NTP REPORT ON THE

TOXICITY STUDIES OF

ETHYLBENZENE

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park NC 27709

March 1992

NTP TOX 10
NIH Publication No. 92-3129

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

These studies were supported in part by funds from the Comprehensive Environmental Response, Compensation, and Liability Act trust fund, by interagency agreement with the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

FOREWARD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July, 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from the Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

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

Anyone who is aware of related ongoing or published studies not mentioned in this report, or of any errors in this report, is encouraged to make this information known to the NTP. Comments and questions should be directed to Dr. J.R. Bucher, NIEHS, P.O. Box 12233, Research Triangle Park NC 27709 (919) 541-4532).

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's toxic potential. Single copies of this report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3991).

TOXICITY STUDIES
of
ETHYLBENZENE
(CAS No. 100-41-4)
IN F344/N RATS AND B6C3F1 MICE
(INHALATION STUDIES)

Po Chan, Ph.D.
(Study Scientist)

NATIONAL TOXICOLOGY PROGRAM
P. O. Box 12233
Research Triangle Park, NC 27709

March 1992

NTP TOX 10
NIH Publication No. 92-3129

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

The NTP report on the toxicity studies of ethylbenzene is based primarily on 13-week studies conducted between March 29 and June 30, 1988, at IIT Research Institute, Chicago, IL.

National Toxicology Program
(Evaluated Experiment, Interpreted Results, and Reported Findings)

Po Chan, Ph.D., Study Scientist

John R. Bucher, Ph.D.
Leo T. Burka, Ph.D.
Michael R. Elwell, D.V.M., Ph.D.
Charles W. Jameson, Ph.D.
H.B. Matthews, Ph.D.
Morrow Thompson, D.V.M., Ph.D.
Errol Zeiger, Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report)

Paul K. Hildebrandt, D.V.M., Chairperson, PATHCO, Inc.

Gary Boorman, D.V.M., Ph.D., NTP
Michael R. Elwell, D.V.M., Ph.D., NTP
Scot L. Eustis, D.V.M., Ph.D., NTP
Katharina Heider, D.V.M., CIBA-GEIGY, Switzerland
Joel Leininger, D.V.M., Ph.D., NTP

Principal Contributors at IIT Research Institute
(Conducted Studies)

Catherine Aranyi, M.S.
James Fenter, Ph.D.
Charles L. Gaworski, D.V.M.
Richard E. Long, D.V.M.

Principal Contributor at Experimental Pathology Laboratories
(Provided Pathology Quality Assessment)

Jerry F. Hardisty, D.V.M.

Principal Contributors at Environmental Health Research and Testing, Inc.
(Contractor for Sperm Morphology and Vaginal Cytology Evaluation)

Dushant K. Gulati, Ph.D. Teresa Cocanougher, B.A. Susan Russell, B.A.

Analytical Sciences, Inc.
(Contractor for Statistical Analysis)

Steven Seilkop, M.S.
Janet Teague, M.S.

Principal Contributors at NTP for Report Preparation

Jane Lambert, B.S. Diane Overstreet, B.S.
Kristine Witt, M.S. (Oak Ridge Associated Universities)

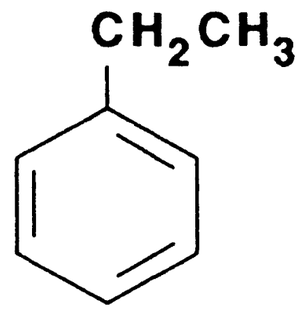
These studies were supported in part by funds from the Comprehensive Environmental Response, Compensation, and Liability Act trust fund, by interagency agreement with the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

CONTENTS

TABLES

FIGURES

ETHYLBENZENE



Molecular formula: C_8H_{10}

CAS Number: 100-41-4

Molecular Weight: 106.16

Synonyms: EB, ethyl benzene, ethylbenzol, phenylethane

ABSTRACT

Ethylbenzene is commonly used as a solvent and chemical intermediate and as an additive in some motor fuel formulations. Inhalation toxicology studies of ethylbenzene (99% pure) were conducted by exposing groups of F344/N rats and B6C3F$_1$ mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 hours per day, 5 days per week for 13 weeks.

No rats or mice died during the 13-week exposure. Body weight gains were slightly lower in the high dose groups of male and female rats, but the differences were not statistically significant. Absolute and relative kidney, liver, and lung weights were increased in the exposed rats, while weight increases occurred only in the livers of exposed mice. Chemically related histopathologic changes were not observed in any tissues of rats or mice. No changes were observed in the evaluation of sperm or vaginal cytology in rats or mice. Ethylbenzene was not mutagenic in *Salmonella* and did not induce chromosomal aberrations or sister chromatid exchanges in Chinese hamster ovary (CHO) cells *in vitro*, though it did induce trifluorothymidine resistance in mouse lymphoma cells at the highest concentration tested. Micronuclei assays in peripheral blood of mice were negative. Thus, there appears to be only minimal evidence of toxicity in F344/N rats and B6C3F$_1$ mice exposed to ethylbenzene by inhalation at concentrations as high as to 1000 ppm for 13 weeks.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies on ethylbenzene on November 20, 1990, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

NATIONAL TOXICOLOGY PROGRAM'S BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

Robert A. Scala, Ph.D., Chair
Medicine and Environmental Health Dept.
Research and Environmental Health Division
Exxon Corp.
East Millstone, NJ

Jay I. Goodman, Ph.D.
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

Daniel S. Longnecker, M.D.
Department of Pathology
Dartmouth Medical School
Hanover, NH

Ellen K. Silbergeld, Ph.D.
University of Maryland Medical School
Baltimore, MD

AD HOC SUBCOMMITTEE PANEL OF EXPERTS

John Ashby, Ph.D.
Central Toxicology Laboratory
Imperial Chemical Industries, PLC
Alderley Park, England

Gary P. Carlson, Ph.D.
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN

Harold Davis, D.V.M., Ph.D.
School of Aerospace Medicine
Brooks Air Force Base, TX

Robert H. Garman, D.V.M.
Consultants in Veterinary Pathology
Murrysville, PA

Lois Swirsky Gold, Ph.D.
Lawrence Berkeley Laboratory
University of California
Berkeley, CA

David W. Hayden, D.V.M., Ph.D.
Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota
St. Paul, MN

Curtis D. Klaasen, Ph.D.
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Barbara McKnight, Ph.D.
Department of Biostatistics
University of Washington
Seattle, WA

Lauren Zeise, Ph.D.
California Department of Health Services
Berkeley, CA

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICITY STUDIES OF ETHYLBENZENE

On November 20, 1990, the draft report on toxicity studies of ethylbenzene received public review by the Technical Reports Review Subcommittee and associated Panel of Experts of the National Toxicology Program's Board of Scientific Counselors. The review meeting was held at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina.

Dr Po Chan, NIEHS, began the discussion by reviewing the design and findings of the ethylbenzene studies. Dr. Hayden noted sex-related differences in serum alkaline phosphatase changes in rats exposed to benzene, and he asked that a reference be included to support the attribution of decreases in the serum activity of this enzyme to reduced food intake.

Dr. Ashby noted differences among the genotoxic and carcinogenic effects of benzene and a series of substituted benzene compounds; he predicted that ethylbenzene would not be positive for cancer induction in 2-year studies.

Dr. Chan and Dr. Thompson discussed the program experiences with changes in alkaline phosphatase and agreed to add a reference to the discussion section.

After further discussion of editorial and other matters, Dr. Scala accepted the report on behalf of the panel.

I. INTRODUCTION

Physical Properties, Production, Uses, and Exposure

Ethylbenzene is a colorless, aromatic liquid with a melting point of -95.0°C, a boiling point of 136.2°C, and density of 0.866. Its vapor pressure is 10 mm at 25.9°C. It is insoluble in water (0.014 g/100 ml) but is soluble in most organic solvents (Budavari *et al.*, 1989; Verschueren, 1983).

U.S. production of ethylbenzene was 7.56 billion pounds in 1984 (U.S. International Trade Commission, 1985) and 8.5 billion pounds in 1986 (Heylin, 1987). Current production figures are not available. Ethylbenzene is used mainly in the manufacture of styrene. It also is a major component (15-20%) of mixed xylenes (Toftgard and Nilsen, 1982), which are used as solvents in agricultural and household insecticide sprays, in rubber and chemical manufacturing industries, household degreasing cleaners, paints, adhesives, and rust preventives (Fishbein, 1985). The U.S. produced 6.49 billion pounds of mixed xylenes in 1984 (USITC, 1985). Ethylbenzene also is used as an anti-knock agent in motor and aviation fuels (NIOSH, 1979).

Because it is so often used in fuels and as a solvent, ethylbenzene is distributed widely in the environment. It has been detected in ambient air, in surface and ground water, and in human breast milk (National Research Council, 1981). Ethylbenzene has been found at concentrations up to 7 µg/L in samples of potable water in Canada (Otson *et al.*, 1982), and it has been detected at concentrations up to 10-26 µg/L in the Missouri River (STORET, 1986). Ethylbenzene also has been found in effluents from wood pulp mills (Nestmann *et al.*, 1980). Wallace *et al.* (1984) identified ethylbenzene in a water sample as well as in each of 8 air samples and 12 human breath samples from New Jersey. Atmospheric samples obtained in the Los Angeles basin contained ethylbenzene originating from vehicle exhaust (Lonneman *et al.*, 1968). No evidence of ethylbenzene bioaccumulation was found in studies of environmental samples of Manila clams (Nunes and Beaville, 1979), Coho salmon or starry flounder (Roubal *et al.*, 1978).

The exposure limit of ethylbenzene, set by OSHA and ACGIH (1989), is 100 ppm.

Absorption, Metabolism, and Excretion

Human exposure to ethylbenzene is mainly through inhalation of vapors and mists. To a lesser extent, exposure occurs by ingestion or dermal contact with either ethylbenzene or liquids that contain it. (Dutkiewicz and Tyras, 1967). Ethylbenzene is absorbed readily from the atmosphere through the lung. Results of 2 studies (Gromiec and Piotrowski, 1984; Bardodej and Bardodejova, 1970) indicated that humans retained 49-64% of ethylbenzene inhaled. When rats were exposed to ethylbenzene by inhalation at 1 mg/L for 6 hours, it was estimated that 44% of the ^{14}C-ethylbenzene inhaled was retained (Chin *et al.*, 1980). Forty-two hours following a 6-hour inhalation exposure of rats to radio-labelled ethylbenzene, radioactivity was distributed in liver, adipose, and gastrointestinal tissues (Chin *et al.*, 1980). A single oral

administration of labeled ethylbenzene to rats resulted in distribution of ethylbenzene-derived radioactivity primarily in the intestine, liver, and kidney (Climie *et al.*, 1983). Ethylbenzene is absorbed through the skin relatively rapidly, compared to similar hydrocarbon compounds such as benzene or styrene (Dutkiewicz and Tyras, 1967).

