those of the controls. All male rats in the 1.24 mmol/kg group were thin and lethargic; four males in this group had diarrhea, and three of these four rats also had abnormal breathing and ruffled fur.

The absolute and relative liver weights of male rats administered 0.62 mmol/kg were greater than those of the controls. There were no significant differences in urinalysis parameters between males in the 0.62 mmol/kg group and the controls.

The right kidneys of only the control and 0.62 mmol/kg groups were evaluated due to the 100% mortality of rats in the 1.24 mmol/kg group. No changes attributable to 1,1,2,2-TCE were observed. The amount, size, and shape of tubule hyaline droplets in the 0.62 mmol/kg group were similar to those of droplets in the controls; similarly, the mean PCNA labeling index for cortical tubule cells in dosed rats was similar to that of the controls.

Cytoplasmic vacuolization of hepatocytes occurred in all rats in the 0.62 mmol/kg group. This change was of mild to moderate severity and was similar to the effect seen in the liver of PCE-treated rats, consisting of multifocal areas of hepatocytes with clear droplets within the cytoplasm.

1,1,2,2-TBE

All male rats administered 1,1,2,2-TBE died or were killed moribund by Day 11 of the study. Males in the 0.62 mmol/kg group had nasal/eye discharge, and four males in this group were thin and lethargic and had ruffled fur. All males in the 1.24 mmol/kg group were lethargic and had abnormal breathing, ruffled fur, and nasal/eye discharge.

Absolute and relative right kidney weights of male rats in the 0.62 mmol/kg group were significantly greater than those of the controls. Other statistically significant differences in organ weights were considered secondary to the lower final mean body weight of this group.

Urinalysis parameters were not measured for rats that received 1,1,2,2-TBE because all rats died before the end of the study.

The right kidneys and livers of the four rats in the 0.62 mmol/kg group that survived through Day 11 were evaluated microscopically. In the kidney, no changes clearly attributable to 1,1,2,2-TBE administration were present. The incidence of minimal tubule regeneration was greater in the 0.62 mmol/kg group (4 of 4 examined) than in the controls (1/5); this increased incidence was considered equivocal evidence of a treatment effect. However, the amount, size, and shape of tubule hyaline droplets were similar those of droplets observed in the controls. PCNA staining was not performed due to the lack of detectable hyaline droplet accumulation in routine sections.

At the 0.62 mmol/kg dose level, cytoplasmic vacuolization of hepatocytes occurred in all rats examined. This change was of minimal to mild severity and was similar to the effect observed in the liver of rats treated with PCE or 1,1,2,2-TCE, consisting of multifocal areas of hepatocytes with clear droplets within the cytoplasm.

1,1,1-TriC

One male receiving 1.24 mmol/kg 1,1,1-TriC died on Day 2 of the study. The final mean body weights and mean body weight gains of dosed males were similar to those of the controls. There were no clinical signs of toxicity.

The relative liver weight of males in the 1.24 mmol/kg group was slightly greater than that of the controls. The urinary protein output and AST activity of males in the 1.24 mmol/kg group were greater than those of the controls.

Although the clinical pathology findings were highly suggestive of renal injury, no microscopic effects attributable to 1,1,1-TriC administration were present in either the kidney or the liver at either dose level. PCNA staining was not performed due to the absence of a detectable treatment effect in routine sections.

1,1,1-TriC-2,2,2-TFE

All male rats administered 1,1,1-TriC-2,2,2-TFE survived until the end of the study. The final mean body weights and mean body weight gains of dosed males were similar to those of the controls. There were no clinical signs of toxicity.

There were no significant differences in organ weights or urinalysis parameters between dosed and control males.

No microscopic effects attributable to 1,1,1-TriC-2,2,2-TFE administration were present in either the kidney or the liver at either dose level. PCNA staining was not performed due to the absence of a detectable treatment effect in routine sections.

1,1,2,2-TC-1,2-DFE

All male rats administered 1,1,2,2-TC-1,2-DFE survived until the end of the study. The final mean body weights and mean body weight gains of dosed males were similar to those of the controls. There were no clinical signs of toxicity.

There were no significant differences in organ weights between dosed and control males. Males in the 0.62 and 1.24 mmol/kg groups had a greater urine AST activity than the controls, and the 1.24 mmol/kg group also had slightly higher NAG activity than the controls.

No microscopic effects attributable to 1,1,2,2-TC-1,2-DFE administration were present in either the kidney or the liver at either dose level. PCNA staining was not performed due to the absence of a detectable treatment effect in routine sections.

1,2-DC-1,1-DFE

All male rats administered 1,2-DC-1,1-DFE survived until the end of the study. The final mean body weights and mean body weight gains of dosed males were similar to those of the controls. There were no clinical signs of toxicity.

Absolute and relative liver weights of male rats receiving 0.62 or 1.24 mmol/kg were greater than those of the controls. The relative right kidney weight of males in the 1.24 mmol/kg group was also slightly greater than that of the controls. There were no significant differences in urinalysis parameters between dosed and control males.

No microscopic effects attributable to 1,2-DC-1,1-DFE administration were present in either the kidney or the liver at either dose level. PCNA staining was not performed due to the absence of a detectable treatment effect in routine sections.

PBE

All male rats administered 1.24 mmol/kg PBE were killed moribund on Day 4 of the study. The mean body weight gain of males in the 0.62 mmol/kg group was less than that of the controls. All males in the 1.24 mmol/kg group were lethargic and had abnormal breathing, ruffled fur, nasal/eye discharge, and diarrhea.

Absolute and relative right kidney and liver weights of males in the 0.62 mmol/kg PBE group were greater than those of the controls. Males in the 0.62 mmol/kg PBE group had a higher urine glucose output and volume than the controls.

Only right kidneys of the control and 0.62 mmol/kg groups were evaluated due to the 100% mortality in the 1.24 mmol/kg group. Most of the halogenated ethanes tested caused either an increase or no change in the amount of tubule hyaline droplets relative to the number in control animals; in contrast, treatment with 0.62 mmol/kg PBE was associated with a reduced accumulation of tubule hyaline droplets. In routine sections, there were no other renal effects. For PCNA-stained sections from the 0.62 mmol/kg group, the mean labeling index was higher than that of the controls; however, this difference was primarily due to a markedly elevated index in one dosed rat and was not statistically significant.

The livers of all animals treated with 0.62 mmol/kg PBE had cytoplasmic vacuolization of hepatocytes. This change was similar to the effect in animals treated with PCE, 1,1,2,2-TCE, or 1,1,2,2-TBE; the severity, however, was greater in rats administered PBE.

1,1,1,2-TBE

All male rats in the 1.24 mmol/kg group died or were killed moribund on Day 5, and one male in the 0.62 mmol/kg group died on Day 21 of the study. The mean body weight gain of males in the 0.62 mmol/kg group was less than that of the controls; males in this group lost weight during the study. Males that received 1,1,1,2-TBE were thin and lethargic and had diarrhea, ruffled fur, and nasal/eye discharge; four males in the 1.24 mmol/kg group had abnormal breathing.

Absolute and relative right kidney weights of male rats in the 0.62 mmol/kg group were significantly greater than those of the controls. Other statistically significant differences in organ weights were considered secondary to the reduced body weight gain of this group.

The urinary creatinine output and specific gravity of males in the 0.62 mmol/kg group were significantly lower than those of the controls.

Only the right kidneys of the control and 0.62 mmol/kg groups were evaluated due to the 100% mortality of rats in the 1.24 mmol/kg group. Similar to PBE, treatment with 0.62 mmol/kg 1,1,1,2-TBE was associated with a reduced accumulation of hyaline droplets in the kidney. No other renal effects were observed. PCNA staining was not performed.

The livers of the four males treated with 0.62 mmol/kg that survived to the end of the study had cytoplasmic vacuolization of hepatocytes. This change was mild to moderate in severity and was similar to the effect in animals treated with PCE, 1,1,2,2-TCE, PBE, or 1,1,2,2-TBE.

TABLE 5 Survival and Body Weights of F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

Dose (mmol/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)
		Initial	Final	Change	
MALE					
Part A					
Vehicle control	5/5	308	327	19	
1,1,2,2-TCE					
0.62	5/5	305	312	7	95
1.24	0/5[3]	303	)	)	)
1,1,1,2-TCE					
0.62	5/5	305	331	27	101
1.24	5/5	305	324	19	99
PCE					
0.62	5/5	305	323	17	99
1.24	5/5	303	317	14	97
Part B					
Vehicle control	5/5	291	323	33	
1,1,2,2-TC-1,2-DFE					
0.62	5/5	292	328	36	101
1.24	5/5	292	327	35	101
1,1,1-TriC-2,2,2-TFE					
0.62	5/5	288	328	40	101
1.24	5/5	286	320	34	99
1,2-DC-1,1-DFE					
0.62	5/5	290	321	31	99
1.24	5/5	290	320	30	99
1,1,1-TriC					
0.62	5/5	290	332	41	103
1.24	4/5[4]	292	318	25	98
Part C					
Vehicle control	5/5	289	316	26	
HCE					
0.62	5/5	287	307	20	97
1.24	5/5	291	307	17	97
1,1,2,2-TBE					
0.62	0/5[5]	291	)	)	)
1.24	0/5[6]	288	)	)	)
PBE					
0.62	5/5	288	298	10	94
1.24	0/5[6]	290	)	)	)
1,1,1,2-TBE					
0.62	4/5[7]	287	282	−7	89
1.24	0/5[8]	293	)	)	)
FEMALE (Part A)					
Vehicle control	5/5	185	194	9	
PCE control					
1.24	5/5	183	188	5	97

[1] Number surviving at 3 weeks/number of animals per dose group.
[2] (Dose group mean/control group mean) × 100.
[3] Day of death: 13, 13, 14, 14, 14.
[4] Day of death: 2.
[5] Day of death: 5, 11, 11, 11, 11.
[6] Day of death: all on Day 4.
[7] Day of death: 21.
[8] Day of death: all on Day 5.

