BIOASSAY OF

1,2-DICHLOROETHANE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 78-1361

REPORT ON THE BIOASSAY OF 1,2-DICHLOROETHANE
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 1,2-dichloroethane conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected environmental chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 1,2-dichloroethane was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. M. B. Powers (3), Dr. R. W. Voelker (3), Dr. W. A. Olson (3,4) and Dr. W. M. Weatherholtz (3). Chemical analysis was performed by Dr. C. L. Guyton (3,5) and the analytical results were reviewed by Dr. N. Zimmerman (6); the technical supervisor of animal treatment and observation was Ms. K. J. Petrovics (3).

Histopathologic examinations were performed by Dr. R. H. Habermann (3) and reviewed by Dr. R. W. Voelker (3) at the Hazleton Laboratories America, Inc., and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (7).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (8); the

statistical analysis was performed by Mr. W. W. Belew (6) and Dr. J. R. Joiner (7), using methods selected for the Bioassay Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (6) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (6), the task leader, Dr. M. R. Kornreich (6), the senior biologist, Ms. P. Walker (6), and the technical editor, Ms. P. A. Miller (6). The final report was reviewed by members of the participating organizations.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of the NCI: Dr. J. J. Gart (9), Mr. J. Nam (9), Dr. H. M. Pettigrew (9), and Dr. R. E. Tarone (9).

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1), Dr. R. A. Griesemer (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,10), and Dr. J. M. Ward (1).

---

1. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

2. Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammon House Road, Valhalla, New York.

3. Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia.

4. Now with the Center for Regulatory Services, 2347 Paddock Lane, Reston, Virginia.

5. Now with Rhodia, Inc., 23 Belmont Drive, Somerset, New Jersey.

6. The MITRE Corporation, METREK Division, 1820 Dolley Madison Boulevard, McLean, Virginia.

7. Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

8. EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

9. Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

10. Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of technical-grade 1,2-dichloroethane for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F1 mice. 1,2-Dichloroethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The 78-week period of chemical administration was followed by an observation period of 32 weeks for the low dose rats of both sexes. The last high dose male rat died after 23 weeks of observation and the last high dose female rat died after 15 weeks of observation. All treated groups of mice were observed for an additional 12 or 13 weeks following chemical administration.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The time-weighted average high and low doses of 1,2-dichloroethane in the chronic study were 95 and 47 mg/kg/day, respectively, for rats of both sexes. The high and low time-weighted average doses for the male mice were 195 and 97 mg/kg/day, respectively, and 299 and 149 mg/kg/day, respectively, for the female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same times that dosed animals were gavaged with the 1,2-dichloroethane mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

A statistically significant positive association between dosage and the incidence of squamous-cell carcinomas of the forestomach and hemangiosarcomas of the circulatory system occurred in the male rats, but not in the females. There was also a significantly increased incidence of adenocarcinomas of the mammary gland in female rats.

The incidences of mammary adenocarcinomas in female mice were statistically significant. There was a statistically significant positive association between chemical administration and the combined incidences of endometrial stromal polyps and endometrial stromal sarcomas in female mice. The incidence of alveolar/bronchiolar adenomas in both male and female mice was also statistically significant.

Under the conditions of this study, 1,2-dichloroethane was carcinogenic to Osborne-Mendel rats, causing squamous-cell carcinomas of the forestomach, hemangiosarcomas, and subcutaneous fibromas in male

rats and causing mammary adenocarcinomas in female rats. This compound was also found to be carcinogenic to B6C3F1 mice, causing mammary adenocarcinomas and endometrial tumors in female mice, and causing alveolar/bronchiolar adenomas in mice of both sexes.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

1,2-Dichloroethane (NCI No. C00511), a chlorinated aliphatic hydrocarbon, is one of several halogenated solvents selected for bioassay by the National Cancer Institute.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 1,2-dichloroethane.* It is also known as ethylene chloride; ethylene dichloride; and alpha beta dichloroethane.

Although the major use of 1,2-dichloroethane is as an intermediate in the synthesis of vinyl chloride (approximately 80 percent of U.S. 1,2-dichloroethane production in 1974 [Chemical Economics Newsletter, 1975]), the compound also finds application as a constituent in lead-containing antiknock preparations, as an ingredient in fumigant-insecticide formulations and, to a more limited extent, as a component of metal degreasing mixtures (U.S. Department of Health, Education, and Welfare, 1976). 1,2-Dichloroethane is additionally employed as an intermediate in the synthesis of the chlorinated solvents 1,1,1-trichloroethane, trichloroethylene, and perchloroethylene (Chemical Economics Newsletter, 1975) and as a constituent of rubber cements and acrylic-type adhesive formulations (U.S. Department of Health, Education, and Welfare, 1976).

*The CAS registry number is 107-06-2.

According to the U.S. International Trade Commission (1976) approximately 9.2×10^9 pounds of 1,2-dichloroethane were produced in the United States in 1974; however, additional quantities of the compound are believed to have been generated *in situ* during vinyl chloride production, thus narrowing the gap between actual production and reported production capacity of 13.8×10^9 pounds (*Chemical Economics Newsletter*, 1975).

Occupational exposure to 1,2-dichloroethane may occur at vinyl chloride production facilities and among formulators of insecticide, antiknock and metal-degreasing mixtures. Agricultural workers engaged in the fumigation of crops, and industrial workers using 1,2-dichloroethane-containing mixtures for cleaning metal parts may also experience significant contact with the chemical. The National Institute for Occupational Safety and Health estimates that approximately 34,000 workers in the United States are exposed full-time to 1,2-dichloroethane (U.S. Department of Health, Education, and Welfare, 1977). Full-time exposures were defined as those lasting more than four hours a day.

The general population may be exposed to 1,2-dichloroethane through a variety of routes. Ingestion of the compound may occur due to the persistence of residual quantities on grain and other food crops following fumigation of stored crops with one of at least 45 1,2-dichloroethane-containing insecticide formulations (Ehrenberg et al., 1974; U.S. Department of Health, Education, and Welfare, 1976).