Ethylbenzene is excreted by rats primarily in urine, following oxidative metabolism to mandelic acid and phenylgloxylic acid (Engstrom, 1984; Bardodej and Bardodejova, 1970; Gromiec and Piotrowski, 1984). Aromatic ring hydroxylation of ethylbenzene by mixed function oxidases results in the formation of small amounts of 2- and 4-ethylphenol which also are excreted in urine (Angerer and Lehnert, 1979; Engstrom, 1984; Pyykko *et al.*, 1987).

Toxic Effects

The oral LD_{50} for ethylbenzene in Wistar rats (both sexes) was estimated to be 3.5 g/kg (Wolf *et al.*, 1956). Cragg *et al.* (1989) reported that all male F344/N rats and $B6C3F_1$ mice died within 4 days when exposed by inhalation to ethylbenzene at 2400 ppm for 6 hours per day. At 1200 ppm, 4 of 5 mice died; the surviving mouse and all exposed rats showed respiratory distress, lacrimation, salivation, prostration, and anogenital staining.

Ethylbenzene is a mucous membrane irritant. Guinea pigs exposed for one minute to 0.2% ethylbenzene vapor experienced moderate eye and nasal irritation. A 0.1% exposure produced slight nasal irritation that ceased after 30 minutes. Similarly, human volunteers breathing 0.1% vapor reported severe eye irritation which gradually decreased over time. Extreme eye, nose, and throat irritation occurred within 6 minutes of exposure to a 0.2% atmosphere (Yant *et al.*, 1930).

Oral administration of ethylbenzene to female rats at 408 or 680 mg/kg/d, or inhalation exposures at 1250 or 2200 ppm, 7-8 hours per day, 5 days a week for 6 months, induced slight increases in kidney and liver weights, with cloudy swelling of the tubular epithelium of the kidney and hepatocytes of the liver (Wolf *et al.*, 1956). An electron microscopy study attributed the histologic changes to an increase in smooth endoplasmic reticulum in both liver and kidney which was associated with increased mixed function oxidase and other enzyme activity (Elovaara *et al.*, 1985).

Male Wistar rats exposed to ethylbenzene vapor at 300 or 600 ppm 6 hours per day, 5 days a week for 16 weeks, exhibited elevated liver microsomal enzyme activities, including NADPH cytochrome *c* reductase, 7-ethoxycoumarin O-deethylase, UDP-glucuronosyl-transferase, and D-glucurono-lactone dehydrogenase. The activities of 7-ethoxycoumarin O-deethylase and UDP-glucuronosyl transferase also were increased in the kidney (Elovaara *et al.*, 1985).

Genetic Toxicity

Ethylbenzene was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98 when tested up to toxic doses (1000 µg/plate) in the presence and absence of exogenous metabolic activation (S9) (Appendix B, Table B1). It also was reported negative for gene mutation induction, with and without S9, in *Salmonella* strains TA1537 and TA1538

(Nestmann *et al.*, 1980), in *E. coli* WP2 and WP2uvrA, and in *Saccharomyces cerevisiae* JD1 (Dean *et al.*, 1985). No induction of sister chromatid exchanges (SCE) or chromosomal aberrations were observed in Chinese hamster ovary cells treated with ethylbenzene in the presence or absence of S9 (Appendix B., Table B3 and B4), but a weakly positive response was reported for SCE induction in cultured human lymphocytes with S9 (Norppa and Vainio, 1983). An increase in trifluorothymidine-resistant colonies of L5178Y/TK$^{\pm}$ mouse lymphoma cells was observed at the highest nonlethal dose (80 µg/ml) of ethylbenzene tested without S9 (McGregor *et al.*, 1988, Appendix B, Table B2). No induction of micronuclei was observed in peripheral blood erythrocytes of male and female mice sampled at the termination of the 13-week inhalation study (Appendix B, Table B5).

Reproductive Toxicity

The offspring of female Wistar rats exposed to ethylbenzene at 1000 ppm, 7 hours per day, 5 days a week, for 3 weeks before mating to normal males, then exposed daily through 19 days of gestation, had a higher incidence of extra ribs (Hardin *et al.*, 1981). Similar findings were reported in the offspring of CFY rats which were exposed to ethylbenzene at 554 ppm, 24 hours per day, from day 7 to day 15 of gestation (Tatri *et al.*, 1982). Maternal toxicity was manifested as an increase in liver, kidney, and spleen weights.

Rationale for Conducting Studies

Ethylbenzene was nominated for study by the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health. It was selected for study by representatives from the Consumer Products Safety Commission, the Environmental Protection Agency, the National Institute of Occupational Safety and Health, and the National Toxicology Program, because of the chemical's potential for widespread consumer exposure and because of its structural similarity to the carcinogen benzene. The present studies were undertaken after ethylbenzene was designated a priority chemical for toxicologic testing by the Interagency Agreement (Superfund Project) between the National Toxicology Program and the Agency for Toxic Substances and Disease Registry. These studies were designed to determine the toxicologic effects of ethylbenzene in rats and mice after a 90-day inhalation exposure. Inhalation was chosen as the route of administration because most human exposure to ethylbenzene is by inhalation. Exposure concentrations used in the study were based on preliminary dose-range finding studies reported by Cragg *et al.* (1989).

II. MATERIALS AND METHODS

Procurement and Characterization of Ethylbenzene

Ethylbenzene was obtained in two lots (Nos. K061786 and K050988) from Koch Chemical Company, Corpus Christi, TX, and stored at 5°C until use, when it was allowed to warm to room temperature over a 4-hour period. A frozen reference standard from Midwest Research Institute (Kansas City, MO) was stored at -20 °C. Analyses (infrared, ultraviolet/visible and NMR spectroscopy, Karl Fisher water analysis, elemental analysis, and gas chromatography) by Midwest Research Institute confirmed the identity of the chemical and indicated its purity was greater than 99%. Gas chromatography/mass spectrometry identified cumene as the major impurity present in both lots of study material; it was present in 0.1% of lot K061786 and 0.06% in lot K050988. Periodic analysis of the chemical by gas chromatography and UV spectroscopy at the study laboratory indicated no degradation over the course of the studies.

Generation and Measurement of Chamber Atmospheric Concentrations

Vapor Generation System

Ethylbenzene vapor was generated using a gas dispersion-type system in which zero-grade nitrogen was passed through liquid ethylbenzene. The vapor then entered a heated stainless steel line maintained at 70°C until it entered the airstream near the top of the chamber (Hazleton 2000 Lab Products Inc., Maywood, NJ), where it was mixed in the chamber plenum before entering the exposure area of the chamber. For vapor generation purposes, one bubbler was used in the 100 ppm chamber, and multiple bubblers were connected in parallel for the remaining target concentrations in order to supply the proper vapor output.

Vapor Concentration Monitoring

Concentrations of ethylbenzene in the inhalation chambers were monitored by an automatic sampling system coupled to a gas chromatograph (Hewlett Packard Model 5880A) equipped with a flame ionization detector and a 10% SP1000 column. The gas chromatographic system was standardized daily by manually injecting standard gas mixtures of ethylbenzene in nitrogen (Union Carbide Corp., Somerset, NJ). Samples of the study chamber atmospheres were pulled from the chambers by a vacuum pump. During the 13-week studies, each study chamber atmosphere was analyzed at least once per hour during the 6-hour exposure. Daily mean exposures for the 13-week studies are given in Table 1.

Chamber Characterization

The uniformity of the vapor concentration in each exposure chamber, with and without animals present, was measured once before the study start (without animals present) and once during the 13-week study, using samples obtained from 2 points at each of the 4 shelf locations in the chambers. The between-port variability, expressed as a percentage of relative deviation, was less than 4.1% for all measurements.

TABLE 1 Mean Chamber Concentrations in the 13-Week Inhalation Studies of Ethylbenzene

Target Concentration (ppm)	Determined Concentration[a]	Maximum Concentration	Minimum Concentration	Percentage within Range
0	b	b	b	
100	99.4 ± 4.4	113	52.3	99
250	246 ± 9.3	311	104	98
500	498 ± 16	705	380	99
750	740 ± 20	847	466	99
1000	975 ± 40	1139	256	97

[a] Mean ± standard deviation for approximately 400 determinations; values within 10% of target chamber concentration.

[b] Less than the detectable value of 1 ppm.

Build-up and clearance times were similar for all chamber concentrations. The time to reach 90% of the target chamber concentration (T90) was determined to be 10 minutes; the time to clear the chamber atmospheres of ethylbenzene following an exposure was 20 minutes.

The test atmospheres of the 100 and 1000 ppm chambers were analyzed by gas chromatography for degradation products at the beginning of the 13-week study. These analyses indicated there was no build up of degradation products during the course of the 6-hour exposure period. Any impurities seen were present at concentrations of less than 0.1% of the ethylbenzene concentration.

The generator reservoirs of the 100 and 1000 ppm chambers were sampled at the beginning of the 13-week study immediately prior to and after a 6-hour exposure. Gas chromatographic analysis of both sets of samples indicated no change in the study chemical composition during the course of the 6-hour generation period.

The possible presence of an aerosol of the study material in the 1000 ppm chamber was determined by drawing the ethylbenzene atmosphere through a 10-stage, Quartz Crystal Microbalance-based Cascade Impactor (QCM, California Measurements Inc., Sierra Madre, CA). The total mass collected from the chamber samples taken during the simulated exposure was compared to that of background samples taken prior to exposure. No aerosol was present on any given stage at or below the background sensitivity of the instrument, indicating that aerosol was not forming in the generation process.

13-Week Study Design

Male and female F344/N rats and B6C3F$_1$ mice used in this study were produced under strict barrier conditions at Taconic Farms, Inc. (Germantown, NY). Animals were progeny of defined, microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats and mice were shipped to the study laboratory at approximately 5 weeks of age,

quarantined at the study laboratory for 12 (rats) or 15 (mice) days, and placed on study at 7 weeks of age.