TABLE 6 Kidney Effects in Male F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

	Hyaline Droplet Nephropathy[1]	Tubule Regeneration[2]	Granular Casts[2]
PCE			
0.62	++	5/5 (2.2)	5/5 (3.0)
1.24	++	5/5 (1.6)	5/5 (1.8)
HCE			
0.62	++	3/5 (2.0)	4/5 (1.2)
1.24	++	5/5 (1.8)	3/5 (1.3)
1,1,1,2-TCE			
0.62	+	3/5 (1.0)	0/5
1.24	+	5/5 (1.6)	5/5 (1.4)
1,1,2,2-TCE			
0.62	0	0/5	0/5
1.24	)[3]	)	)
1,1,2,2-TBE			
0.62[4]	0	4/4 (1.0)	0/4
1.24	)	)	)
1,1,1-TriC			
0.62	0	0/5	0/5
1.24	0	0/5	0/5
1,1,1-TriC-2,2,2-TFE			
0.62	0	0/5	0/5
1.24	0	0/5	0/5
1,1,2,2-TC-1,2-DFE			
0.62	0	0/5	0/5
1.24	0	0/5	0/5
1,2-DC-1,1-DFE			
0.62	0	0/5	0/5
1.24	0	0/5	0/5
PBE			
0.62	↓	0/5	0/5
1.24	)	)	)
1,1,1,2-TBE			
0.62	↓	0/4	0/4
1.24	)	)	)

[1] + = hyaline droplet accumulation one severity grade above controls; ++ = hyaline droplet accumulation two severity grades above controls; 0 = no difference from the controls; ↓ = hyaline droplet accumulation less than in the controls.

[2] Incidences are given as number of animals with lesion/number of animals with kidney examined microscopically. Average severity (in parentheses) is based on the number of animals with lesions: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, and 5 = markedly severe.

[3] Not examined due to 100% mortality in group.

[4] The four rats in this group that survived until Day 11 were examined.

TABLE 7 Proliferating Cell Nuclear Antigen Labeling Indexes for F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes[1]

	Dose (mmol/kg)		
	Vehicle Control	0.62	1.24
MALE			
Part A			
1,1,2,2-TCE	0.40 ± 0.10	0.067 ± 0.018	)[2]
1,1,1,2-TCE		0.33 ± 0.05	0.89 ± 0.10*
PCE		1.4 ± 0.1*	1.3 ± 0.1*
Part C			
HCE	0.13 ± 0.02	0.74 ± 0.19*	1.2 ± 0.2*
PBE		0.82 ± 0.52	)

	Vehicle Control	1.24 mmol/kg PCE Control
FEMALE (Part A)	0.14 ± 0.04	0.38 ± 0.08*

[1] Labeling index = percentage of proximal and distal tubule epithelial cells in S-phase. At least 4,000 renal epithelial cells were scored per animal. Only rats from groups showing evidence of a change in protein droplet accumulation were evaluated.

[2] No animals in this group were examined microscopically.

* Significantly different ($P \leq 0.05$) from the control group by Student's *t*-test.

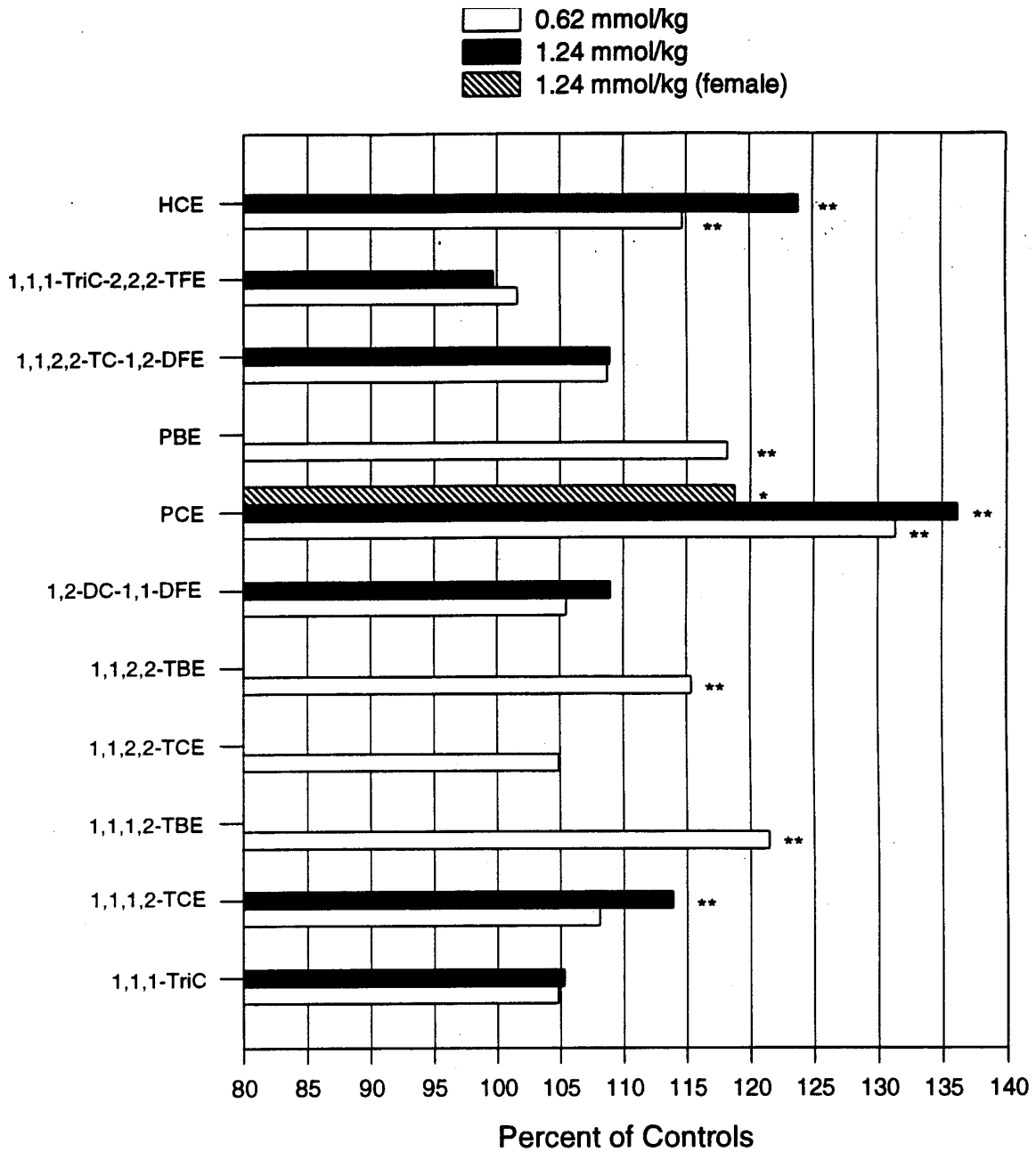

FIGURE 1 Absolute Right Kidney Weights of F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

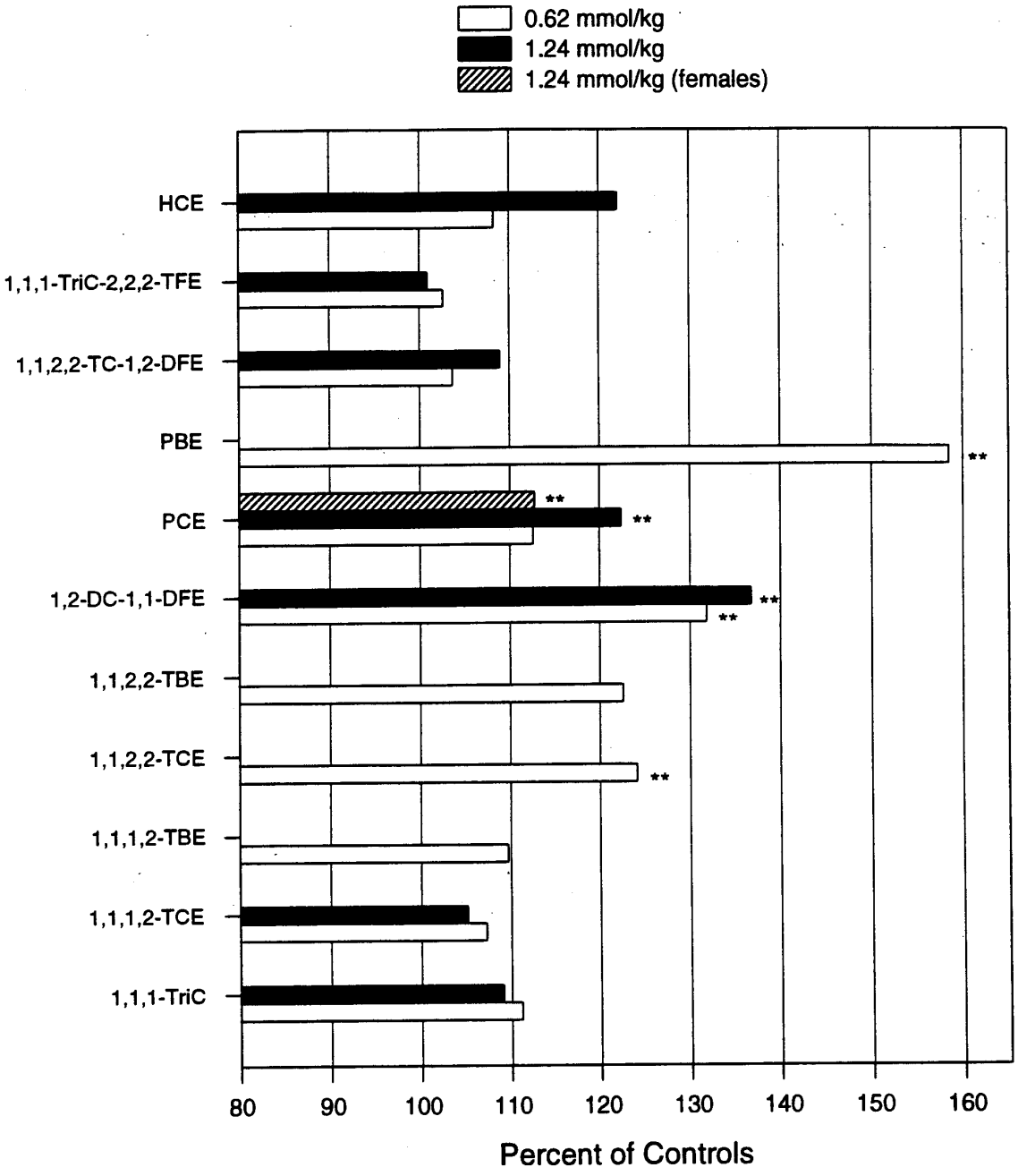

FIGURE 2 Absolute Liver Weights of F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