In addition, 1,2-dichloroethane may accumulate in cows' milk when cows are fed contaminated grain feed (Sykes and Klein, 1957).

Atmospheric contamination with 1,2-dichloroethane and subsequent inhalation may result following use of the compound in the field as a soil fumigant. Evaporating gasoline or incompletely burned vehicle exhaust fumes may also result in release of significant quantities to the atmosphere, since 1,2-dichloroethane constitutes as much as 20 percent by weight of at least six antiknock fuel additives (Ehrenberg et al., 1974; U.S. Department of Health, Education, and Welfare, 1976).

1,2-Dichloroethane can be toxic to humans following ingestion, inhalation, and dermal absorption. Symptoms of acute poisoning include central nervous system effects (e.g., headache, dizziness, feelings of drunkenness and sometimes unconsciousness) and circulatory damage. The latter manifests itself as hyperemia and hemorrhaging into the visceral organs. Exposure via inhalation or ingestion is often fatal, with death resulting from respiratory and circulatory failure (U.S. Department of Health, Education, and Welfare, 1976).

Repeated exposure of humans to 1,2-dichloroethane has resulted in neurological changes, anorexia, nausea, vomiting, epigastric pain, irritation of the mucous membranes, possible liver and kidney dysfunction, and death (U.S. Department of Health, Education, and Welfare, 1976).

1,2-Dichloroethane exhibited weak mutagenic activity when assayed for its effectiveness in reverting a highly sensitive tester

strain of Salmonella typhimurium (the Ames Test using strain TA 100) (McCann et al., 1975). Although chloroacetic acid, a known product of 1,2-dichloroethane metabolism, was inactive in this test, chloroacetaldehyde, a possible metabolite, proved strongly mutagenic while chloroethanol, a second likely metabolite and precursor of chloroacetaldehyde, exhibited significantly enhanced mutagenic activity following activation with human liver homogenates.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade 1,2-dichloroethane was purchased from Dow Chemical Company, Midland, Michigan. Analysis was performed by Hazleton Laboratories America, Inc., Vienna, Virginia. Gas-liquid chromatography suggested a purity greater than 90 percent and showed the presence of 11 minor contaminants. Infrared analysis was consistent with the structure of the compound. Two analyses, performed about 14 and 18 months later, suggested no significant decomposition.

Throughout this report the term 1,2-dichloroethane is used to represent this technical-grade material.

B. Dosage Preparation

Fresh solutions of 1,2-dichloroethane in Duke's® corn oil (S. F. Sauer Company, Richmond, Virginia) were prepared weekly, sealed, and stored in dark bottles at 1°C. These solutions were considered generally stable for 10 days under the indicated storage conditions. The concentrations of 1,2-dichloroethane in corn oil were 5 to 7.5 percent for the rat bioassay and 1 to 4 percent for the mice bioassay.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. The Osborne-Mendel rat was selected on the basis of a comparative study of the tumorigenic responsiveness to carbon tetrachloride of five different strains of rats (Reuber and Glover, 1970).

The B6C3F1 mouse was selected because it has been used by the NCI for carcinogenesis bioassays and has proved satisfactory in this capacity.

Rats and mice of both sexes were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Both the Osborne-Mendel rats and the B6C3F1 mice were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Upon receipt, animals were quarantined for at least 10 days, observed for visible signs of disease or parasites, and assigned to the various dosed and control groups.

D. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 20° to 24°C and the relative humidity was maintained between 45 and 55 percent. The air conditioning system in the laboratory provided filtered air at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

The rats were individually housed in suspended galvanized-steel wire-mesh cages with perforated floors, while mice were housed by sex in groups of 10 in solid-bottom polypropylene cages equipped with filter tops. Sanitized cages with fresh bedding (Sanichips®, Pinewood Sawdust Company, Moonachie, New Jersey) were provided once each week for mice. Rats received sanitized cages with no bedding with the same frequency. Food hoppers were changed and heat-sterilized once a week for the first 10 weeks and once a month thereafter. Fresh

heat-sterilized glass water bottles were provided three times a week. Food (Wayne Lab-Blox®, Allied Mills, Inc., Chicago, Illinois) and water were available ad libitum.

The rats treated with 1,2-dichloroethane and the vehicle control rats were housed in the same room as other rats intubated with* 1,1-dichloroethane (75-34-3), dibromochloropropane (96-12-8), trichloroethylene (79-01-6), and carbon disulfide (75-15-0). Untreated control rats were housed in a different room along with other rats intubated with 1,1,2-trichloroethane (79-00-5), and tetrachloroethylene (127-18-4).

All mice used in the 1,2-dichloroethane study were housed in the same room as other mice intubated with 1,1,2,2-tetrachloroethane (79-34-5), chloroform (67-66-3), allyl chloride (107-05-1), chloropicrin (76-06-2), dibromochloropropane (96-12-8), 1,2-dibromoethane (106-93-4), 1,1-dichloroethane (75-34-3), trichloroethylene (79-01-6), 3-sulfolene (77-79-2), iodoform (75-47-8), methylchloroform (71-55-6), 1,1,2-trichloroethane (79-00-5), tetrachloroethylene (127-18-4), hexachloroethane (67-72-1), carbon disulfide (75-15-0), trichlorofluoromethane (75-69-4), and carbon tetrachloride (56-23-5).

E. Gastric Intubation

Intubation was performed for five consecutive days per week on a mg/kg body weight basis utilizing the most recently observed group mean body weight as a guide for determining the dose. Mean body weights for each group were recorded at weekly intervals for the

*CAS registry numbers are given in parentheses.

first 10 weeks and at monthly intervals thereafter. All animals of one sex within a treated group received the same dose. Animals were gavaged with the test solution under a hood to minimize extraneous exposure of other animals and laboratory personnel to the chemical.

F. Selection of Initial Dose Levels

In order to establish the maximum tolerated dosages of 1,2-dichloroethane for administration to treated animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. 1,2-Dichloroethane mixed with corn oil was introduced by gavage to five of the six rat groups at dosages of 40, 63, 100, 159, and 251 mg/kg/day and to five of the six mouse groups at dosages of 159, 251, 398, 631 and 1000 mg/kg/day. The sixth group of each species served as a control group, receiving only the corn oil by gavage. Intubation was performed 5 consecutive days per week for 6 weeks, followed by a 2-week observation period to detect any delayed toxicity.