Groups of 10 rats and 10 mice of each sex were exposed to ethylbenzene in the chambers for 6 hours (plus T90) per day, 5 days per week for 92 (female rats), 93 (male rats), 97 (female mice) or 98 (male mice) days, at 0, 100, 250, 500, 750, or 1000 ppm. Controls were exposed to filtered air. Ten additional rats/sex were included at each exposure level to provide blood samples for clinical pathology (after blood collection on day 23, these rats were killed, and no tissues were retained). The concentrations of ethylbenzene were selected based on preliminary information from a study subsequently published by Cragg *et al.* (1989), which demonstrated respiratory distress and/or death of F344 rats and $B6C3F_1$ mice exposed to 1000 ppm ethylbenzene for 4 days. Additional details concerning study design and performance are listed in Table 2.

Blood for clinical pathology studies was collected on study days 4 and 23, and at week 13 from the retroorbital sinus of male and female rats anesthetized with CO_2. Animals surviving to the end of the studies were killed humanely with CO_2. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Hematologic analyses included erythrocyte count, leukocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hemoglobin, hematocrit, leukocyte differential count, erythrocyte morphologic assessment, reticulocyte count, platelet count and platelet morphologic assessment. All data except those for reticulocyte and differential counts were obtained by using a Baker 9000 hematology analyzer. Clinical chemistry assays were performed with a Baker Centrifichem 500 automated analyzer using standard methods developed for this instrument. Serum chemistry analyses included total protein (TP), albumin (ALB), urea nitrogen (UN), creatinine (CREA), alanine aminotransferase (ALT), alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), total bile acids (TBA), and creatine kinase (CK).

Sperm morphology and vaginal cytology evaluations were performed for rats and mice exposed to 0, 100, 500, and 1000 ppm, according to methods described by Morrissey *et al.* (1988) as briefly outlined in Appendix A1.

A necropsy was performed on all core study animals. Organs and tissues were examined for gross lesions. Tissues were preserved in 10% neutral buffered formalin and routinely processed for preparation of histologic sections for microscopic examination. Tissues and groups examined for rats and mice, both sexes, are listed in Table 2.

Upon completion of the histologic evaluation by the laboratory pathologist, 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, and the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

Statistical Methods

Analysis of organ weight, serum chemistry, hematologic, and male reproductive system data was carried out to assess the significance of pairwise comparisons between dosed and chamber control groups, using the nonparametric multiple comparison procedures of Dunn (1964), Shirley (1977), and Williams (1986). Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends and to determine whether Dunn's or Shirley's test was more appropriate for pairwise comparisons.

The proportion of time spent in each stage of the estrous cycle was compared by using the Wilks criterion statistic of the multivariate analysis of variance procedure, which was performed after an arc sine transformation of the data.

Quality Assurance

The studies of ethylbenzene were performed in compliance with FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of IIT Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The operations of the Quality Assurance Unit were monitored by the NTP, including a site visit during the period of study performance.

TABLE 2 Experimental Design and Materials and Methods in the 13-Week Inhalation Studies of Ethylbenzene

Date of Studies
March 29-June 30, 1988

Animal Source
Taconic Farms, Inc., Germantown, NY

Chemical Source
Koch Chemical Company, Corpus Christi, TX

Study Laboratory
IIT Research Institute, Chicago, IL

Size of Study Groups
10 males and 10 females of each species, individually caged

Concentrations
0, 100, 250, 500, 750, or 1000 ppm ethylbenzene

Duration of Dosing
Rats--6 h/d, 5 d/wk for 92 (females) and 93 (males) days
Mice--6 h/d, 5 d/wk for 97 (females) and 98 (males) days

Strain and Species
F344/N rats; $B6C3F_1$ mice

Method of Animal Distribution
Animals assigned to groups using a stratified weight method and then assigned to study groups in random order

Diet
NIH 07 Open Formula Diet, Zeigler Bros., Inc., Gardners, PA; available *ad libitum* except during exposure

Animal Room Environment
Temp.--75 ±3°F; fluorescent light 12 h/d

Time Held Before Study
Rats--12 days; mice--15 days

Age When Placed on Study
7 weeks

Age When Killed
Rats--20 weeks
Mice--21 weeks

Type and Frequency of Observation
Observed 2 x d for mortality /moribundity; 1 x wk for clinical signs of toxicity; weighed initially, on day 8, 1 x wk, and at necropsy.

Necropsy and Histologic Examinations
Necropsy was performed on all animals. The following tissues were examined microscopically for all controls and 1000 ppm groups: adrenal glands, brain, bronchial lymph nodes, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur (including marrow), gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx, liver, lungs with mainstem bronchi, mammary gland and adjacent skin, mandibular and mesenteric lymph nodes, mediastinal lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pharnyx (if grossly abnormal), pituitary gland, preputial or clitoral glands (rat), rectum, salivary glands, spinal cord and sciatic nerve (if neurologic signs were present), spleen, stomach (including forestomach and glandular stomach), thigh muscle, thymus, thyroid gland, trachea, and urinary bladder.

Tissues examined in all other dose groups included: lung, bronchial lymph node, mediastinal lymph node, and kidney for male rats; and lung, bronchial lymph node, and mediastinal lymph node for female rats.

Organ weights (to the nearest mg) obtained from all core study animals included: liver, thymus, right kidney, right testis, heart, and lungs.

Hematologic and serum chemical analyses were performed; sperm morphology and vaginal cytology evaluated in rats and mice exposed to 0, 100, 500, and 1000 ppm.

III. RESULTS

13-Week Inhalation Study in F344/N Rats

There were no deaths among rats in any of the exposure groups during this study, and no significant clinical signs of toxicity were identified in the rats exposed to ethylbenzene. Male rats exposed to 1000 ppm ethylbenzene exhibited a mild weight depression (5-7%) compared to their respective controls, but the difference was not statistically significant. Group mean body weights for male and female rats measured over the course of the study period are shown in Figure 1.

Serum alkaline phosphatase activity was decreased in a dose-related manner for both male and female rats (Table 3). Hematology parameters (not shown) and other measures of clinical pathology were not consistently affected by ethylbenzene exposure. Increases in absolute and/or relative weights of kidney, liver, and lung were seen in the exposed rats (Table 4). Weights of heart, thymus, and testis were not affected by ethylbenzene. There were no treatment-related microscopic lesions associated with the increased liver and kidney weights. Enlarged bronchial and/or mediastinal lymph nodes were observed grossly in rats exposed to 250 ppm or higher concentrations. In all groups except the 100 ppm and control exposure groups, there was lymphoid hyperplasia in the bronchial and mediastinal lymph nodes, and inflammatory cell infiltrates around vessels, with foci of inflammatory cells in septae and lumen of alveoli in the lung. Although most rats were affected at exposure concentrations of 250 ppm and above, the severity of these lesions was not dose related. In the 250 ppm exposure groups the average severity of the lung lesions was greater than in rats exposed to ethylbenzene concentrations of 500 or 750 ppm (Table 5). Serum samples taken from 5 male and 5 female control rats at terminal sacrifice were negative for antibodies of respiratory tract viruses common to rodents (Sendai, Pneumonia Virus of Mice, and Rat Corona/Sialodacryoadenitis Viruses).

There were no effects observed on sperm, testicular morphology, or the length of the estrous cycle in rats exposed to ethylbenzene (Appendix Table A1). The decrease in epididymal weight in the high dose group (Appendix Table A2) was not considered biologically significant since spermatid counts, sperm motility, and caudal weight were normal.

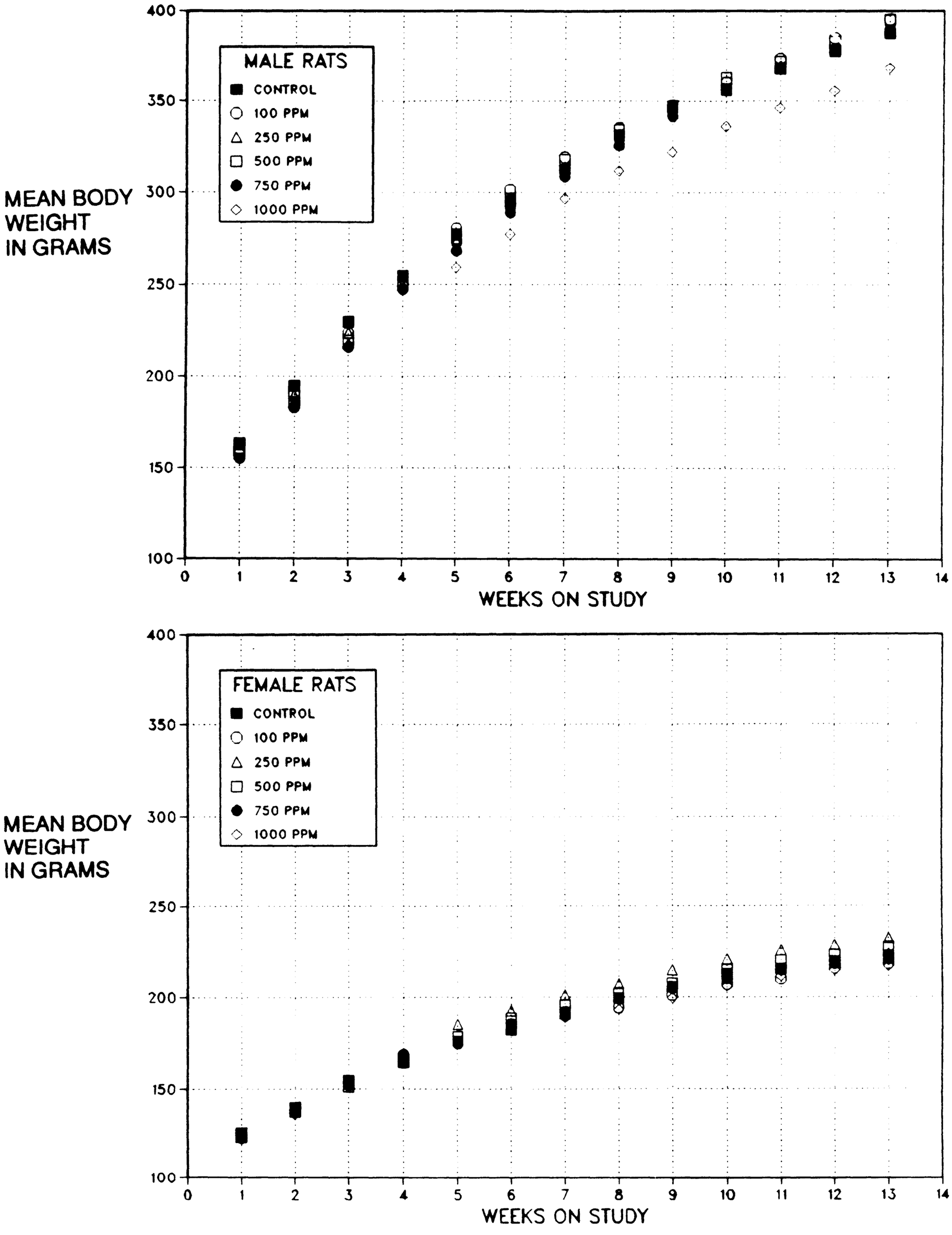


FIGURE 1. Body Weights of Rats Exposed to Ethylbenzene by Inhalation for 13-Weeks