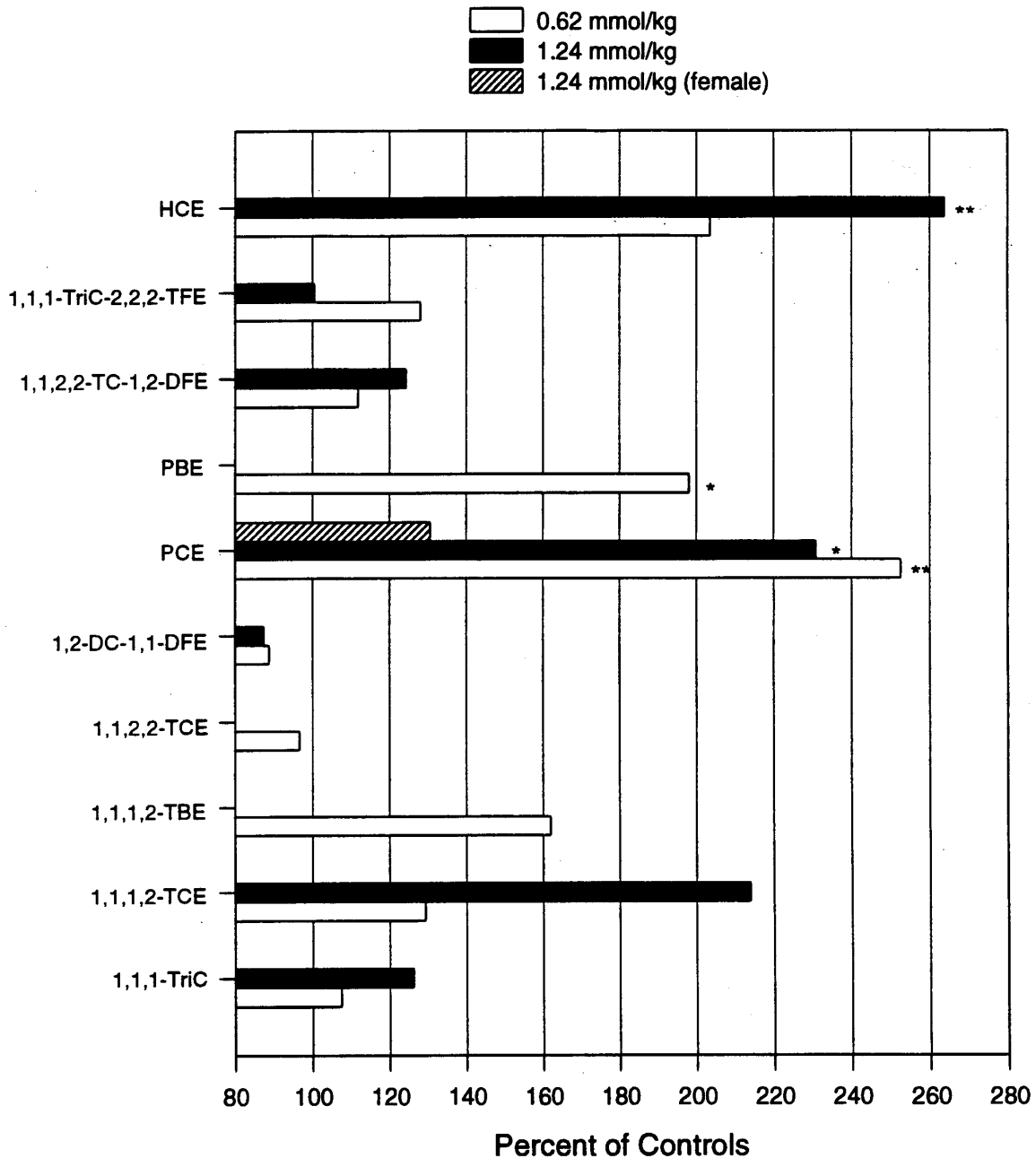

FIGURE 3 Glucose Output in the Urine of F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

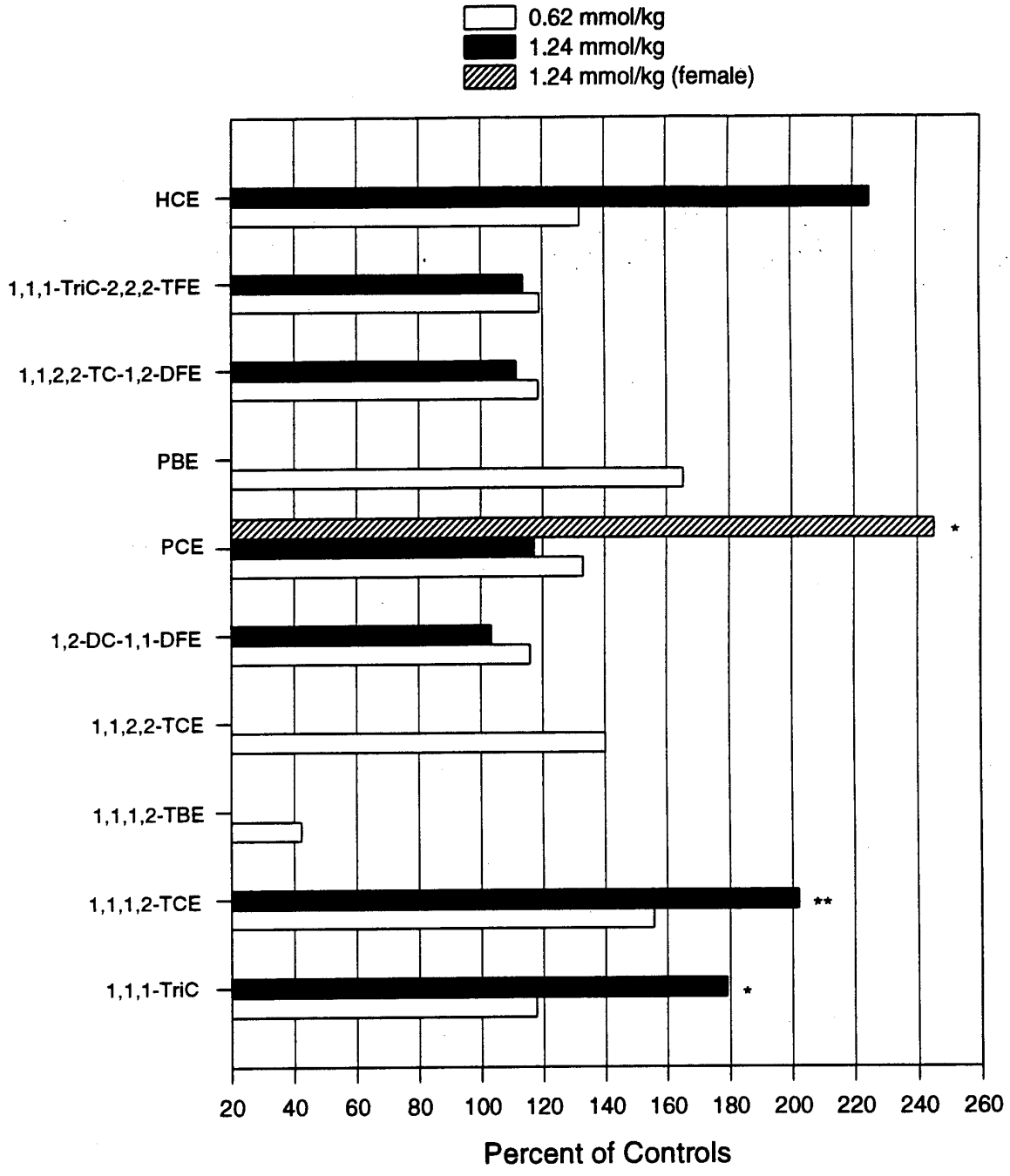

FIGURE 4 Protein Output in the Urine of F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