A dosage inducing no mortality and resulting in a depression in mean group body weight of approximately 20 percent relative to controls was selected as the initial high dose. When weight gain criteria were not applicable, mortality data alone were utilized.

At 159 mg/kg/day, all the rats survived for the entire 8 weeks. However, at 251 mg/kg/day three male rats and one female rat died before the end of the experiment. Mean body weight depression was

not significant in males, except in those receiving 251 mg/kg/day. At this level mean body weight depression was 50 percent. In females, mean body weight depression progressed from 10 percent at 40 mg/kg/day to 17 percent at 100 mg/kg/day and 32 percent at 159 mg/kg/day. The initial high dose selected for both male and female rats was 100 mg/kg/day.

All male mice receiving doses of 159 mg/kg/day survived to the end of the experiment and all females dosed with 251 mg/kg/day or less survived to the end of the experiment. No males survived to termination of the experiment when treated with 398 mg/kg/day, and no females survived when treated with 631 mg/kg/day. Body weights for the treated mice, when compared to the body weights of the control mice, were greater or approximately the same for all groups except the female mice receiving 398 mg/kg/day. This group experienced drastic weight loss. The initial high doses selected for use in the chronic study were 150 and 250 mg/kg/day for male and female mice, respectively.

G. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, dosages administered, duration of treated and untreated observation periods, and the time-weighted average dosages) are summarized in Tables 1 and 2.

The treated and vehicle control rats were all approximately 9 weeks old at the time the experiment began. The untreated controls,

TABLE 1

DESIGN SUMMARY FOR OSBORNE-MENDEL RATS
1,2-DICHLOROETHANE GAVAGE EXPERIMENT

	INITIAL NUMBER OF ANIMALS	1,2-DICHLORO-ETHANE DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE DOSAGE OVER A 78-WEEK PERIOD[b]
MALE					
UNTREATED CONTROL	20	--	--	106	--
VEHICLE CONTROL	20	0	78	32	0
LOW DOSE	50	50	7		47
		75	10		
		50	18		
		50[c]	34	9	
		0		32	
HIGH DOSE[d]	50	100	7		95
		150	10		
		100	18		
		100[c]	34	9	
		0		23	
FEMALE					
UNTREATED CONTROL	20	--	--	106	--
VEHICLE CONTROL	20	0	78	32	0
LOW DOSE	50	50	7		47
		75	10		
		50	18		
		50[c]	34	9	
		0		32	
HIGH DOSE[d]	50	100	7		95
		150	10		
		100	18		
		100[c]	34	9	
		0		15	

[a] Dosage, given in mg/kg body weight, was administered by gavage five consecutive days per week.

[b] Time-weighted average dosage = $\frac{\Sigma(\text{dosage X weeks received})}{78 \text{ weeks}}$

[c] These dosages were cyclically administered with a pattern of 1 dosage-free week followed by 4 weeks (5 days per week) of dosage at the level indicated.

[d] All animals in this group died before the bioassay was terminated.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
1,2-DICHLOROETHANE GAVAGE EXPERIMENT

	INITIAL NUMBER OF ANIMALS	1,2-DICHLOROETHANE DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE DOSAGE[b]
MALE					
UNTREATED CONTROL	20	--	--	90	--
VEHICLE CONTROL	20	0	78	12	0
LOW DOSE	50	75	8		97
		100	70		
		0		12	
HIGH DOSE	50	150	8		195
		200	70		
		0		13	
FEMALE					
UNTREATED CONTROL	20	--	--	91	--
VEHICLE CONTROL	20	0	78	90	0
LOW DOSE	50	125	8		149
		200	3		
		150	67		
		0		13	
HIGH DOSE	50	250	8		299
		400	3		
		300	67		
		0		13	

[a] Dosage, given in mg/kg body weight, was administered by gavage five consecutive days per week.

[b] Time-weighted average dosage = $\frac{\Sigma(\text{dosage X weeks received})}{\Sigma(\text{weeks receiving chemical})}$

which did not have the same median birth date as the other three groups, were included in the test from 9 weeks after the first doses were administered until 14 weeks after the final observations were made on the treated groups. The initial doses utilized for the first 7 weeks of the experiment for rats of both sexes were 100 and 50 mg/kg/day. Throughout this report those rat groups receiving initial dosages of 100 mg/kg/day are referred to as the high dose groups and those receiving initial dosages of 50 mg/kg/day are referred to as the low dose groups. The high and low doses were increased to 150 and 75 mg/kg/day, respectively, for the next 10 weeks and then decreased again, to 100 and 50 mg/kg/day for the last 61 weeks of chemical administration. In week 36, intubation ceased for all treated animals for 1 week, followed by 4 weeks of dose administration. This cyclic pattern of dosage administration continued for the remainder of the dosing period. The last high dose male rat died during week 23 of the observation period following chemical administration and the last high dose female rat died during week 15 of the observation period. Low dose and vehicle control rats were observed for 32 weeks after dose administration.

The vehicle control and treated mice were all approximately 5 weeks old on the day the first dose was administered. The untreated control mice had a median birth date approximately 2 weeks later than the treated mice and were started on test 2 weeks after the other groups and were observed a corresponding 2 weeks longer at the end

of the bioassay. Throughout this report those male mice receiving initial dosages of 150 mg/kg/day and those female mice receiving initial dosages of 250 mg/kg/day are referred to as the high dose groups and those male mice receiving initial dosages of 75 mg/kg/day and those female mice receiving initial dosages of 125 mg/kg/day are referred to as the low dose groups. High and low dose male mice received 150 and 75 mg/kg/day, respectively, of 1,2-dichloroethane for the first 8 weeks of the study, and 200 and 100 mg/kg/day thereafter. High and low dose females, respectively, received 250 and 125 mg/kg/day for the first 8 weeks, 400 and 200 mg/kg/day for the next 3 weeks, and 300 and 150 mg/kg/day for the final 67 weeks of dosage administration. Observation continued for 13 weeks after the dosing period.