TABLE 3 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Studies of Ethylbenzene[a]

Concentration (ppm)	0	100	250	500	750	1000
DAY 5						
MALE						
UN (mg/dL)	21.7 ± 1.10	20.6 ± 0.73	18.5 ± 0.79	20.1 ± 0.48	19.1 ± 0.36	20.8 ± 1.08
CREA (mg/dL)	0.65 ± 0.02	0.60 ± 0.02	0.64 ± 0.02	0.61 ± 0.02	0.59 ± 0.02**	0.58 ± 0.02*
ALP (IU/L)	512 ± 7.5	510 ± 12	461 ± 11**	447 ± 15**	383 ± 8.2**	389 ± 12**
ALT (IU/L)	35 ± 1.3	35 ± 1.4	36 ± 1.1	34 ± 0.9	37 ± 1.9	36 ± 1.2
ALB (g/dL)	4.0 ± 0.01	4.0 ± 0.04	3.9 ± 0.05	4.1 ± 0.06	4.1 ± 0.06	4.1 ± 0.04
TP (g/dL)	5.9 ± 0.12	6.0 ± 0.07	5.9 ± 0.076	6.0 ± 0.06	5.9 ± 0.07	6.0 ± 0.06
CK (IU/L)	121 ± 22.2	128 ± 16.4	134 ± 24.6	106 ± 9.72	157 ± 17.3	119 ± 15.6
TBA (µmol/L)	28 ± 1.7	27 ± 2.0	30 ± 1.0	27 ± 1.3	27 ± 2.5	25 ± 2.5
SDH (IU/L)	7 ± 0.8	7 ± 0.4	6 ± 0.8	8 ± 1	8 ± 0.9	8 ± 1
FEMALE						
UN (mg/dL)	24.7 ± 1.21	22.6 ± 0.89	24.0 ± 0.53	23.6 ± 2.79	22.1 ± 0.88	22.4 ± 1.07
CREA (mg/dL)	0.66 ± 0.02	0.68 ± 0.03	0.72 ± 0.02	0.73 ± 0.03	0.66 ± 0.02	0.67 ± 0.02
ALP (IU/L)	425 ± 13	382 ± 17	414 ± 16	396 ± 12	374 ± 14*	350 ± 10**
ALT (IU/L)	33 ± 0.7	32 ± 0.9	34 ± 1.3	37 ± 0.9*	34 ± 0.8	35 ± 1.3
ALB (g/dL)	3.9 ± 0.04	4.0 ± 0.04	3.89 ± 0.05	4.0 ± 0.06	4.1 ± 0.06	4.1± 0.04
TP (g/dL)	5.7 ± 0.05	5.7 ± 0.05	5.9 ± 0.07	5.8 ± 0.12	5.9 ± 0.0.4	5.7 ± 0.08
CK (IU/L)	98 ± 10.0	162 ± 21.6	123 ± 11.1	236 ± 33.6**	110 ± 10.5	120 ± 19.5
TBA (µmol/L)	22 ± 1.2	21 ± 1.6	24 ± 2.5	26 ± 1.3	26 ± 3.2	29 ± 3.2*
SDH (IU/L)	8 ± 0.6	10 ± 0.5	10 ± 0.5	9 ± 0.6	10 ± 0.3	10 ± 0.3
DAY 23						
MALE						
UN (mg/dL)	21.1 ± 0.29	21.2 ± 0.69	20.3 ± 0.52	20.8 ± 0.43	21.4 ± 0.61	21.0 ± 0.42
CREA (mg/dL)	0.73 ± 0.01	0.75 ± 0.02	0.77 ± 0.01	0.75 ± 0.02	0.78 ± 0.03	0.73 ± 0.02
ALP (IU/L	376 ± 7	388 ± 12	357 ± 7.7	327 ± 10**	321 ± 6.9**	302 ± 6.3**
ALT (IU/L)	51 ± 3.4	50 ± 3.7	54 ± 4.5	42 ± 1.5	56 ± 4.1	53 ± 4.0
ALB (g/dL)	4.2 ± 0.05	4.1 ± 0.04	4.2 ± 0.03	4.2 ± 0.04	4.2 ± 0.04	4.2 ± 0.03
TP (g/dL)	6.3 ± 0.09	6.1 ± 0.07	6.2 ± 0.06	6.2 ± 0.05	6.3 ± 0.07	6.3 ± 0.05
CK (IU/L)	199 ± 47	149 ± 19	138 ± 17	164 ± 35	160 ± 30	165 ± 31
TBA (µmol/L)	26 ± 1.3	25 ± 1.0	26 ± 1.3	26 ± 1.0	24 ± 0.5	25 ± 0.6
SDH (IU/L)	19 ± 2	17 ± 2	21 ± 1	18 ± 1	21 ± 2	22 ± 1
FEMALE						
UN (mg/dL)	19.8 ± 0.61	21.1 ±1.12	20.5 ± 1.03	20.7 ± 0.80	19.2 ± 0.62	8.4 ± 0.53
CREA (mg/dL)	0.67 ± 0.02	0.72 ± 0.02	0.67± 0.02	0.67 ± 0.02	0.65 ± 0.01	0.66 ± 0.02
ALP (IU/L)	283 ± 6.3	297 ± 6.2	286 ± 11.0	269 ± 6.5	233 ± 6.5**	220 ± 5.5**
ALT (IU/L)	34 ± 2.8	31 ± 0.8	30 ± 0.7	29 ± 0.1*	29 ± 1.3	29 ± 0.8
ALB (g/dL)	4.3 ± 0.03	4.2 ± 0.05	4.2 ± 0.04	4.2 ± 0.05	4.2 ± 0.05	4.2 ± 0.04
TP (g/dL)	6.2 ± 0.08	6.0 ± 0.08	6.1 ± 0.06	6.2 ± 0.07	6.2 ± 0.10	6.1 ± 0.06
CK (IU/L)	169 ± 28	184 ± 44	95 ± 7	140 ± 20	185 ± 32	130 ± 33
TBA (µmol/L)	21 ± 1.2	25 ± 1.9	21 ± 0.9	24 ± 1.5	25 ± 1.9	29 ± 2.7*
SDH (IU/L)	15 ± 0.8	15 ± 0.5	15 ± 0.3	14 ± 0.5	16 ± 0.8	14 ± 0.6
13 WEEKS						
MALE						
UN (mg/dL)	20.7 ± 0.43	20.1 ± 0.65	19.5 ± 0.75	18.6 ± 0.44*	18.7 ± 0.38*	19.0 ± 0.49*
CREA (mg/dL)	0.79 ± 0.03	0.78 ± 0.02	0.76 ± 0.02	0.82 ± 0.03	0.79 ± 0.02	0.78 ± 0.01
ALP (IU/L)	196 ± 8.0	195 ± 4.7	182 ± 4.0	175 ± 2.8*	172 ± 3.4*	172 ± 6.0*
ALT (IU/L)	76 ± 10	99 ± 13	100 ± 21	72 ± 8	79 ± 12	68 ± 9
ALB (g/dL)	4.2 ± 0.04	4.2 ± 0.05	4.1 ± 0.05	4.3 ± 0.13	4.3 ± 0.04	4.2 ± 0.05
TP (g/dL)	6.5 ± 0.09	6.5 ± 0.05	6.4 ± 0.03	6.6 ± 0.04	6.8 ± 0.08**	6.7 ± 0.05*
CK (IU/L)	78 ± 17	89 ± 16	97 ± 16	95 ± 15	96 ± 18	85 ± 22
TBA (µmol/L)	30 ± 1.2	39 ± 3.5	36 ± 3.4	37 ± 3.2	32 ± 1.6	29 ± 1.1
SDH (IU/L)	32 ± 6	48 ± 6	48 ± 10	34 ± 4	34 ± 5	30 ± 5
FEMALE						
UN (mg/dL)	19.4 ± 0.83	18.2 ± 1.17	18.6 ± 0.72	19.0 ± 1.01	17.3 ± 0.53	17.3 ± 0.67
CREA (mg/dL)	0.71 ± 0.03	0.69 ± 0.02	0.71 ± 0.02	0.70 ± 0.03	0.67 ± 0.02	0.66 ± 0.02
ALP (IU/L)	183 ± 7.7	154 ± 4.7**	150 ± 6.7**	136 ± 2.7**	131 ± 3.6**	120 ± 3.0**
ALT (IU/L)	34 ± 2.2	40 ± 3.3	42 ± 5.9	38 ± 3.4	35 ± 2.7	30 ± 2.0
ALB (g/dL)	4.7 ± 0.04	4.6 ± 0.05	4.4 ± 0.07	4.7 ± 0.06	4.7 ± 0.05	4.6 ± 0.06
TP (g/dL)	6.7 ± 0.06	6.8 ± 0.08	6.6 ± 0.13	6.9 ± 0.09	7.0 ± 0.09	6.9 ± 0.08
CK (IU/L)	139 ± 54	98 ± 20	175 ± 37	114 ± 20	81 ± 17	108 ± 31
TBA (µmol/L)	24 ± 2.2	22 ± 1.8	30 ± 4.3	32 ± 2.6	30 ± 1.7*	27 ± 2.3
SDH (IU/L)	12 ± 1	14 ± 1	17 ± 5	13 ± 2	13 ± 2	12 ± 2

a Groups of ten animals each sex; all animals survived.
* Significantly different from controls using Dunnett's test ($p < 0.05$).
** Significantly different from controls using Dunnett's test ($p < 0.01$).