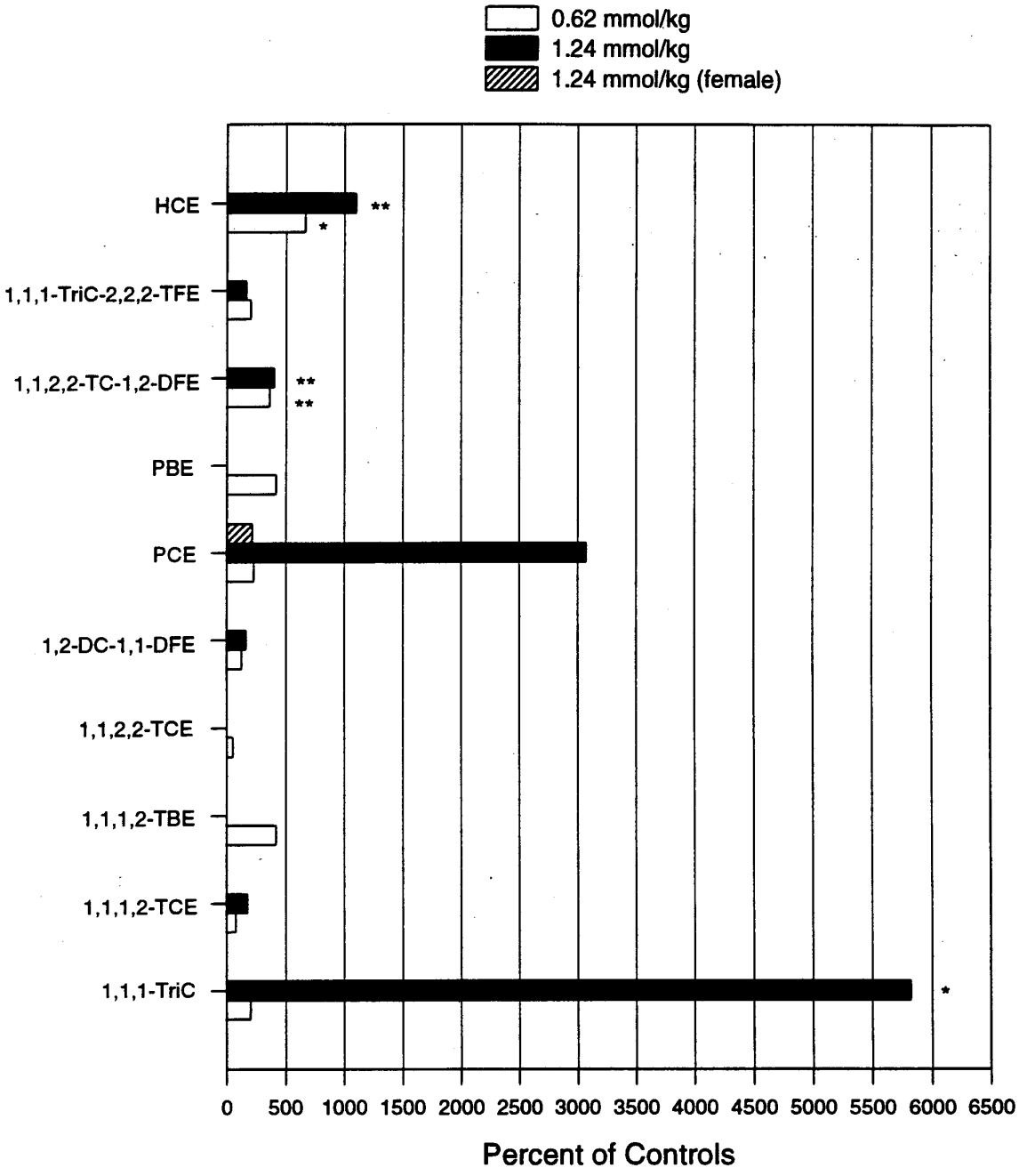

FIGURE 5 Urinary Aspartate Aminotransferase Activity in F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

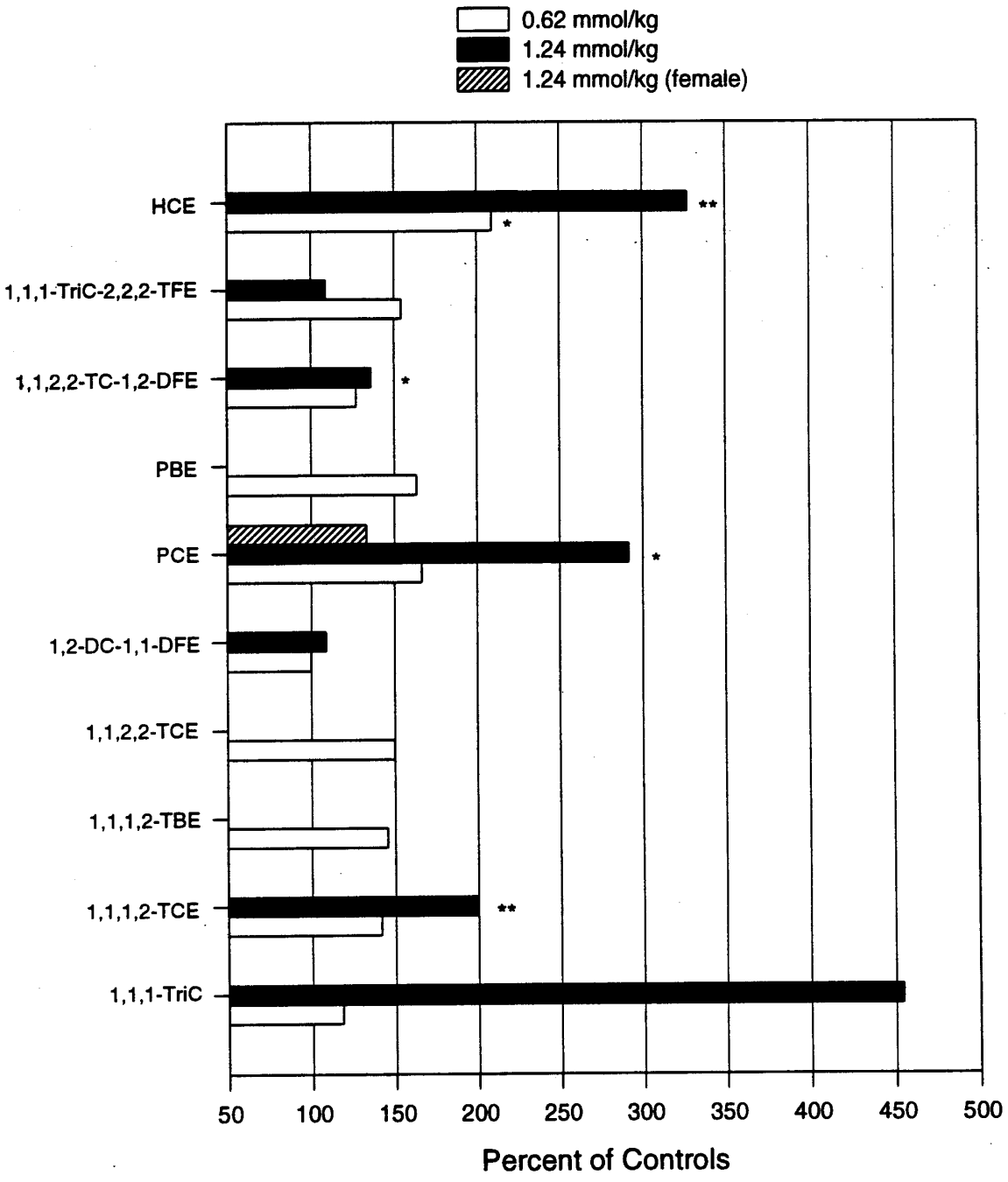

FIGURE 6 Urinary *N*-acetyl-β-D-glucosaminidase Activity in F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

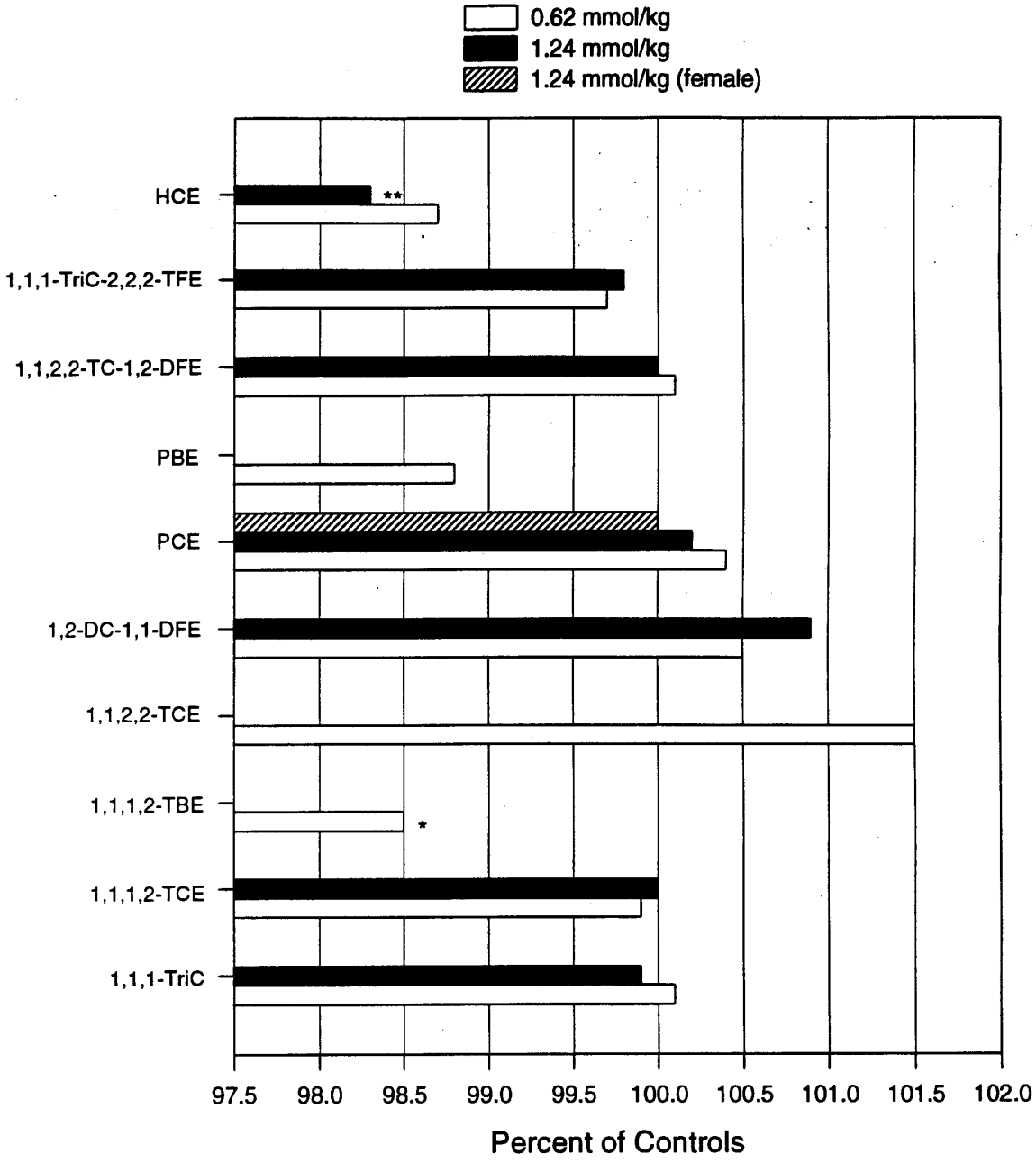

FIGURE 7 Urine Specific Gravity of F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes

PLATE 1

Kidney of a male rat administered 0.62 mmol/kg pentachloroethane by gavage for 3 weeks. Numerous cortical tubules are dark staining (arrows) due to the presence of intracellular hyaline droplets. Compare to the control rat kidney in Plate 2. Mallory-Heidenhain 60×.