The untreated controls received no 1,2-dichloroethane or corn oil, while the vehicle controls were intubated with pure corn oil.

H. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected daily for mortality. Body weights, food consumption, and data concerning appearance, behavior, signs of toxic effects, and incidence, size, and location of tissue masses were recorded at weekly intervals for the first 10 weeks and at monthly intervals thereafter. The presence of tissue masses was determined by observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by exsanguination under sodium pentobarbital anesthesia, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, or gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, tunica vaginalis, uterus, mammary gland, and ovary.

Tissues for which slides were prepared were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined

microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

I. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and used Tarone's (1975) extensions of Cox's methods for testing a dose-related trend. One-tailed P-values have been reported

for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing

these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it

can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

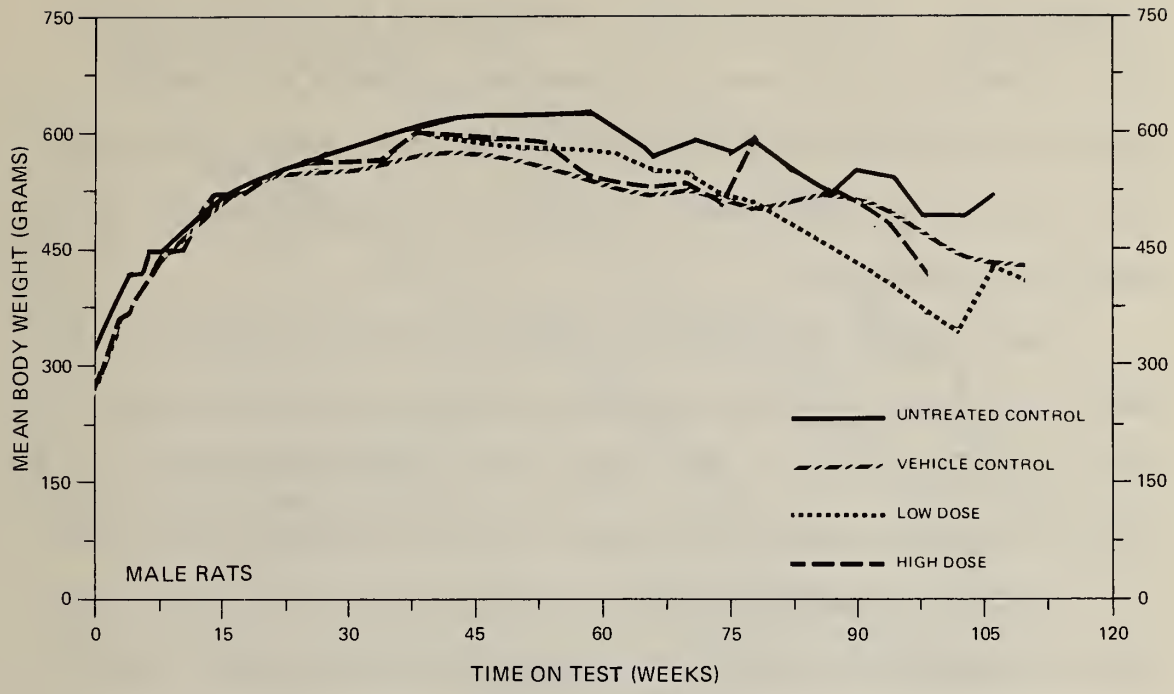
III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

No distinct dose-related mean body weight depression was apparent in either male or female rats relative to vehicle controls (Figure 1). The untreated controls, however, did weigh more than other groups after the first 6 months of the study. Fluctuations in the growth curves may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

The effect of the compound on survival was evident as early as week 2, gradually increasing as the study progressed. The difference in survival between low and high dose rats of both sexes became substantial after the first year (54 weeks). During the last 26 weeks of the study, the decreased survival for the controls was probably due to chronic respiratory and renal involvement.

Beginning with week 6 of the study, several rats in both treated groups started to show a hunched appearance and transient labored respiration. One or two control rats also began to exhibit these signs but, as the study progressed, the incidence was considerably greater in the treated groups than in the controls. Other signs such as abdominal urine stains, cloudy or squinted eyes, or eyes with a reddish crust were also observed with greater frequency in the treated groups than in the controls through the first year. Thereafter, all signs were observed at comparable rates in treated and