TABLE 4 Organ Weights of F344/N Rats in the 13-Week Inhalation Studies of Ethylbenzene[a]

Concentration (ppm)	0	100	250	500	750	1000
MALE						
Body weight[b]	397 ± 18	406 ± 18	401 ± 25	406 ± 16	403 ± 20	379 ± 32
Right Kidney						
Absolute [c]	1.21 ± 0.05	1.25 ± 0.06	1.29 ± 0.10	1.31 ± 0.07*	1.34 ± 0.08**	1.30 ± 0.11
Relative [d]	3.05 ± 0.13	3.08 ± 0.13	3.19 ± 0.19	3.23 ± 0.18*	3.33 ± 0.05**	3.43 ± 0.19**
Liver						
Absolute	12.7 ± 0.88	13.4 ± 1.1	14.3 ± 1.2*	14.9 ± 1.1**	15.5 ± 1.1**	15.7 ± 1.2**
Relative	32.0 ± 1.5	33.0 ± 1.7	35.7 ± 1.7	36.7 ± 2.2	38.5 ± 1.4**	41.4 ± 2.1**
Lung						
Absolute	1.55 ± 0.17	1.57 ± 0.23	1.82 ± 0.26	1.76 ± 0.26	1.79 ± 0.25	1.75 ± 0.30
Relative	3.90 ± 0.32	3.87 ± 0.48	4.54 ± 0.52	4.33 ± 0.60	4.44 ± 0.49	4.62 ± 0.71*
FEMALE						
Body Weight	226 ± 14	224 ± 14	238 ± 16	231± 12	227 ± 9.2	223 ± 9.0
Right Kidney						
Absolute	0.73 ± 0.05	0.75 ± 0.03	0.76 ± 0.05	0.77 ± 0.05	79 ± 0.04**	0.79 ± 0.05
Relative	3.21 ± 0.16	3.34 ± 0.19	3.20 ± 0.17	3.31 ± 0.16	3.48 ± 0.24	3.53 ± 0.15
Liver						
Absolute	6.84 ± 0.60	6.83 ± 0.55	7.57 ± 0.61	7.65 ± 0.70*	7.63 ± 0.58*	7.92 ± 0.70**
Relative	30.3 ± 1.3	30.5 ± 2.2	31.8 ± 1.6	33.1 ± 2.1	33.6 ± 1.8	35.5 ± 2.1
Lung						
Absolute	0.97 ± 0.10	0.94 ± 0.04	1.22 ± 0.16**	1.07 ± 0.11	1.11 ± 0.11*	1.23 ± 0.17**
Relative	4.27 ± 0.55	4.21 ± 0.37	5.13 ± 0.77**	4.63 ± 0.48	4.89 ± 0.40	5.52 ± 0.63**

a Groups of ten animals each sex ; all animals survived.
b Body weights are presented as mean ± standard error in grams.
c Absolute weights are presented as mean ± standard error in milligrams.
d Relative weights are presented as mean ± standard error in milligrams per gram of body weight.
* Significantly different from control groups using ANOVA and DUNNETT'S TEST ($p<0.05$).
** Significantly different from control groups using ANOVA and DUNNETT'S TEST ($p<0.01$).

TABLE 5 Incidence and Severity of Inflammation in the Lung of Rats

Concentration (ppm)	0	100	250	500	750	1000
MALE	0	0	9 (1.7)*	9 (1.5)	9 (1.1)	9 (1.9)
FEMALE	0	0	10 (2.1)	10 (1.5)	10 (1.0)	10 (2.5)

* Number of rats affected (average severity); 10 rats per group. Average severity score based on a scale of 1= minimal, 2= mild, 3=moderate, 4= marked.

13-Week Inhalation Study in B6C3F$_1$ Mice

There were no deaths among mice in any of the exposure groups during the study, and no significant clinical signs of toxicity were identified in the mice exposed to ethylbenzene. No significant, adverse effects on male or female mouse body weights were observed that were related to ethylbenzene exposure. Group mean body weights for male and female mice measured over the course of the study period are shown in Figure 2. No exposure-related gross observations were noted in mice at terminal necropsy. Dose-related increases in both absolute and relative liver weights were seen in both sexes of mice exposed to 750 or 1000 ppm (Table 6), and the relative kidney weight of female mice exposed at 1000 ppm was greater than that of the controls. These small but statistically significant weight changes were not accompanied by histopathologic lesions, and no chemically related histopathologic changes were identified in any organs.

Results of sperm morphology and vaginal cytology evaluations for mice were negative. (Appendix Table A2).

TABLE 6 Organ Weights of B6C3F$_1$ Mice in the 13-Week Inhalation Studies of Ethylbenzene [a]

Concentration (ppm)	0	100	250	500	750	1000
MALE						
Body Weight [b]	37.8 ± 3.0	39.7 ± 1.9	37.4 ± 3.3	37.2 ± 3.0	38.3 ± 1.7	38.0 ± 2.4
Right Kidney						
Absolute[c]	325 ± 34	342 ± 35	327 ± 31	339 ± 29	334 ± 24	342 ± 28
Relative[d]	8.60 ± 1.2	8.61 ± 0.91	8.74 ± 1.5	9.11 ± 1.3	8.72 ± 0.79	9.00 ± 0.74
Liver						
Absolute	1720 ± 200	1800 ± 190	1850 ± 190	1850 ± 170	2060 ± 170**	2150 ± 180**
Relative	45.5 ± 3.4	45.3 ± 5.1	49.5 ± 5.8	49.7 ± 4.2	53.8 ± 4.1	56.6 ± 4.7
Lung						
Absolute	201 ± 45	199 ± 23	199 ± 55	195 ± 21	206 ± 44	196 ± 23
Relative	5.32 ± 1.1	5.01 ± 0.56	5.32 ± 1.3	5.24 ± 0.72	5.38 ± 1.1	5.16 ± 0.81
FEMALE						
Body Weight	34.7 ± 3.0	33.1 ± 4.0	38.7 ± 3.2*	34.7 ± 2.7	37.5 ± 2.3	33.9 ± 2.3
Right Kidney						
Absolute	211 ± 17	220 ± 12	221 ± 12	227 ± 21	229 ± 14	232 ± 17
Relative	6.1 ± 0.77	6.6 ± 0.60	6.7 ± 0.25	6.5 ± 0.61	6.1 ± 0.31	6.8 ± 0.71*
Liver						
Absolute	1590 ± 110	1560 ± 190	1720 ± 190	1800 ± 240	2070 ± 220**	2040 ± 180**
Relative	45.8 ± 5.2	47.1 ± 3.9	52.0 ± 3.8	51.0 ± 6.5	55.2 ± 4.6	60.2 ± 4.3
Lung						
Absolute	182 ± 50	170 ± 19	189 ± 48	200 ± 53	192 ± 27	209 ± 52
Relative	5.2 ± 0.98	5.1 ± 0.34	5.7 ± 1.0	5.8 ± 1.4	5.1 ± 0.78	6.2 ± 1.7

a Groups of ten animals each sex ; all animals survived.
b Body weights are presented as mean ± standard error in grams.
c Absolute weights are presented as mean ± standard error in milligrams.
d Relative weights are presented as mean ± standard error in milligrams per gram of body weight.
* Significantly different from control groups using ANOVA and DUNNETT'S TEST ($p<0.05$).
** Significantly different from control groups using ANOVA and DUNNETT'S TEST ($p<0.01$).

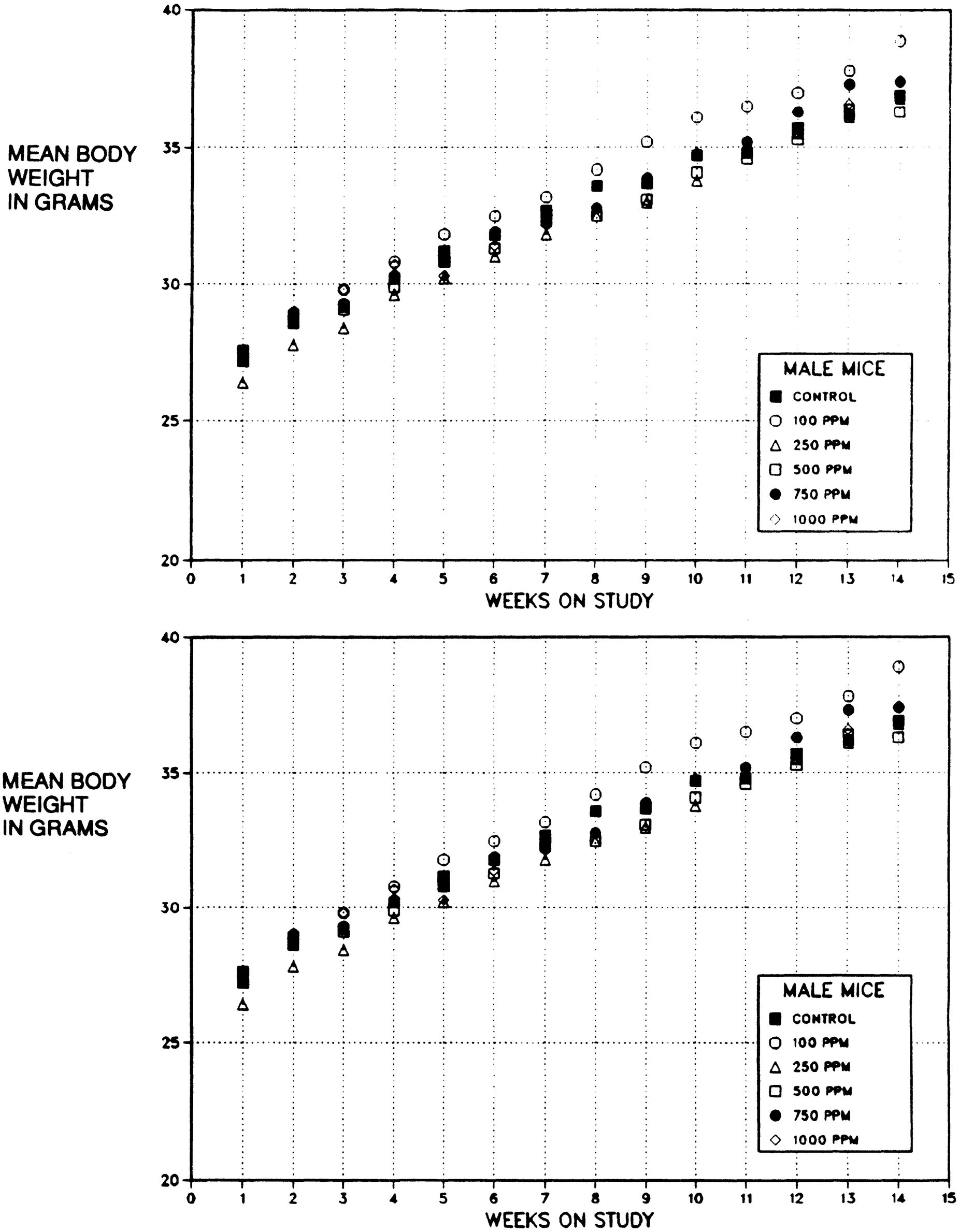


FIGURE 2. Body Weights of B6C3F$_1$ Mice Exposed to Ethylbenzene by Inhalation for 13-Weeks