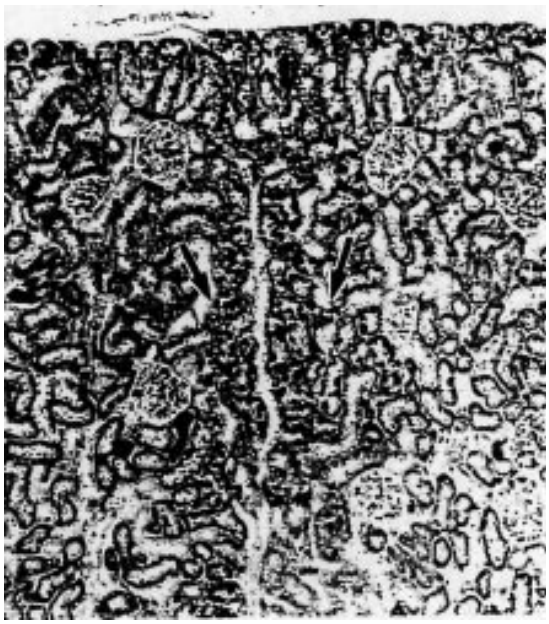

PLATE 2

Kidney of a control male rat. Tubules containing hyaline droplets (arrows) are less extensive and less prominent than in the treated rat. Mallory Heidenhain 60×.

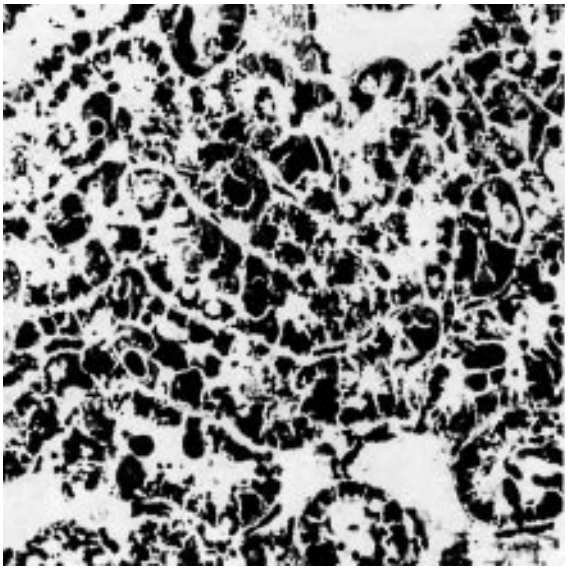

PLATE 3

Higher magnification of a kidney of a male rat administered 0.62 mmol/kg pentachloroethane by gavag for 3 weeks. Dark-staining hyaline droplets of various sizes and shapes fill the cytoplasm of the cortical covoluted tubules. Compare to normal kidney convoluted tubules shown in Plates 4. Mallory-Heidenhain 260×.

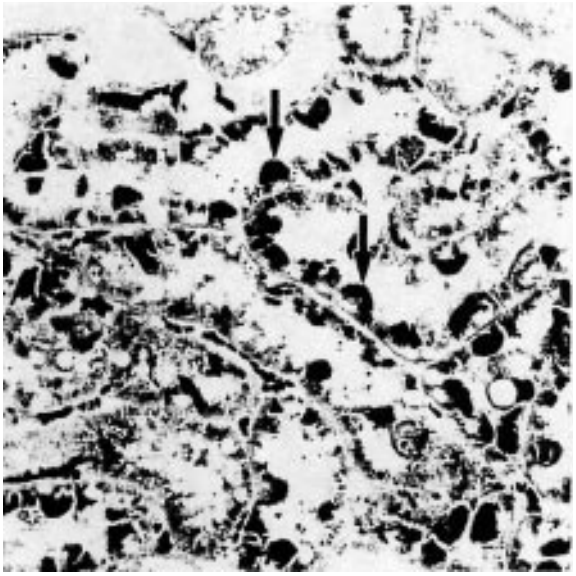

PLATE 4

Kidney convoluted tubules o a control male rat. Hyaline droplets are mostly single and small or occur as clusters in the apical cytoplasm of tubule epithelial cells (arrows). Mallory-Heidenhain 260×.

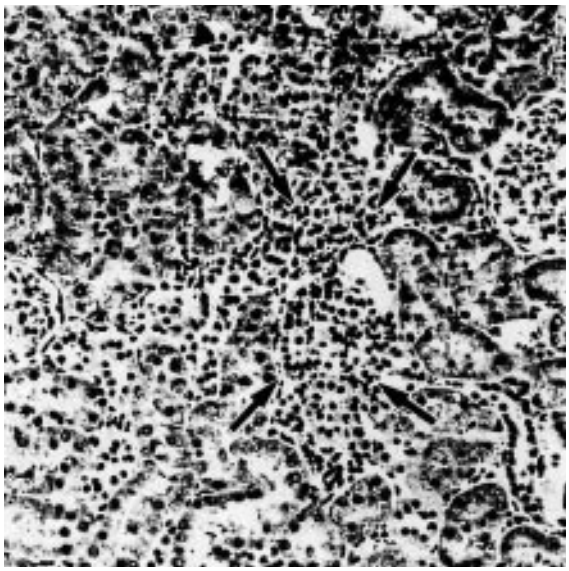

PLATE 5

A focus of tubule regeneration in the kidney of a male rat administered 1.24 mmol/kg pentachloroethane by gavage for 3 weeks. The focus is characterized by an area of increased cell density compared to surrounding tubule parenchyma, with immature tubule epithelial cells and inapparent tubule lumens. H&E 160×.

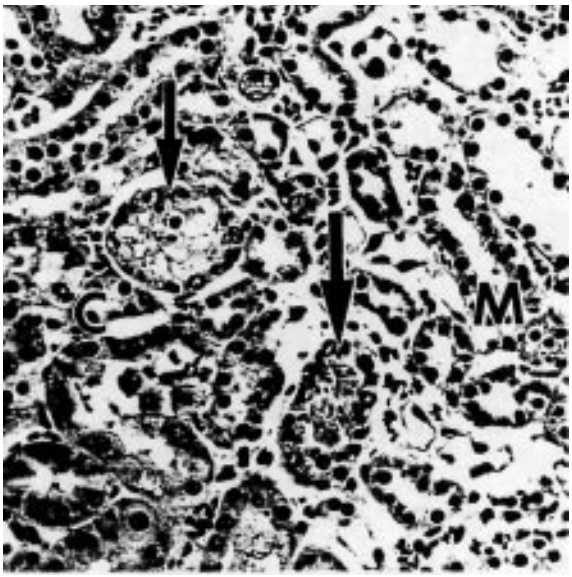

PLATE 6

Granular casts in the kidney of a male rat administered 1.24 mmol/kg pentachloroethane by gavage for 3 weeks. Granular material fills the lumens of several tubules (arrows) located at the junction between the cortex (C) and medulla (M). H&E 260×.

DISCUSSION

Ethanes containing four or more halogens, excluding iodine and astatine, were selected for the study presented in this report. Initial observations indicated that penta- and hexachloroethane clearly induced hyaline droplet nephropathy in male rats (NTP, 1983b, 1989c). Induction of this lesion by pentachloroethane has been attributed specifically to increased α_{2u}-globulin accumulation in protein droplets in the cytoplasm of cells in the P2 segment of the proximal tubule (Goldsworthy *et al.*, 1988). Some basic structural requirements for binding to α_{2u}-globulin have been described (Bomhard *et al.*, 1990; Borghoff *et al.*, 1991). However, binding to α_{2u}-globulin, as determined in competitive binding experiments *in vitro*, does not fully account for the extent of hyaline droplet accumulation produced for all chemicals studied *in vivo*. This discrepancy may be attributed in part to the involvement of metabolites in the *in vivo* studies (Borghoff *et al.*, 1991).

Penta- and hexachloroethane are metabolized in various species to a variety of chemicals, including trichloroethanol, trichloroacetic acid, 1,1,2,2-tetrachloroethane, tetrachloroethylene, and others (NTP, 1983b, 1989c). It is not known which of the parent chemicals or metabolites specifically contribute to the hyaline droplet accumulation, but comparative studies of pentachloroethane, perchloroethylene, and trichloroethylene indicated that pentachloroethane was approximately 10-fold more potent at inducing protein droplets than the unsaturated metabolites (Goldsworthy *et al.*, 1988). This finding suggests that the parent halogenated ethanes might be directly involved in α_{2u}-globulin binding and that study of various highly halogenated ethanes might provide further insight into structural requirements.