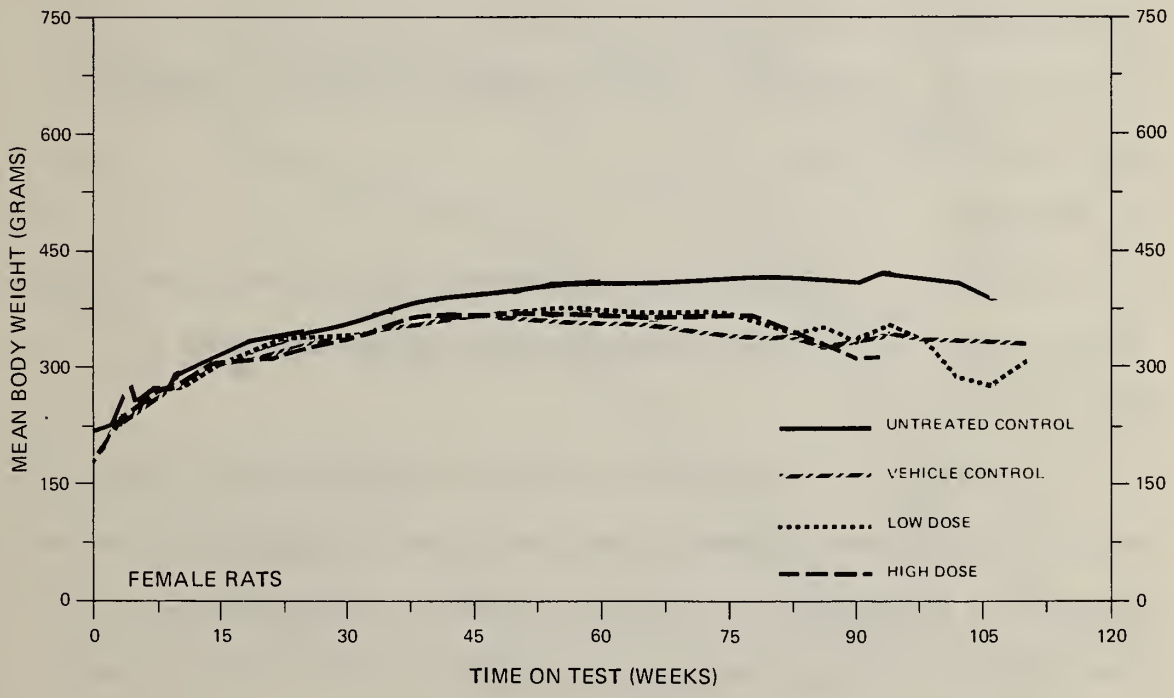


FIGURE 1
GROWTH CURVES FOR 1,2-DICHLOROETHANE CHRONIC STUDY RATS

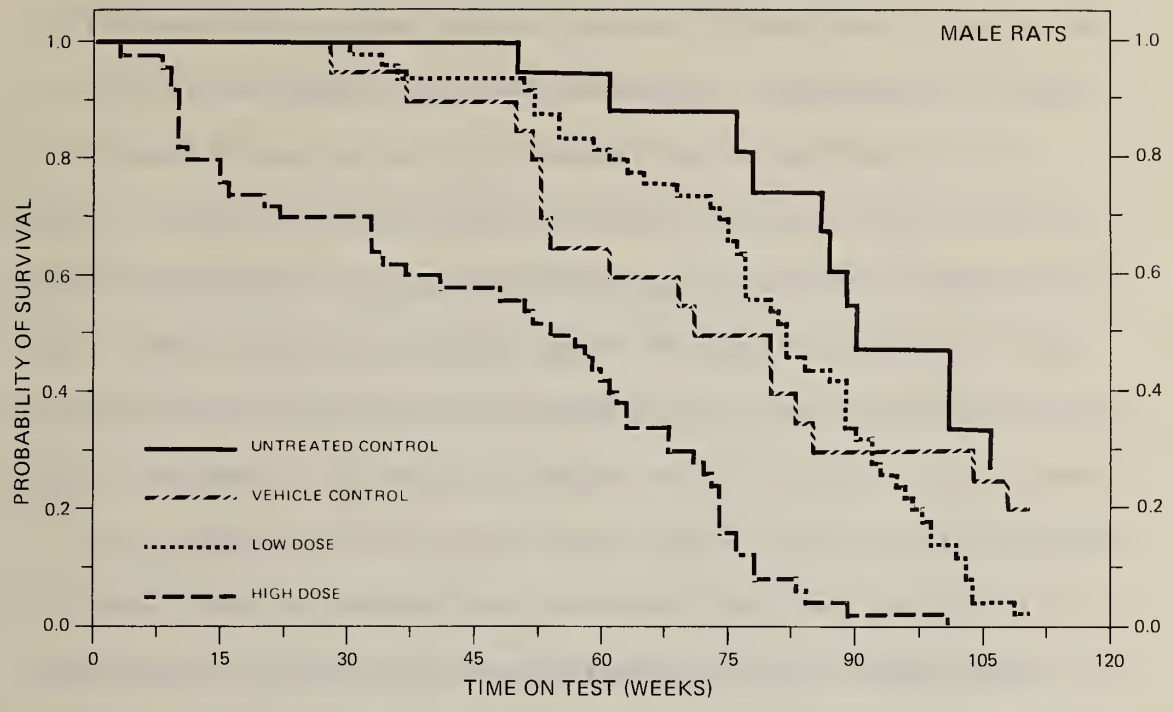
control rats. Respiratory signs, characterized by labored respiration, wheezing, and/or nasal discharge were observed at a low to moderate incidence in all groups during the early part of the second year, but were the predominant observations in all survivors at termination of the study.

Signs associated with aging in the group-housed laboratory rat were noted with comparable frequency in treated and control rats during the second year of the study. Such signs included sores on the tail and other parts of the body, localized alopecia, discolored or stained fur, bloated appearance, and/or swollen areas. The incidence of palpable nodules and/or tissue masses was slightly greater in the treated than in the control animals. Isolated signs observed intermittently in a few animals included circling or head tilt, and abnormal gait. A low dose female was observed to have a broken leg and subsequently died.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 1,2-dichloroethane-dosed groups are shown in Figure 2.

For male rats the Tarone test showed a significant ($P < 0.001$) association between increased dosage and elevated mortality. However, there was a significant ($P = 0.002$) departure from linear trend, partially due to the sharp increase in mortality in the high dose males