IV. DISCUSSION

Inhalation exposure to ethylbenzene at concentrations up to 1,000 ppm did not adversely affect the survival or body weight gains of F344/N rats or B6C3F$_1$ mice. No significant clinical indications of toxicity, nor microscopic changes attributable to ethylbenzene exposure, were observed in rats or mice.

In the present study, there were apparent dose-related increases in absolute kidney and liver weights and liver to body weight ratios in both sexes of rats and mice, as well as an increase in the kidney/body weight ratio of female mice exposed to 1,000 ppm. Increases in liver and kidney weights were reported in rats exposed by inhalation to ethylbenzene at 400 ppm, 7 hours per day, 5 days per week, for 5-7 months, in studies by Wolf *et al.* (1956). Cragg *et al.* (1989) also observed an increase in relative liver weights in F344/N rats and B6C3F$_1$ mice of both sexes exposed to ethylbenzene at 782 ppm. Previous investigators have speculated that the liver weight increase is related to adaptive induction of hepatic mixed function oxidases (Toftgard and Nilsen, 1982; Elovaara *et al.*, 1985).

Wolf *et al.* (1956) observed histopathologic changes (cloudy swelling) in the kidney and liver of female rats exposed orally to $\geq$ 408 mg/kg, or by inhalation of $\geq$ 400 ppm, 5 days per week for 6 months. No treatment-related microscopic lesions were observed in the liver and kidney of rats and mice exposed to ethylbenzene in the present inhalation study.

Dose-related decreases of serum alkaline phosphatase activity were observed in both male and female rats in the present study, but activities of other serum enzymes were not affected. Decreased serum alkaline phosphatase activity may be related to reduced food and water intake (Hoffmann, *et al.*, 1989).

Although ethylbenzene is reported to be a mucous membrane irritant for the eye, nose, and throat (Yant *et al.*, 1930), morphologic changes in the lung or upper respiratory tract did not occur in rats, mice, or rabbits exposed to ethylbenzene by the inhalation route at concentrations up to 782 ppm for 4 weeks (Cragg *et al.*, 1989). In the present study, inflammation that occurred in the lungs of animals exposed to doses of 250 ppm and above was not attributed to toxicity of ethylbenzene. The morphologic appearance and anatomic distribution of the inflammatory lesions in the lung and the lymphoid hyperplasia in the lymph nodes of the respiratory tract are characteristic of a response to an infectious agent. The lack of a dose-related response in the severity of the lung lesions, and the absence of lesions in the upper respiratory tract, are not typical findings for an inhaled toxicant. In both sexes of rats there was a decreased severity of inflammation in the 750 ppm groups, as compared to rats exposed to the 2 lower concentrations of ethylbenzene. The occurrence and severity of inflammation corresponds to observed increases in lung weights. Lesions morphologically indistinguishable from those in this study have been seen in lung and respiratory tract lymph nodes of control and treatment groups of rats from other inhalation and dosed feed studies. Thus, the inflammatory lung lesions observed in rats in the present study were probably unrelated to ethylbenzene exposure. Antibodies to common rodent respiratory tract viruses

were not detected; however, this may have been the result of serum-sampling only those rats from the control chambers which did not have inflammatory lesions in the lung. The absence of respiratory tract lesions in mice housed in the same chambers with rats during the exposure period suggests sendai and pneumonia virus infections are unlikely causes of the lung lesions in rats.

In conclusion, our data indicate that ethylbenzene is neither mutagenic nor clastogenic. Inhalation exposures up to 1000 ppm did not induce adverse clinical or histopathologic changes in rats and mice other than an increase in liver and kidney weights. These findings are consistent with those recently reported by Cragg *et al.* (1989).

V. REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH) (1989) *Threshold Limit Values and Biological Exposure Indices.* Cincinnati: ACGIH

Angerer, J., and Lehnert, T. (1979) Occupational chronic exposure to organic solvents. VIII. Phenolic compounds -- metabolites of alkylbenzenes in man. Simultaneous exposure to ethylbenzene and xylenes. *Int. Arch. Occup. Environ. Health* **43**, 145-150.

Bardodej, Z., and Bardodejova, E. (1970) Biotransformation of ethylbenzene, styrene, and alpha-methylstyrene in man. *Am. Ind. Hyg. Assoc. J.* **31**, 206-209.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985) Quality Assurance in pathology for rodent carcinogenicity studies, in: Milman, H., and Weisburger, E. (eds.), *Handbook of Carcinogen Testing.* Park Ridge, NJ: Noyes Publications, pp. 345-357.

Budavari, S., O'Neil, M.J., Smith, A., and Heckelman, P.E. (eds.) (1989) *The Merck Index,* 11th ed. Rahway, NJ: Merck & Co., Inc.

Chin, B.H., McKelvey, J.A., Tyler, T.R., Calisti, L.R., Kozbelt, S.J., and Sullivan, L.J. (1980) Absorption, distribution, and excretion of ethylbenzene, ethylcyclohexane, and methylethylbenzene isomers in rats. *Bull. Environ. Contam. Toxicol.* **24,** 477-483.

Climie, I.J.G., Hutson, D.H., and Stoydin, G. (1983) The metabolism of ethylbenzene hydroperoxide in the rat. *Xenobiotica* **13**, 611-618.

Cragg, S.T., Clarke, E.A., Daly, I.W., Miller, R.R., Terrill, J.B., Ouellette, R.E. (1989) Subchronic inhalation toxicity of ethylbenzene in mice, rats, and rabbits. *Fund. Appl. Toxicol.* **13**, 399-408.

Dean, B.J., Brooks, T.M., Walker, G.H., and Hutson, D.H. (1985) Genetic toxicity testing of 41 industrial chemicals. *Mutat. Res.* **153**, 57-77.

Dunn, O.J. (1964) Multiple Comparisons Using Rank Sums. *Technometrics* **6**, 241-252.

Dunnett, W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1095-1121.

Dutkiewicz, T., and Tyras, H. (1967) A study of the skin absorption of ethylbenzene in man. *Brit. J. Ind. Med.* **24**, 330-332.

Elovaara, E., Engstrom, K., Nickels, J., Aitio, A., and Vainio, H. (1985) Biochemical and morphological effects of long term inhalation exposure of rats to ethylbenzene. *Xenobiotica* **15**, 299-308.

Engstrom, J., and Bjurstrom, R. (1978) Exposure to xylene and ethylbenzene. II. Concentration in subcutaneous adipose tissue. *Scand. J. Work Environ. and Health* **4**,195-203.

Engstrom, K.M. (1984) Metabolism of inhaled ethylbenzene in rats. *Scand. J. Work Environ. and Health* **10**, 83-87.

Federal Register (1987) Twentieth report of the Interagency Testing Committee to the administrator; receipt of report and request for comments regarding priority list of chemicals. Federal Register **52**, 19020-19026.

Federal Register (1989) Ethyl Benzene. Federal Register **54**, 2460-2461.

Fishbein, L. (1985) An overview of environmental and toxicological aspects of aromatic hydrocarbons. IV. Ethylbenzene. *Sci. of Total Environ.* **44**, 269-287.

Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P., and Niemeier, R.W. (1981) Testing of selected workplace chemicals for teratogenic potential. *Scand. J. Work Environ. and Health* **7**, 66-75.

Heylin, M. (1987) Facts & figures for the chemical industry. *Chem. Eng. News* **65**, 23-76.

Hoffmann, W.E., Kramer, J., Main, A.R., and Torres, J.L. (1989) Clinical Enzymology, in: Loeb, W.F., and Quimby, F.W. (eds.), *The Clinical Chemistry of Laboratory Animals.* New York: Pergamon Press, p. 251-252.

Jonckheere, A. (1954) A distribution-free k-sample test against ordered alternatives. *Biometrica* **41**, 133-145.

Lonneman, W.A., Bellar, T.A., and Altshuller, A.P. (1968) Aromatic hydrocarbons in the atmosphere of the Los Angeles basin. *Environ. Sci. Tech.* **2**, 1017-1020.

Maltoni, C., Conti, B., Cotti, G., and Belpoggi, F. (1985) Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: Current results and ongoing research. *Am. J. Ind. Med.* **7**, 415-446.

Maronpot, R.R., and Boorman, G.A. (1982) Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

McGregor, D., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C., and Caspary, W. (1988) Responses of the L5178Y/tk$^{\pm}$ mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ. Molec. Mutagen.* **12**, 85-154.

Morrissey, R.E., Schwetz, B.A., Lamb, J.C., IV, Ross, M.C., Teague. J.L., and Morris, R.W. (1988) Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. *Fund. Appl. Toxicol.* **11**, 343-358.

National Research Council (1981) *The Alkyl Benzenes.* Washington, D.C.: National Academy Press.

Nestmann, E.R., Lee, E.G.-H., Matula, T.I., Douglas, G.R., and Mueller, J.C. (1980) Mutagenicity of constituents identified in pulp and paper mill effluents using the *Salmonella*/mammalian-microsome assay. *Mutat. Res.* **79**, 203-212.