The chemicals evaluated in the present study fell into three distinct groups: those that induced hyaline droplet nephropathy, those that did not, and those that appeared to suppress renal droplet accumulation. Pentachloroethane gave the expected results in male rats, *i.e.*, a clear hyaline droplet nephropathy, an increase in kidney weight, increased urinary glucose output and *N*-acetyl-β-D-glucosaminidase (NAG) activity, and a slight but not significant increase in urinary aspartate aminotransferase (AST) activity. The assessment of renal tubule cell proliferating cell nuclear antigen (PCNA) indicated an approximately threefold increase over the controls; however, this was not dose related. Pentachloroethane administration also caused an increase in the right kidney weight of females without histologic evidence of hyaline droplet nephropathy or the typical F344 rat nephropathy. Urinary protein output by dosed female rats was also greater than that by the controls,

and liver weights and the incidences of cytoplasmic vacuolization were greater in dosed males and females than in the controls.

Hexachloroethane produced nearly the same renal toxicity profile as pentachloroethane. There was clear hyaline droplet nephropathy, with increased labeling of proliferating tubule cells, and urinary glucose output and AST and NAG activities were increased. While both penta- and hexachloroethane caused renal tubule cell neoplasms in 2-year studies (NTP, 1983b, 1989c), hexachloroethane gave an equal or somewhat greater neoplasm response at lower doses than pentachloroethane (Table 8).

Table 8 Incidence of Renal Tubule Cell Neoplasms in Male F344/N Rats in the 2-Year Gavage Studies of Selected Halogenated Ethanes[1]

Ethane	Dose (mg/kg)	Adenoma	Carcinoma
Pentachloroethane	0	0/50	1/50
	75	1/49	1/49
	150	4/50	0/50
Hexachloroethane	0	1/50	0/50
	10	2/50	0/50
	20	4/50	3/50
1,1,2,2-Tetrachloroethane	0	0/40	0/40
	50[2]	0/50	1/50[3]
	100[2]	1/50	0/50
1,1,1,2-Tetrachloroethane	0	0/48	0/48
	125	0/50	0/50
	250	1/48	0/48

[1] NCI, 1978e; NTP, 1983a,b, 1989c
[2] Doses were increased to 65 and 130 mg/kg after 15 weeks.
[3] Described as a mixed-cell malignant tumor.

The only other chemical in this series of halogenated ethanes that caused lesions consistent with hyaline droplet nephropathy was 1,1,1,2-tetrachloroethane. The nephropathy was less marked than that seen with penta- and hexachloroethane, but there were increases in the incidence of PCNA labeling of tubule cells, kidney weights, and urinary protein output and NAG activity at the highest dose (1.24 mmol/kg).

The placement of two chlorine atoms on each carbon (1,1,2,2-tetrachloroethane) results in a more acutely toxic chemical than 1,1,1,2-tetrachloroethane, but there was no evidence of hyaline droplet nephropathy in rats that died early or in rats administered the low dose of 1,1,2,2-tetrachloroethane

that survived to the end of the study. In fact, the only evidence of toxicity in rats in the low-dose group included increases in liver weight and in the incidence of cytoplasmic vacuolization. The urinary protein output of rats administered the low dose was similar to that of the controls, suggesting that the synthesis of plasma proteins by the liver was not affected at this dose.

The restriction of chlorine atoms to only one carbon (1,1,1-tetrachloroethane) also prevented the induction of hyaline droplet nephropathy. While there was no microscopic evidence of kidney injury, urinary protein output and AST activity were elevated, suggesting mild renal toxicity.

The chlorofluorocarbons 1,1,1-trichloro-2,2,2-trifluoroethane, 1,1,2,2-tetrachloro-1,2-difluoroethane, and 1,2-dichloro-1,1-difluoroethane also did not induce hyaline droplet nephropathy, and rats administered these compounds had no appreciable signs of toxicity except for a modest increase in urinary AST and NAG activities with 1,1,2,2,tetrachloro-1,2-difluoroethane. Renal toxicity of some fluorinated chemicals such as methoxyflurane has been reported and attributed, at least in part, to fluoride ions released during metabolism (Mazze, 1976), although studies of other ethane-based chlorofluorocarbons generally do not indicate the kidney as a target of toxicity (WHO, 1992).

Replacement of chlorine with bromine, as in 1,1,1,2- and 1,1,2,2-tetrabromoethane and pentabromoethane, also prevented the induction of hyaline droplet nephropathy. The tetrabromoethanes were more acutely toxic than the tetrachloroethanes; only four low-dose rats given 1,1,1,2-tetrabromoethane and no rats administered 1,1,2,2-tetrabromoethane survived to the end of the study. Pentabromoethane also did not cause hyaline droplet nephropathy and, in fact, caused a marked decrease in the amount of renal tubule cell protein droplets. This effect, along with low urinary protein output, was also seen in rats administered 1,1,1,2-tetrabromoethane, suggesting that protein production by the liver may have been impaired in these animals. There was evidence of minimal to mild liver cytoplasmic vacuolization with both chemicals, although this did not seem to be markedly different from that seen with other chemicals in this study where liver protein synthesis apparently was not affected.

The hypothesis of α_{2u}-globulin binding accounting for the apparent male-rat-specific renal neoplasm response to a variety of chemicals has been debated in a recent series of papers. Melnick has pointed out a number of apparent inconsistencies in the data cited to support the theory (Melnick, 1992, 1993, 1995), and Borghoff *et al.* (1993) have provided counterarguments. To the extent that the current findings can contribute to the debate, they would appear to support at least a qualitative association between the induction of hyaline droplet nephropathy and renal neoplasms in the male rat. Although

it was recognized previously that penta- and hexachloroethane induce hyaline droplet nephropathy, the ability of 1,1,1,2- and 1,1,2,2-tetrachloroethane to induce this nephropathy could not clearly be determined from the prechronic studies of those chemicals. The present study has shown that hyaline droplet nephropathy can be induced by 1,1,1,2-tetrachloroethane, but not 1,1,2,2-tetrachloroethane (or the metabolites of either compound). While neither tetrachloroethane was found to induce renal tubule cell neoplasms, it is possible that the hyaline droplet nephropathy induced by 1,1,1,2-tetrachloroethane was insufficient to cause increased incidences of renal neoplasms, as the nephropathy response was less than that seen with penta- or hexachloroethane. Alternatively, the lack of concordance with 1,1,1,2-tetrachloroethane may place this chemical in the same category with gabapentin and lindane, chemicals that produced no renal neoplasms in 2-year studies at any dose or at some doses that induced hyaline droplet nephropathy.

The complete absence of hyaline droplet nephropathy in the studies of the bromo- and chlorofluoroethanes is surprising. This suggests either that the α_{2u}-globulin:chemical interaction is sufficiently structurally specific as to require an ethane molecule with the bulk associated with substituted chlorines rather than larger bromines or smaller fluorines or, more likely, that a common metabolite of the chlorinated ethanes is responsible for the nephropathy. Studies of the metabolism of the highly chlorinated ethanes have been insufficient to allow the prediction of which metabolite or metabolites may be primarily involved.

This study was designed to screen a number of halogenated ethanes for any indications of a capability to induce hyaline droplet nephropathy. As a screen, the study had a number of limitations. First, the duration was relatively short, and the study evaluated only two doses, and in some cases one dose, of each compound. A more complete dose-response study of longer duration might have provided more definitive information. Second, the limited assessment by the PCNA staining method did not allow a full consideration of the relationship of renal injury, involving or not involving hyaline droplet nephropathy, with the induction of cell proliferation, which is an important component of the α_{2u}-globulin syndrome. The continuous labeling of cells undergoing DNA synthesis with bromodeoxyuridine would have been preferable to more fully explore this relationship. Third, the actual doses of the ethanes and their metabolites in the critical tissue, the kidney, were not assessed; reliance on the equivalent molar dose to the animal may not be a sufficient basis for comparison. Finally, definitive immunological identification of the protein droplets observed as accumulations of α_{2u}-globulin was not done. Notwithstanding these limitations, it would appear that, of the halogenated ethanes studied, the capacity to induce hyaline droplet nephropathy in male rats was restricted to ethanes containing four or more halogens, and only the chlorinated ethanes were active.

If the ability to induce hyaline droplet nephropathy is the determining factor in the induction of renal tubule cell neoplasms with this class of chemicals, then an absence of kidney neoplasms in male rats would be predicted in the event that 2-year studies were performed with the bromo- or chlorofluoroethanes.

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APPENDIX A

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes (Part A)[1]

	Vehicle Control	0.62 mmol/kg	1.24 mmol/kg
MALE			
Necropsy body wt			
1,1,2,2-TCE	327 ± 5	312 ± 8	)[2]
1,1,1,2-TCE		331 ± 9	324 ± 6
PCE		323 ± 5	317 ± 5
Right kidney			
Absolute			
1,1,2,2-TCE	1.085 ± 0.022	1.138 ± 0.044	)
1,1,1,2-TCE		1.173 ± 0.055	1.236 ± 0.034*
PCE		1.426 ± 0.033**	1.478 ± 0.038**
Relative			
1,1,2,2-TCE	3.32 ± 0.07	3.64 ± 0.05	)
1,1,1,2-TCE		3.54 ± 0.07	3.82 ± 0.10**
PCE		4.42 ± 0.12**	4.67 ± 0.13**
Liver			
Absolute			
1,1,2,2-TCE	11.586 ± 0.303	14.380 ± 0.803**	)
1,1,1,2-TCE		12.431 ± 0.549	12.194 ± 0.284
PCE		13.051 ± 0.379	14.180 ± 0.387**
Relative			
1,1,2,2-TCE	35.42 ± 0.63	45.93 ± 1.59**	)
1,1,1,2-TCE		37.49 ± 0.75	37.66 ± 0.48
PCE		40.43 ± 0.75**	44.78 ± 0.78**
Right testis			
Absolute			
1,1,2,2-TCE	1.502 ± 0.039	1.413 ± 0.033	)
1,1,1,2-TCE		1.444 ± 0.044	1.435 ± 0.025
PCE		1.442 ± 0.010	1.419 ± 0.059
Relative			
1,1,2,2-TCE	4.59 ± 0.10	4.53 ± 0.05	)
1,1,1,2-TCE		4.36 ± 0.07	4.44 ± 0.14
PCE		4.47 ± 0.05	4.49 ± 0.18

	Vehicle Control	1.24 mmol/kg PCE Control
FEMALE		
Necropsy body wt	194 ± 3	188 ± 2
Right kidney		
Absolute	0.669 ± 0.023	0.795 ± 0.027*
Relative	3.45 ± 0.08	4.22 ± 0.14**
Liver		
Absolute	6.598 ± 0.192	7.443 ± 0.065**
Relative	34.06 ± 0.82	39.55 ± 0.27**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error). For all groups, n=5 unless otherwise specified.