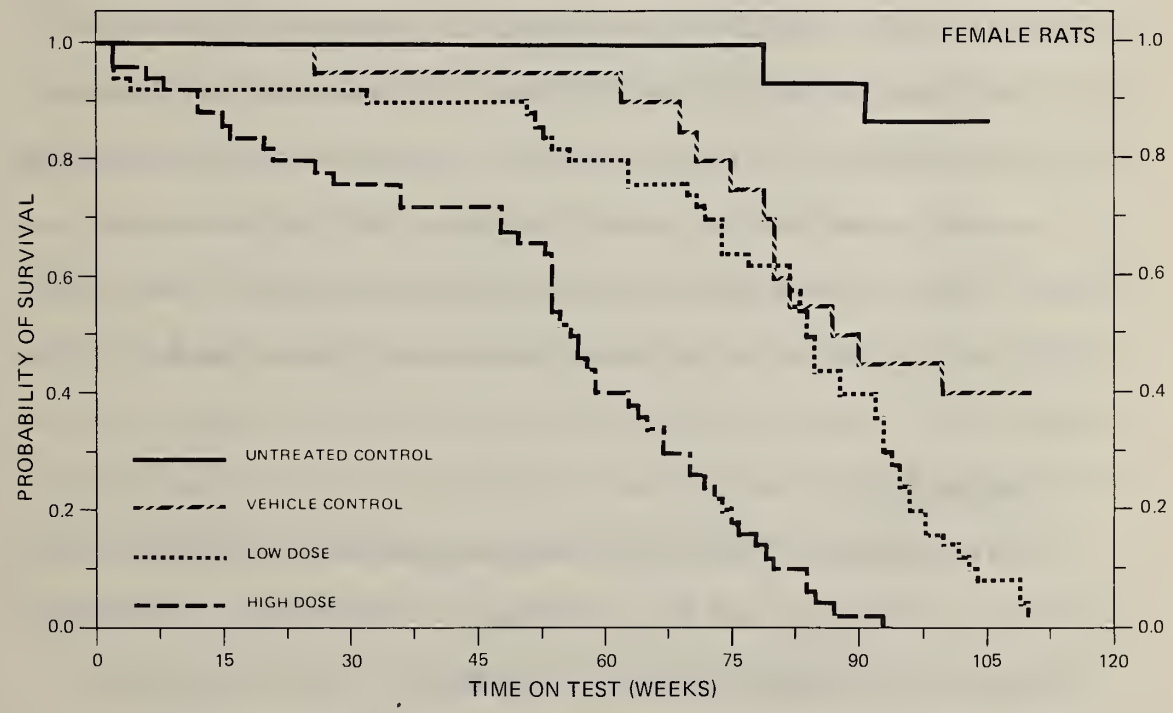


FIGURE 2
SURVIVAL COMPARISONS OF 1,2-DICHLOROETHANE CHRONIC STUDY RATS

and partially because the vehicle controls had a greater mortality than the low dose males during the early portion of the study.

For the high dose male rats survival was low, as 50 percent (25/50) were dead by week 55 and 84 percent (42/50) were dead by week 75. Survival was higher in the other groups, as 52 percent (26/50) of the rats lived at least 82 weeks in the low dose group and 50 percent (10/20) lived at least 72 weeks in the vehicle control group. Despite the sacrifice of five animals in week 57, 50 percent (10/20) of the untreated control group survived at least 87 weeks.

For female rats the Tarone test again showed a significant ($P < 0.001$) dose-mortality association. The departure from linear trend was significant ($P = 0.018$), primarily because of the elevated mortality in the high dose group--where 50 percent (25/50) of the rats were dead by week 57 and 80 percent (40/50) were dead by week 75. Fifty percent (25/50) of the rats survived at least 85 weeks in the low dose group and at least 88 weeks in the vehicle control group. Despite the sacrifice of five females in week 57, 65 percent (13/20) of the untreated control group survived until the end of the study.

C. Pathology

Histopathologic findings on neoplasms in rats are tabulated in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are tabulated in Appendix C (Tables C1 and C2).

Squamous-cell carcinomas of the forestomach occurred in 3/50 (6 percent) low dose males, 9/50 (18 percent) high dose males, and

1/49 (2 percent) low dose females. None of these tumors occurred in controls. These lesions were characterized microscopically by acanthosis and hyperkeratosis in the superficial areas. The basal epithelial layer contained papillary cords and nests of anaplastic squamous epithelium supported by dense bands of fibrous connective tissue. The carcinoma extended through the muscularis mucosa, submucosa, muscular layers, and serosa and metastasized (transcoelomic) to adjacent tissues in one high dose male.

Hemangiosarcomas occurred in the spleen of 6/49 (12 percent) low dose males, 2/49 (4 percent) high dose males, 2/50 (4 percent) low dose females, and 1/50 (2 percent) high dose females. Hemangiosarcomas were diagnosed at a variety of other body sites including: the liver in 1/50 (2 percent) low dose males and 1/50 (2 percent) high dose females; the adrenal in 1/49 (2 percent) high dose males; the pancreas in 1/50 (2 percent) low dose males, 1/48 (2 percent) high dose males and 1/50 (2 percent) high dose females; the stomach in 2/50 (4 percent) low dose males; the large intestine in 1/48 (2 percent) low dose females; the subcutaneous tissue in 2/50 (4 percent) low dose females and 1/50 (2 percent) high dose females; and the abdominal cavity in 1/50 (2 percent) low dose and 1/50 (2 percent) high dose males. No hemangiosarcomas were found in male or female controls.

Microscopically, the hemangiosarcomas consisted of capillaries and cavernous vascular spaces lined with hyperchromatic, plump anaplastic endothelial cells. There was piling up of these cells around

the vascular spaces. These cells invaded and replaced the normal architecture of the spleen and other tissues involved.

Adenocarcinomas were observed in the mammary gland of 1/20 (5 percent) untreated control males, 1/20 (5 percent) vehicle control males, 2/50 (4 percent) low dose males, 2/20 (10 percent) untreated control females, 1/50 (2 percent) low dose females, and 18/50 (36 percent) high dose females. Microscopically, mammary adenocarcinomas were characterized by irregular acini lined by anaplastic epithelium and supported by a dense fibrous stroma. Acini were frequently lined by multiple layers of epithelium, and papillary infoldings or projections were present. Larger hyperchromatic cells were present and mitoses were frequent in the anaplastic adenocarcinomas. Fibroadenomas of the mammary gland occurred in 2/20 (10 percent) untreated control females, 14/50 (28 percent) low dose females, 8/50 (16 percent) high dose females, and 1/20 (5 percent) untreated control males.

Subcutaneous fibromas were observed in 5/50 (10 percent) low dose males, 6/50 (12 percent) high dose males, 1/20 (5 percent) vehicle control females, 1/50 (2 percent) low dose females, and 2/50 (4 percent) high dose females.

Other unusual tumors observed include: renal tubular-cell adenocarcinomas in 1/50 (2 percent) high dose males and 1/50 (2 percent) high dose females; tubular-cell adenomas in 1/50 (2 percent) high dose males and 2/50 (4 percent) high dose females; a leiomyosarcoma of the stomach in 1/50 (2 percent) high dose males; and an adenocarcinoma of the small intestine in 1/50 (2 percent) high dose males.