National Institute of Occupational Safety and Health (NIOSH) (1979) *Ethylbenzene data sheet for prioritized NIOSH/OSHA substances for the National Toxicology Program.* Rockville, MD: National Institute for Occupational Safety and Health.

Norppa, H., and Vainio, H. (1983) Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes. *Mutat. Res.* **116**, 379-387.

Nunes, P., and Benville, P.E., Jr. (1979) Uptake and depuration of petroleum hydrocarbons in the manila clam; tapes semidecussata Reeve. *Bull. Environ. Contam. Toxicol.* **21**, 719.

Otson, R., Williams, D.T., and Bothwell, P.D. (1982) Volatile organic compounds in water at thirty Canadian potable water treatment facilities. *J. Assoc. Off. Anal. Chem.* **65**, 1370-1374.

Pyykko, K., Paavilainen, S., Metsa-Ketela, T., and Laustiola, K. (1987) The increasing and decreasing effects of aromatic hydrocarbon solvents on pulmonary and hepatic cytochrome P-450 in the rat. *Pharmacol. Toxicol.* **60**, 288-293.

Roubal, N.I., Stranahan, S.I., and Malins, D.C. (1978) The accumulation of low molecular weight aromatic hydrocarbons of crude oil by Coho salmon (*Oncorhyrchus kisutch*) and starry flounder (*Platichlys stellatus*). *Arch. Environ. Contam. Toxicol.* **7**, 237.

Shirley, E. (1977) A Non-Parametric Equivalent of Williams' Test for Contrasting Increasing Dose Levels of a Treatment. *Biometrics* **33**, 386-389.

Shirley, E. (1977) A Non-Parametric Equivalent of Williams' Test for Contrasting Increasing Dose Levels of a Treatment. *Biometrics* **33**, 386-389.

Smyth, N.F., Jr., Carpenter, C.P., Weil, M.A., Pozzani, U.C., and Striegel, J.A. (1962) Range-finding toxicity data: List VI. *Am. Ind. Hyg. Assoc. J.* **23**, 95-107.

STORET (1986) *Water Quality Control Information System* [database]. Washington, D.C.: U.S. Environmental Protection Agency.

Toftgard R., and Nilsen, O.G. (1982) Effects of xylene and xylene isomers on cytochrome P-450 and *in vitro* enzymatic activities in rat liver, kidney, and lung. *Toxicology* **23**, 197-212.

U.S. International Trade Commission (1985) *Synthetic Organic Chemicals, United States Production and Sales, 1984.* Washington, D.C.: U.S. Government Printing Office, Publication No. 1745, p.25.

Ungvary, G., and Tatrai, E. (1985) On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats, and rabbits. *Arch. Toxicol.*, **8 (Suppl)**, 425-430.

Verschueren, K. (1983) *Handbook of Environmental Data on Organic Chemicals*, 2nd ed. New York: Van Nostrand and Reinhold Co., p. 628.

Wallace, L., Pellizzari, E., Hartwell, T., Rosenzweig, M., Erickson, M., Sparacino, C., and Zelon, H. (1984) Personal exposure to volatile organic compounds. I. Direct measurements in breathing-zone air, drinking water, food and exhaled breath. *Environ. Res.* **35**, 293-319.

Williams, D.A. (1986) A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Wolf, M.A., Rowe, V.K., McCollister, R.L., and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. *A.M.A. Arch. Ind. Hlth.* **14**, 387-398.

Wolf, M.S., Daum, S.M., Lorimer, W.V., Selikoff, I.J., and Aubrey, B.B. (1977) Styrene and related hydrocarbons in subcutaneous fat from polymerization workers. *J. Toxicol. Environ. Health* **2**, 997-1005.

Yant, W.P., Schrenk, H.H., Waite, C.P., and Patty, F.A. (1930) Acute response of guinea pigs to vapors of some new commercial organic compounds. II. Ethylbenzene. *Public Health Reports* **45**, 1241-1250.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988) *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Molec. Mutagen.* **11(Suppl. 12)**, 1-158.

APPENDIX A

RESULTS OF REPRODUCTIVE ANALYSES IN THE 13-WEEK INHALATION STUDIES OF ETHYLBENZENE

APPENDIX A

Methods for Sperm Motility and Vaginal Cytology Evaluation

Vaginal Cytology

For the 12 days prior to sacrifice, females were subject to vaginal lavage with saline. The aspirated cells were air-dried onto slides, stained with Toluidine Blue O, and coverslipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrous cycle.

Sperm Motility

The left epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, and weighed. Tyrodes buffer (mice, 80 µl) or test yolk buffer (rats, 80 µl) was applied to 2 pre-warmed slides, and a small cut made in the distal cauda epididymis. The sperm that were removed from the epididymis were dispersed throughout the solution, coverslipped, and counted immediately on a warmed microscope stage. The number of moving and non-moving sperm were counted in 5 fields of 30 sperm or less on each slide.

After sperm sampling for motility estimation, the cauda was placed in phosphate buffered saline (PBS) and gently chopped with a razor blade and allowed to sit for 15 minutes. The remaining clumps of tissue were removed, the solution mixed gently, and heat-fixed at 65°C. Sperm counts were then determined using a hemocytometer.

The left testis was frozen and stored. After thawing, testicular spermatid head count was determined after removing the tunica albuginea and homogenizing the testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis and per gram of testis.

TABLE A1 Reproductive System Data for F344/N Rats in the 13-Week Inhalation Studies of Ethylbenzene[a]

Concentration (ppm)	0	100	500	1000
MALE				
Left caudal weight (mg)	209 ± 7	217 ± 9	216 ± 6	211 ± 10
Left epididymal weight (mg)	492 ± 8	509 ± 12	497 ± 6	497 ± 18
Sperm count (X10^6)/gram testis	85.2 ± 0.39	81.2 ± 0.30	78.2 ± 0.40	80.7 ± 0.31
Sperm motility (%)	94.1 ± 0.90	95.6 ± 0.86	95.31 ± 0.73	93.4 ± 0.66
FEMALE				
Estrous stage (%)				
Proestrus	17.5	15.8	15.0	10.0
Estrus	26.7	25.8	28.3	30.8
Metestrus	20.8	22.5	21.7	19.2
Diestrus	35.0	35.8	35.0	40.0
Cycle Length (days)	4.95 ± 0.09	4.70 ± 0.13	4.95 ± 0.09	4.75 ± 0.11

a Mean ± standard error for groups of 10 animals; no significant differences vs. the controls by Dunn's test (Dunn, 1964).

TABLE A2 Reproductive System Data for B6C3F_1 Mice in the 13-Week Inhalation Studies of Ethylbenzene[a]

Concentration (ppm)	0	100	500	1000
MALE				
Left caudal weight (mg)	20 ± 1	20 ± 1	17 ± 1	18 ± 1
Left epididymal weight (mg)	53 ± 2	49 ± 1	47 ± 1	47 ± 1*
Spermatids (X10^6)/gram testis	149 ± 0.54	167 ± 0.55	156 ± 0.47	148 ± 0.87
Sperm motility (%)	94.7 ± 0.51	93.98 ± 0.62	94.24 ± 0.70	94.02 ± 0.74
FEMALE				
Estrous stage (percent)				
Proestrus	22.5	20.8	25.0	21.7
Estrus	31.7	27.5	25.8	26.7
Metestrus	17.5	23.3	21.7	20.8
Diestrus	28.3	28.3	27.5	30.8
Cycle Length (days)	4.67 ± 0.14[b]	4.50 ± 0.40	4.20 ± 0.11	4.35 ± 0.11

a Mean ± standard error for groups of 10 animals.

b Estrous cycle longer than twelve days or unclear in 1/10 animals; data presented are for the remaining animals.

* Significantly different from control group by Shirley's Test (Shirley, 1977), $p < 0.05$

APPENDIX B

RESULTS OF MUTAGENESIS ANALYSES IN THE 13-WEEK INHALATION STUDIES OF ETHYLBENZENE

TABLE B1 Mutagenicity of Ethylbenzene in *Salmonella typhimurium* [a]

Strain	Dose (µg/plate)	Revertants/plate (b) (-) S9 Trial 1	(-) S9 Trial 2	Hamster S9 10%	Hamster S9 30%	Rat S9 10%	Rat S9 30%
TA100							
	0	112 ± 9.3	147 ± 4.0	114 ± 8.2	136 ± 3.3	111 ± 2.1	154 ± 7.8
	10	104 ± 0.9	161 ± 5.8	120 ± 11.5	138 ± 9.5	100 ± 5.0	155 ± 9.0
	33	100 ± 4.4	147 ± 4.1	137 ± 22.7	140 ± 11.5	110 ± 8.1	155 ± 9.3
	100	97 ± 4.8	157 ± 3.2	109 ± 7.1	138 ± 12.2	105 ± 2.3	161 ± 14.5
	333	97 ± 6.9	118 ± 11.5	97 ± 7.1	137 ± 1.2	111 ± 4.7	127 ± 13.2
	666	76 ± 6.2	74 ± 4.0[c]				
	1000			98 ± 1.7	112 ± 6.1	77 ± 8.2	109 ± 8.8
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive Control[e]		375 ± 12.3	394 ± 32.5	873 ± 46.0	740 ± 18.0	1304 ± 306.0	352 ± 19.8
TA1535							
	0	14 ± 3.7	29 ± 3.8	7 ± 1.5	11 ± 2.3	9 ± 2.0	12 ± 1.2
	10	19 ± 1.3	26 ± 3.2	9 ± 1.3	14 ± 1.5	8 ± 0.7	13 ± 2.5
	33	21 ± 4.6	19 ± 2.5	6 ± 0.7	11 ± 1.5	9 ± 3.0	8 ± 0.6
	100	16 ± 1.5	25 ± 2.5	8 ± 1.5	10 ± 2.4	5 ± 0.6	10 ± 1.5
	333	16 ± 2.1	14 ± 0.3	9 ± 1.2	9 ± 2.7	8 ± 2.4	6 ± 0.9
	666	0 ± 0.0[d]	0 ± 0.0[c]				
	1000			5 ± 1.8	11 ± 1.9	5 ± 1.5	9 ± 1.5
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[e]		418 ± 23.1	520 ± 20.0	703 ± 16.5	431 ± 36.9	393 ± 72.0	101 ± 11.4
TA97							
	0	182 ± 1.5	111 ± 9.5	195 ± 12.3	184 ± 18.2	200 ± 10.0	218 ± 6.5
	10	203 ± 1.8	120 ± 16.3	194 ± 10.3	210 ± 22.5	190 ± 15.1	249 ± 20.2
	33	198 ± 6.9	144 ± 2.4	195 ± 3.5	186 ± 22.4	193 ± 5.3	227 ± 16.5
	100	195 ± 9.9	124 ± 5.2	191 ± 7.1	227 ± 1.8	179 ± 7.8	12 ± 13.0
	333	188 ± 5.7	108 ± 9.1	173 ± 3.5	202 ± 8.3	211 ± 3.3	211 ± 6.4
	666	103 ± 1.5	6 ± 5.7[c]				
	1000			124 ± 9.6	180 ± 15.9	189 ± 23.4	195 ± 15.3
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[e]		856 ± 20.8	954 ± 47.1	1587 ± 146.1	1123 ± 30.4	647 ± 154.3	540 ± 12.7
TA98							
	0	26 ± 1.8	29 ± 5.5	24 ± 3.2	35 ± 3.8	34 ± 3.3	34 ± 7.2
	10	16 ± 2.3	27 ± 4.4	29 ± 1.8	34 ± 4.7	26 ± 1.8	32 ± 4.1
	33	22 ± 4.8	35 ± 7.8	26 ± 0.6	34 ± 4.5	34 ± 3.5	32 ± 2.3
	100	21 ± 2.4	16 ± 2.1	28 ± 4.7	26 ± 1.2	32 ± 2.3	30 ± 4.2
	333	18 ± 1.5	20 ± 8.4	23 ± 3.0	30 ± 0.7	30 ± 2.3	28 ± 5.6
	666	13 ± 1.2	27 ± 14.5[c]				
	1000			21 ± 2.3	30 ± 0.9	26 ± 1.5	30 ± 3.5
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[e]		845 ± 69.2	566 ± 45.0	1082 ± 174.8	285 ± 32.9	784 ± 214.8	149 ± 10.7