[2] n=0.

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes (Part B)[1]

	Vehicle Control	0.62 mmol/kg	1.24 mmol/kg
Necropsy body wt			
1,1,2,2-TC-1,2-DFE	323 ± 3	328 ± 6	327 ± 6
1,1,1-TriC-2,2,2-TFE		328 ± 6	320 ± 7
1,2-DC-1,1-DFE		321 ± 8	320 ± 6
1,1,1-TriC		332 ± 4	318 ± 10[2]
Right kidney			
Absolute			
1,1,2,2-TC-1,2-DFE	1.031 ± 0.024	1.121 ± 0.019	1.123 ± 0.041
1,1,1-TriC-2,2,2-TFE		1.048 ± 0.023	1.028 ± 0.040
1,2-DC-1,1-DFE		1.088 ± 0.026	1.124 ± 0.013
1,1,1-TriC		1.081 ± 0.011	1.086 ± 0.023[2]
Relative			
1,1,2,2-TC-1,2-DFE	3.19 ± 0.05	3.43 ± 0.05	3.43 ± 0.09
1,1,1-TriC-2,2,2-TFE		3.19 ± 0.05	3.21 ± 0.10
1,2-DC-1,1-DFE		3.39 ± 0.04	3.52 ± 0.07*
1,1,1-TriC		3.26 ± 0.10	3.42 ± 0.15[2]
Liver			
Absolute			
1,1,2,2-TC-1,2-DFE	11.611 ± 0.103	12.045 ± 0.459	12.661 ± 0.453
1,1,1-TriC-2,2,2-TFE		11.920 ± 0.284	11.720 ± 0.524
1,2-DC-1,1-DFE		15.312 ± 0.439**	15.882 ± 0.680**
1,1,1-TriC		12.193 ± 0.338	12.670 ± 0.123[2]
Relative			
1,1,2,2-TC-1,2-DFE	35.92 ± 0.46	36.74 ± 0.87	38.71 ± 0.88
1,1,1-TriC-2,2,2-TFE		36.33 ± 0.52	36.61 ± 1.21
1,2-DC-1,1-DFE		47.66 ± 0.82**	49.56 ± 1.38**
1,1,1-TriC		36.77 ± 0.87	39.94 ± 1.40*[2]
Right testis			
Absolute			
1,1,2,2-TC-1,2-DFE	1.435 ± 0.038	1.466 ± 0.013	1.480 ± 0.030
1,1,1-TriC-2,2,2-TFE		1.472 ± 0.025	1.354 ± 0.080
1,2-DC-1,1-DFE		1.360 ± 0.089	1.442 ± 0.014
1,1,1-TriC		1.509 ± 0.033	1.484 ± 0.019[2]
Relative			
1,1,2,2-TC-1,2-DFE	4.44 ± 0.11	4.48 ± 0.09	4.53 ± 0.07
1,1,1-TriC-2,2,2-TFE		4.49 ± 0.10	4.24 ± 0.27
1,2-DC-1,1-DFE		4.24 ± 0.29	4.51 ± 0.07
1,1,1-TriC		4.55 ± 0.10	4.68 ± 0.21[2]

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error). For all groups, n=5 unless otherwise specified.

[2] n=4.

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes (Part C)[1]

	Vehicle Control	0.62 mmol/kg	1.24 mmol/kg
Necropsy body wt			
HCE	316 ± 5	307 ± 6	307 ± 5
1,1,2,2-TBE		244 ± 9**[2]	)[3]
PBE		298 ± 7	)
1,1,1,2-TBE		282 ± 4**[2]	)
Right kidney			
Absolute			
HCE	1.009 ± 0.025	1.157 ± 0.011**	1.250 ± 0.022**
1,1,2,2-TBE		1.164 ± 0.028**[2]	)
PBE		1.193 ± 0.035**	)
1,1,1,2-TBE		1.226 ± 0.053**[2]	)
Relative			
HCE	3.19 ± 0.04	3.77 ± 0.06**	4.07 ± 0.05**
1,1,2,2-TBE		4.79 ± 0.15**[2]	)
PBE		4.02 ± 0.18**	)
1,1,1,2-TBE		4.35 ± 0.16**[2]	)
Liver			
Absolute			
HCE	11.041 ± 0.291[2]	11.959 ± 0.178	13.479 ± 0.390
1,1,2,2-TBE		13.534 ± 1.228[2]	)
PBE		17.503 ± 0.909**	)
1,1,1,2-TBE		12.108 ± 0.847[2]	)
Relative			
HCE	34.82 ± 0.60[2]	39.01 ± 0.92	43.84 ± 0.64**
1,1,2,2-TBE		55.21 ± 2.89**[2]	)
PBE		58.63 ± 1.81**	)
1,1,1,2-TBE		42.99 ± 2.90*[2]	)
Right testis			
Absolute			
HCE	1.412 ± 0.037	1.409 ± 0.023	1.430 ± 0.016
1,1,2,2-TBE		1.235 ± 0.020*[2]	)
PBE		1.239 ± 0.075*	)
1,1,1,2-TBE		1.213 ± 0.032*[2]	)
Relative			
HCE	4.47 ± 0.09	4.60 ± 0.11	4.66 ± 0.05
1,1,2,2-TBE		5.09 ± 0.19*[2]	)
PBE		4.16 ± 0.22	)
1,1,1,2-TBE		4.31 ± 0.12[2]	)

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error). For all groups, n=5 unless otherwise specified.

[2] n=4.

[3] n=0.

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

APPENDIX B

Urinalysis Results

TABLE B1 Urinalysis Data for F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes (Part A)[1]

	Vehicle Control	0.62 mmol/kg	1.24 mmol/kg
MALE			
Creatinine (mg/dL)			
1,1,2,2-TCE	61.24 ± 16.93	61.94 ± 8.61	)[2]
1,1,1,2-TCE		49.64 ± 5.47	46.70 ± 10.08
PCE		64.52 ± 11.72	48.68 ± 7.50
Glucose (µg/mg creatinine)			
1,1,2,2-TCE	184 ± 24	178 ± 13	)
1,1,1,2-TCE		238 ± 18	394 ± 16
PCE		465 ± 27**	425 ± 70*
Protein (µg/mg creatinine)			
1,1,2,2-TCE	1,198 ± 117	1,679 ± 133	)
1,1,1,2-TCE		1,865 ± 191	2,422 ± 258**
PCE		1,596 ± 125	1,405 ± 197
Aspartate aminotransferase (mU/mg creatinine)			
1,1,2,2-TCE	8 ± 2	4 ± 3	)
1,1,1,2-TCE		6 ± 2	14 ± 2
PCE		18 ± 6	246 ± 233
γ-Glutamyltransferase (mU/mg creatinine)			
1,1,2,2-TCE	1,328 ± 136	1,033 ± 49	)
1,1,1,2-TCE		602 ± 157	168 ± 47**
PCE		646 ± 208	665 ± 255
N-acetyl-β-D-glucosaminidase (mU/mg creatinine)			
1,1,2,2-TCE	12 ± 2	18 ± 1	)
1,1,1,2-TCE		17 ± 1	24 ± 2**
PCE		20 ± 1	35 ± 15*
Volume (mL/16 hr)			
1,1,2,2-TCE	10.0 ± 2.5	9.4 ± 0.9	)
1,1,1,2-TCE		11.6 ± 1.8	17.6 ± 7.1
PCE		10.6 ± 2.2	12.3 ± 1.8
Specific gravity			
1,1,2,2-TCE	1.018 ± 0.004	1.033 ± 0.004	)
1,1,1,2-TCE		1.017 ± 0.002	1.018 ± 0.003
PCE		1.022 ± 0.004	1.020 ± 0.002

	Vehicle Control	1.24 mmol/kg PCE Control
FEMALE		
Creatinine (mg/dL)	35.18 ± 6.75	29.80 ± 2.22
Glucose (µg/mg creatinine)	189 ± 27	247 ± 7
Protein (µg/mg creatinine)	117 ± 30	287 ± 96*
Aspartate aminotransferase (mU/mg creatinine)	6 ± 3	13 ± 3
γ-Glutamyltransferase (mU/mg creatinine)	223 ± 65	111 ± 31
N-acetyl-β-D-glucosaminidase (mU/mg creatinine)	12 ± 2	16 ± 2
Volume (mL/16 hr)	10.1 ± 1.7	12.5 ± 0.9
Specific gravity	1.014 ± 0.002	1.014 ± 0.001

[1] Data are given as mean ± standard error. For all groups, n=5 unless otherwise specified.
[2] n=0.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's test.