Other neoplasms that occurred in this study were similar in type and frequency to these occurring naturally in aged Osborne-Mendel rats.

Both acanthosis and hyperkeratosis of the forestomach occurred in 1/20 (5 percent) vehicle control males, 2/50 (4 percent) low dose males, 1/50 (2 percent) high dose males, 1/20 (5 percent) untreated control females, 1/20 (5 percent) vehicle control females, 6/50 (12 percent) low dose females, and 7/50 (14 percent) high dose females. Acanthosis alone occurred in 1/50 (2 percent) low dose males and hyperkeratosis alone occurred in 1/50 (2 percent) high dose males.

Other inflammatory, degenerative, and proliferative lesions were similar in the control and treated animals and consistent with lesions occurring naturally in aged rats.

An increased incidence of the following neoplasms, observed during the histopathologic examinations, provided evidence for the carcinogenicity of 1,2-dichloroethane in Osborne-Mendel rats: squamous-cell carcinomas of the forestomach, hemangiosarcomas of the spleen and other body sites, renal tubular-cell adenomas, and renal tubular-cell adenocarcinomas in rats of both sexes, adenocarcinomas of the mammary gland in female rats, and subcutaneous fibromas in male rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 1,2-DICHLOROETHANE[a]

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	0/60(0.00)	0/20(0.00)	5/50(0.10)	6/50(0.12)
P Values[c]	P = 0.010	N.S.	P = 0.017*	P = 0.007*
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.508	1.912
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.525	0.667
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	75	68
Tunica Vaginalis: Mesothelioma NOS[b]	0/60(0.00)	0/20(0.00)	3/50(0.06)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.010	P = 0.047	---	---
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.718	---
Upper Limit	---	---	Infinite	---
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.250	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	69	---

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Stomach: Squamous-Cell Carcinoma[b]	0/60(0.00)	0/20(0.00)	3/50(0.06)	9/50(0.18)
P Values[c]	P = 0.001	P = 0.010	N.S.	P = 0.001* P = 0.039**
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.718	3.139
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.250	1.096
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	77	51
Circulatory System: Hemangiosarcoma[b]	1/60(0.02)	0/20(0.00)	9/50(0.18)	7/50(0.14)
P Values[c]	P = 0.021	N.S.	P = 0.003* P = 0.039**	P = 0.016*
Relative Risk (Pooled Vehicle Control)[d]	---	---	10.800	8.400
Lower Limit	---	---	1.578	1.134
Upper Limit	---	---	462.176	370.010
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.096	0.809
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	70	---	73	61

TABLE 3 (Concluded)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	3/60(0.05)	2/20(0.10)	1/50(0.02)	4/49(0.08)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[d]	---	---	0.400	1.633
Lower Limit	---	---	0.008	0.289
Upper Limit	---	---	4.786	10.646
Relative Risk (Matched Vehicle Control)[d]	---	---	0.200	0.816
Lower Limit	---	---	0.004	0.131
Upper Limit	---	---	3.682	8.603
Weeks to First Observed Tumor	84	108	95	37

[a]Treated groups received time-weighted average doses of 47 or 95 mg/kg by gavage.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the corresponding control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the pooled vehicle control group (*) or the matched vehicle control group (**) is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 1,2-DICHLOROETHANE[a]

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	13/59(0.22)	7/20(0.35)	7/50(0.14)	5/49(0.10)
P Values[c]	N.S.	P = 0.017(N)	N.S.	P = 0.020**(N)
Relative Risk (Pooled Vehicle Control)[d]	---	---	0.635	0.463
Lower Limit	---	---	0.232	0.138
Upper Limit	---	---	1.569	1.275
Relative Risk (Matched Vehicle Control)[d]	---	---	0.400	0.292
Lower Limit	---	---	0.144	0.087
Upper Limit	---	---	1.187	0.953
Weeks to First Observed Tumor	68	90	53	50
Thyroid: Follicular-Cell Adenoma[b]	0/58(0.00)	0/20(0.00)	3/50(0.06)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.011	P = 0.047	---	---
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.695	---
Upper Limit	---	---	Infinite	---
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.250	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	84	---

TABLE 4 (Continued)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Adenocarcinoma NOS[b]	1/59(0.02)	0/20(0.00)	1/50(0.02)	18/50(0.36)
P Values[c]	P < 0.001	P < 0.001	N.S.	P < 0.001* P = 0.002**
Departure from Linear Trend[e]	P = 0.003	P = 0.021	---	---
Relative Risk (Pooled Vehicle Control)[d]	---	---	1.180	21.240
Lower Limit	---	---	0.015	3.573
Upper Limit	---	---	90.792	857.928
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.022	2.400
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	102	---	74	20
Mammary Gland: Fibroadenoma[b]	5/59(0.08)	0/20(0.00)	14/50(0.28)	8/50(0.16)
P Values[c]	N.S.	N.S.	P = 0.007* P = 0.005**	N.S.
Departure from Linear Trend[e]	P = 0.014	P = 0.008	---	---
Relative Risk (Pooled Vehicle Control)[d]	---	---	3.304	1.888
Lower Limit	---	---	1.218	0.582
Upper Limit	---	---	10.874	6.874
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.819	0.952
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	97	---	77	54

TABLE 4 (Continued)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Adenocarcinoma NOS or Fibroadenoma[b]	6/59(0.10)	0/20(0.00)	15/50(0.30)	24/50(0.48)
P Values[c]	P < 0.001	P < 0.001	P = 0.009* P = 0.003**	P < 0.001* P < 0.001**
Relative Risk (Pooled Vehicle Control)[d]	---	---	2.950	4.720
Lower Limit	---	---	1.178	2.082
Upper Limit	---	---	8.538	12.691
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.965	3.279
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	97	---	74	20
Circulatory System: Hemangiosarcoma[b]	0/59(0.00)	0/20(0.00)	4/50(0.08)	4/50(0.08)
P Values[c]	P = 0.042	N.S.	P = 0.041*	P = 0.041*
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.089	1.089
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.386	0.386
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	93	65

TABLE 4 (Concluded)

[a]Treated groups received time-weighted average doses of 47 or 95 mg/kg by gavage.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the corresponding control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the pooled vehicle control group (*) or the matched vehicle control group (**) is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 1,2-dichloroethane-dosed groups and where such tumors were observed in at least 5 percent of that group.