[a] Study performed at SRI, International. The detailed protocol is presented in Zeiger *et al.* (1988). Cells and study compound or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity or solubility, but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.

[b] Revertants are presented as mean ± the standard error from 3 plates.

[c] Slight toxicity

[d] Precipitate on plate

[e] 2-Aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-o-phenylenediamine was tested on TA98; sodium azide was tested on TA100 and TA1535; and 9-aminoacridine was tested on TA97.

TABLE B2 Induction of Trifluorothymidine Resistance in Mouse L5178Y/TK± Lymphoma Cells by Ethylbenzene[a]

Compound	Concentration (µg/ml)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Colony Count	Mutant Fraction[b]	Average Mutant Fraction
-S9						
Trial 1						
Dimethylsulfoxide						
		84	94	159	63	
		89	106	150	56	
		78	108	155	66	
		87	92	138	53	
						60
Ethylbenzene						
	10	81	103	123	51	
		86	106	157	61	56
	20	81	90	130	54	
		81	93	127	52	53
	40	87	82	175	67	
		73	72	144	66	67
	80	74	36	1235	559	
		71	32	1325	619	589[c]
	160	LETHAL				
Ethylmethane sulfonate[d]						
	250	81	85	357	147	
		83	95	374	150	149[c]
Methylmethane sulfonate[d]						
	15	61	40	251	138	
		52	39	238	152	145[c]

TABLE B2 Induction of Trifluorothymidine Resistance in Mouse L5178Y/TK± Lymphoma Cells by Ethylbenzene (continued)

Compound	Concentration (µg/ml)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Colony Count	Mutant Fraction [b]	Average Mutant Fraction
+S9						
Trial 2						
Dimethylsulfoxide						
		85	106	87	34	
		69	95	63	30	
		82	98	75	30	
		100	101	91	30	31
Ethylbenzene						
	20	83	83	109	44	
		82	83	102	41	42
	40	78	61	75	32	
		73	54	58	27	29
	60	64	37	91	48	
		68	60	79	39	43
	80	48	10	228	159	
		55	15	233	142	150[c]
	100	LETHAL				
Ethylmethane sulfonate[d]						
	250	50	67	302	201	
		51	67	381	250	225[c]
Methylmethane sulfonate[d]						
	15	43	34	122	94	
		42	32	152	120	107[c]

a Study performed at Inveresk Research International. The experimental protocol and these data are presented in detail by McGregor *et al.* (1988). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. Cells (6 x 10^5/ml) were treated for 4 h at 37°C in medium, washed, resuspended in medium, and incubated for 48 h at 37° C (4 replicate flasks for solvent control; 2 replicates for all other treatments). After expression, 3 x 10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency. All data are evaluated statistically for both trend and peak responses. Both responses must be significantly ($P<0.05$) positive for a chemical to be considered mutagenic. If only one of these responses is significant, the call is "questionable"; the absence of both trend and peak response results in a "negative" call.

b Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/10^6 cells treated); MF = mutant fraction .

c Significant positive response ($p < 0.05$); occurs when the relative mutant fraction (average MF of treated culture/ average MF of solvent control) is approximately ≥1.6.

d Positive control

TABLE B3 Induction of Sister-Chromatid Exchanges in Chinese Hamster Ovary Cells by Ethylbenzene[a]

Compound	Dose (µg/ml)	Total Cells	No. of Chromo-somes	No. of SCEs	SCE/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[b]
-S9[c]								
Summary: Negative								
Dimethylsulfoxide		50	1045	555	0.53	11.1	25.5	
Ethylbenzene	75.5	50	1046	551	0.52	11.0	25.5	-0.82
	99.5	50	1049	522	0.49	10.4	25.5	-6.31
	125.0*	50	1033	590	0.57	11.8	25.5	7.54
	151.0*	0					25.5	
Mitomycin-C[e]	0.001	50	1041	773	0.74	15.5	25.5	39.81
	0.010	5	103	220	2.13	44.0	25.5	302.17
				Trend:	0.817			
				Probability:	0.207			
+S9[d]								
Summary: Negative								
Dimethylsulfoxide		50	1047	531	0.50	10.6	25.8	
Ethylbenzene	125	50	1044	561	0.53	11.2	25.8	5.95
	137.5	50	1041	531	0.51	10.6	25.8	0.58
	150*	50	1037	516	0.49	10.3	25.8	-1.89
	175*	0						
Cyclophosphamide[e]	0.35	50	1048	723	0.68	14.5	25.8	36.03
	2	5	108	159	1.47	31.8	25.8	190.29
				Trend:	-0.562			
				Probability:	0.713			

* Precipitate observed at this dose level.

a Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethylsulfoxide) as described in (c) and (d) below, and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

b SCE's/chromosome of culture exposed to study chemical relative to those of culture exposed to solvent.

c In the absence of S9, cells were incubated with study compound or solvent for 2 h at 37°C. Then BrdU was added and incubation was continued for 24 h. Cells were washed; fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 h.

d In the presence of S9, cells were incubated with study compound or solvent for 2 h at 37°C. The cells were then washed, and medium containing BrdU but no test chemical was added. Cells were incubated for a further 26 h, with colcemid present for the final 2-3 h. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

e Positive controls.

f Statistics performed SCE/chromosome values.

Table B4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Ethylbenzene [a]

-S9[b]					+S9[c]				
Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells w/ Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells w/ Abs
Harvest time: 10.5 hours Summary: Negative					Harvest time: 10.5 hours Summary: Negative				
Dimethylsulfoxide									
100	100	3	0.03	3		100	3	0.03	3
Ethylbenzene									
75	100	1	0.01	1	75	100	4	0.04	4
100	100	3	0.03	3	100	100	1	0.01	1
125	100	5	0.05	5	125	100	1	0.01	1
150	0				150	0			
Mitomycin-C[d]					Cyclophosphamide[d]				
1	50	16	0.32	22	50	50	23	0.46	36.0
Trend[e]: 1.034435 Probability: 0.150466					Trend[e]: -1.385584 Probability: 0.917063				

a Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethylsulfoxide) as indicated in (b) and (c). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake off, fixed, and stained in 6% Giemsa.

b In the absence of S9, cells were incubated with study compound or solvent for 8-10 h at 37°C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 h followed by harvest.

c In the presence of S9, cells were incubated with study compound or solvent for 2 h at 37°C. Cells were then washed; medium without test chemical was added, and incubation was continued for 8-10 h. Colcemid was added for the last 2-3 h of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

d Positive controls.

e Statistics performed on % cells with Abs.

TABLE B5 Frequency of Micronuclei in Peripheral Blood Erythrocytes of B6C3F$_1$ Mice Exposed to Ethylbenzene for 13-Weeks[a]

Concentration (ppm)	Micronucleated Cells/1000 Cells[b] PCE	NCE	%PCE	Number of Mice
Male				
0	2.18 ± 0.56	1.54 ± 0.16	2.22 ± 0.10	8
500	2.04 ± 0.31	1.68 ± 0.13	3.13 ± 0.94	10
750	1.90 ± 0.53	1.90 ± 0.13	1.97 ± 0.09	9
1000	1.21 ± 0.20	1.59 ± 0.16	2.02 ± 0.14	10
Trend test[c]	P = 0.928	P = 0.816		
ANOVA[d]			P = 0.278	
Female				
0	1.54 ± 0.56	0.92 ± 0.11	1.74 ± 0.14	10
500	2.64 ± 0.53	1.01 ± 0.12	1.83 ± 0.18	10
750	1.87 ± 0.38	1.32 ± 0.22	1.85 ± 0.15	10
1000	1.01 ± 0.26	1.12 ± 0.12	1.80 ± 0.15	10
Trend test	P = 0.817	P = 0.077		
ANOVA			P = 0.886	
Overall Trend	P = 0.951	P = 0.149		
Overall ANOVA			P = 0.684	

a Smears were prepared from peripheral blood samples obtained by cardiac puncture of dosed and control animals at the termination of the 13-week study. Slides were stained with Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983). At least 2000 polychromatic erythrocytes (PCE) and 10,000 normochromatic erythrocytes (NCE) from each animal were scored for micronuclei. No significant elevation in the frequency of micronucleated erythrocytes was observed in either male or female mice administered ethylbenzene by inhalation.

b Values are mean ± standard error.

c Cochran-Armitage linear regression of proportions for PCE, or linear contrasts from Analysis of Variance for NCE.

d Analysis of variance on ranks.