TABLE B2 Urinalysis Data for F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes (Part B)[1]

	Vehicle Control	0.62 mmol/kg	1.24 mmol/kg
Creatinine (mg/dL)			
1,1,2,2-TC-1,2-DFE	97.46 ± 9.43	96.28 ± 12.06	89.66 ± 13.56
1,1,1-TriC-2,2,2-TFE		72.58 ± 8.61	85.14 ± 16.11
1,2-DC-1,1-DFE		100.90 ± 25.25	100.56 ± 26.39
1,1,1-TriC		92.44 ± 11.57	62.33 ± 10.11[2]
Glucose (µg/mg creatinine)			
1,1,2,2-TC-1,2-DFE	160 ± 6	179 ± 13	199 ± 26
1,1,1-TriC-2,2,2-TFE		205 ± 11	161 ± 12
1,2-DC-1,1-DFE		142 ± 6	140 ± 6
1,1,1-TriC		172 ± 8	202 ± 23[2]
Protein (µg/mg creatinine)			
1,1,2,2-TC-1,2-DFE	1,227 ± 78	1,457 ± 70	1,369 ± 38
1,1,1-TriC-2,2,2-TFE		1,461 ± 102	1,396 ± 75
1,2-DC-1,1-DFE		1,423 ± 121	1,271 ± 66
1,1,1-TriC		1,447 ± 130	2,198 ± 584*[2]
Aspartate aminotransferase (mU/mg creatinine)			
1,1,2,2-TC-1,2-DFE	8 ± 1	29 ± 3**	32 ± 2**
1,1,1-TriC-2,2,2-TFE		16 ± 4	13 ± 1
1,2-DC-1,1-DFE		10 ± 1	13 ± 4
1,1,1-TriC		16 ± 1	466 ± 444*[2]
γ-Glutamyltransferase (mU/mg creatinine)			
1,1,2,2-TC-1,2-DFE	1,384 ± 78	1,729 ± 115	1,619 ± 94
1,1,1-TriC-2,2,2-TFE		1,533 ± 57	1,684 ± 64
1,2-DC-1,1-DFE		995 ± 61	825 ± 139
1,1,1-TriC		1,391 ± 53	1,816 ± 231[2]
N-acetyl-β-D-glucosaminidase (mU/mg creatinine)			
1,1,2,2-TC-1,2-DFE	11 ± 1	14 ± 1	15 ± 0*
1,1,1-TriC-2,2,2-TFE		17 ± 4	12 ± 1
1,2-DC-1,1-DFE		11 ± 1	12 ± 0
1,1,1-TriC		13 ± 2	50 ± 37[2]
Volume (mL/16 hr)			
1,1,2,2-TC-1,2-DFE	7.4 ± 0.9	7.9 ± 1.4	7.4 ± 1.2
1,1,1-TriC-2,2,2-TFE		7.8 ± 1.0	8.3 ± 2.1
1,2-DC-1,1-DFE		9.7 ± 2.8	10.0 ± 3.1
1,1,1-TriC		7.1 ± 1.0	3.2 ± 0.6[2]
Specific gravity			
1,1,2,2-TC-1,2-DFE	1.023 ± 0.002	1.024 ± 0.002	1.023 ± 0.003
1,1,1-TriC-2,2,2-TFE		1.020 ± 0.002	1.021 ± 0.003
1,2-DC-1,1-DFE		1.028 ± 0.007	1.032 ± 0.008
1,1,1-TriC		1.024 ± 0.003	1.022 ± 0.003[2]

[1] Data are given as mean ± standard error. For all groups, n=5 unless otherwise specified.
[2] n=4.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's test.

TABLE B3 Urinalysis Data for F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes (Part C)[1]

	Vehicle Control	0.62 mmol/kg	1.24 mmol/kg
Creatinine (mg/dL)			
HCE	143.22 ± 18.12	79.56 ± 11.01	56.48 ± 3.06**
1,1,2,2-TBE		)[2]	)
PBE		65.54 ± 1.81	)
1,1,1,2-TBE		59.96 ± 3.08*	)
Glucose (µg/mg creatinine)			
HCE	169 ± 3	344 ± 30	446 ± 23**
1,1,2,2-TBE		)	)
PBE		335 ± 16*	)
1,1,1,2-TBE		274 ± 12	)
Protein (µg/mg creatinine)			
HCE	1,322 ± 59	1,748 ± 257	2,980 ± 103
1,1,2,2-TBE		)	)
PBE		2,185 ± 516	)
1,1,1,2-TBE		560 ± 106	)
Aspartate aminotransferase (mU/mg creatinine)			
HCE	6 ± 1	40 ± 6*	66 ± 5**
1,1,2,2-TBE		)	)
PBE		25 ± 16	)
1,1,1,2-TBE		25 ± 7	)
γ-Glutamyltransferase (mU/mg creatinine)			
HCE	1,456 ± 47	1,547 ± 66	1,897 ± 73
1,1,2,2-TBE		)	)
PBE		1,661 ± 163	)
1,1,1,2-TBE		805 ± 87	)
N-acetyl-β-D-glucosaminidase (mU/mg creatinine)			
HCE	11 ± 0	23 ± 2*	36 ± 1**
1,1,2,2-TBE		)	)
PBE		18 ± 2	)
1,1,1,2-TBE		16 ± 1	)
Volume (mL/16 hr)			
HCE	4.2 ± 0.8	7.5 ± 0.9	10.6 ± 1.1**
1,1,2,2-TBE		)	)
PBE		10.1 ± 0.6*	)
1,1,1,2-TBE		9.4 ± 0.8	)
Specific gravity			
HCE	1.038 ± 0.005	1.024 ± 0.003	1.020 ± 0.001**
1,1,2,2-TBE		)	)
PBE		1.026 ± 0.001	)
1,1,1,2-TBE		1.022 ± 0.002*	)

[1] Data are given as mean ± standard error. For all groups, n=5 unless otherwise specified.
[2] n=0.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's test.

APPENDIX C

Cell Proliferation Analysis Results

TABLE C1 Individual Proliferating Cell Nuclear Antigen Labeling Indexes of F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes (Part A)[1]

Chemical	Dose (mmol/kg)	Animal Number	Labeling Index	Mean ± Standard Error
MALE				
Vehicle control		1	0.37	
		2	0.31	
		3	0.75	
		4	0.16	
		5	0.42	0.40 ± 0.10
1,1,2,2-TCE	0.62	16	0.064	
		17	0.13	
		18	0.060	
		19	0.061	
		20	0.021	0.067 ± 0.018
1,1,1,2-TCE	0.62	26	0.26	
		27	0.36	
		28	0.50	
		29	0.28	
		30	0.26	0.33 ± 0.046
	1.24	31	0.53	
		32	0.98	
		33	0.91	
		34	0.93	
		35	1.1	0.89 ± 0.10*
PCE	0.62	36	1.3	
		37	1.5	
		38	1.1	
		39	1.2	
		40	1.7	1.4 ± 0.1*
	1.24	41	0.87	
		42	1.5	
		43	1.6	
		44	1.1	
		45	1.3	1.3 ± 0.1*
FEMALE				
Vehicle control		6	0.041	
		7	0.21	
		8	0.18	
		9	0.060	
		10	0.21	0.14 ± 0.04
PCE control	1.24	11	0.44	
		12	0.23	
		13	0.67	
		14	0.32	
		15	0.25	0.38 ± 0.08*

[1] Labeling index = percentage of proximal and distal tubule epithelial cells in S-phase. At least 4,000 renal epithelial cells were scored per animal.

* Significantly different ($P \leq 0.05$) from the control group by Student's *t*-test.

TABLE C2 Individual Proliferating Cell Nuclear Antigen Labeling Indexes of Male F344/N Rats in the 3-Week Gavage Study of Halogenated Ethanes (Part C)[1]

Chemical	Dose (mmol/kg)	Animal Number	Labeling Index	Mean ± Standard Error
Vehicle control		91	0.18	
		92	0.11	
		93	0.066	
		94	0.18	
		95	0.12	0.13 ± 0.02
HCE	0.62	96	0.83	
		97	1.4	
		98	0.45	
		99	0.32	
		100	0.71	0.74 ± 0.19*
	1.24	101	1.8	
		102	1.0	
		103	1.1	
		104	1.4	
		105	0.82	1.2 ± 0.2*
PBE	0.62	126	0.15	
		127	0.39	
		128	2.9	
		129	0.18	
		130	0.50	0.82 ± 0.52

[1] Labeling index = percentage of proximal and distal tubule epithelial cells in S-phase. At least 4,000 renal epithelial cells were scored per animal.

* Significantly different ($P \leq 0.05$) from the control group by Student's *t*-test.

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	*n*-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	*o*-Cresol *m*-Cresol *p*-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	*p*-Chloro-α,α,α-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	*t*-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	*o*-Nitrotoluene *m*-Nitrotoluene *p*-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
26	Ethylene Glycol Ethers	Drinking Water	93-3349
27	Riddelliine	Gavage	94-3350
28	Tetrachlorophthalic Anhydride	Gavage	93-3351
29	Cupric Sulfate	Drinking Water, Dosed Feed	93-3352
30	Dibutyl Phthalate	Feed	95-3353
31	Isoprene	Inhalation	95-3354

NTP TECHNICAL REPORTS ON TOXICITY STUDIES
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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
32	Methylene Bis(thiocyanate)	Gavage	94-3381
33	2-Chloronitrobenzene 4-Chloronitrobenzene	Inhalation	93-3382
35	Chemical Mixture of 25 Groundwater Contaminants	Drinking Water	93-3384
36	Pesticide/Fertilizer Mixtures	Drinking Water	93-3385
37	Sodium Cyanide	Drinking Water	94-3386
38	Sodium Selenate Sodium Selenite	Drinking Water	94-3387
39	Cadmium Oxide	Inhalation	95-3388
40	β-Bromo-β-nitrostyrene	Gavage	94-3389