Two types of control groups were used for statistical analyses: the vehicle control group (designated in this section as the "matched" vehicle control group) and a pooled vehicle control group which combined the vehicle controls from the studies of 1,2-dichloroethane, 1,1,2-trichloroethane, and trichloroethylene. The pooled control rats were of the same strain, were housed in the same room, were tested concurrently for at least one year, and were diagnosed by the same pathologists. The untreated control group was not used for analyses of tumor incidence because the test conditions of the vehicle controls more closely resembled those of the treated groups.

For male rats the Cochran-Armitage test indicated a significant ($P = 0.010$) positive association between dosage and the incidence of fibromas of the subcutaneous tissue when the dosed groups were compared to the pooled control group. The Fisher exact test confirmed this result in comparing both low dose ($P = 0.017$) and high dose ($P = 0.007$) rats to the pooled control. In historical data compiled by Hazleton Laboratories for the NCI Bioassay Program, 2/200 (1 percent) of the vehicle control male Osborne-Mendel rats had this tumor. Based upon these results the administration of 1,2-dichloroethane to male Osborne-Mendel rats under the conditions of this experiment was

associated with an increased incidence of fibromas of the subcutaneous tissue.

For male rats the Cochran-Armitage test also indicated a significant positive association between dosage and the incidence of squamous-cell carcinomas of the stomach whether comparing to the pooled vehicle control ($P < 0.001$) or the matched vehicle control ($P = 0.010$). The Fisher exact test confirmed these results by a significant ($P = 0.001$) comparison of the high dose to the pooled vehicle control group; the comparison of high dose to matched vehicle control was not significant under the Bonferroni criterion. This tumor was not observed in any of the 200 historical vehicle control male Osborne-Mendel rats. Based upon these results the administration of 1,2-dichloroethane to male rats was associated with an increased incidence of squamous-cell carcinomas of the stomach under the conditions of this experiment.

For the male rats the Cochran-Armitage test indicated a significant ($P = 0.021$) positive association between dosage and the incidence of hemangiosarcomas when comparing dosed groups to the pooled vehicle control group. In comparisons of both the high dose and low dose groups to the pooled control group, the Fisher exact test confirmed these results ($P = 0.016$ and $P = 0.003$, respectively); for the comparison of low dose to matched vehicle control the results were not significant under the Bonferroni criterion. In the historical controls 3/200 (1.5 percent) of the vehicle control males had a hemangiosarcoma. Based upon these results the administration of

1,2-dichloroethane to male Osborne-Mendel rats was associated with an increased incidence of hemangiosarcomas under the conditions of this experiment.

In female rats, adenocarcinomas of the mammary gland were observed at necropsy as early as week 20 in the high dose group. The Cochran-Armitage test detected a significant ($P < 0.001$) positive association between dosage and the incidence of mammary adenocarcinomas in comparing dosed groups to either control. For both comparisons the departure from linear trend was significant due to the elevated incidence in the high dose group. The Fisher exact tests were significant in comparing the high dose group to either the matched vehicle control ($P < 0.001$) or the pooled vehicle control ($P = 0.002$) group. Historically, this tumor was observed in 4/200 (2 percent) of the vehicle control females.

Fibroadenomas of the mammary gland were also noted, especially in the low dose female rats. The Fisher exact test was significant in comparing the low dose group to either the matched vehicle control ($P = 0.005$) group or the pooled vehicle control ($P = 0.007$) group. The incidence in the high dose group, however, was not significantly different from that of the control groups. Historically this tumor was observed in 39/200 (20 percent) of the vehicle controls, but in this bioassay it occurred in 5/59 (8 percent) of the pooled vehicle control females and in none of the 20 matched vehicle control females.

When female rats with either an adenocarcinoma or a fibroadenoma were considered, test results were uniformly significant: The

Cochran-Armitage test was significant ($P < 0.001$) compared to either control group. The Fisher exact test was significant ($P < 0.001$) in comparing either dosed group to either of the control groups.

Based upon these results the administration of 1,2-dichloroethane to female Osborne-Mendel rats was associated with an increased incidence of mammary adenocarcinomas under the conditions of this test.

For female rats the Cochran-Armitage test indicated a significant ($P = 0.042$) positive association between dosage and the incidence of hemangiosarcomas when comparing treated groups to the pooled vehicle control group. In comparisons of both the high dose and low dose groups to the pooled control group, however, the Fisher exact test provided $P = 0.041$, a result that is not significant under the Bonferroni criterion.

The possibility of a negative association between chemical administration and the incidence of pituitary chromophobe adenomas was noted for the female rats. It may be, however, that this result reflected the poor survival in dosed females.

In summary, there were statistically significant incidences of fibromas of the subcutaneous tissue, of squamous-cell carcinomas of the stomach, and of hemangiosarcomas in male rats and statistically significant incidences of mammary adenocarcinomas in female rats.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

No distinct, dose-related mean body weight depression was observed in male mice or low dose female mice (Figure 3). Mean body weight depression for high dose female mice was apparent as early as week 15. Fluctuations in the growth curves may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

Throughout the study the appearance and behavior in the treated mice was generally comparable with that in the control mice, although decreased survival of treated mice was evident during the second year. Sores on the body or extremities and generalized and/or localized alopecia were the predominant signs observed in all groups as early as week 6. These signs were particularly apparent in the males and persisted in all groups throughout the study. Other clinical signs, often associated with aging and group-housed laboratory mice, observed at a comparable frequency in all groups during the second year included a hunched appearance, anal, penile, or vulvar irritation (occasionally with prolapse), rough or stained fur, reddish or brown crust around eyes, and abdominal distension or bloating. Palpable nodules and/or tissue masses and swelling around the abdominal midline were observed with slightly greater frequency in the treated groups than in the controls.

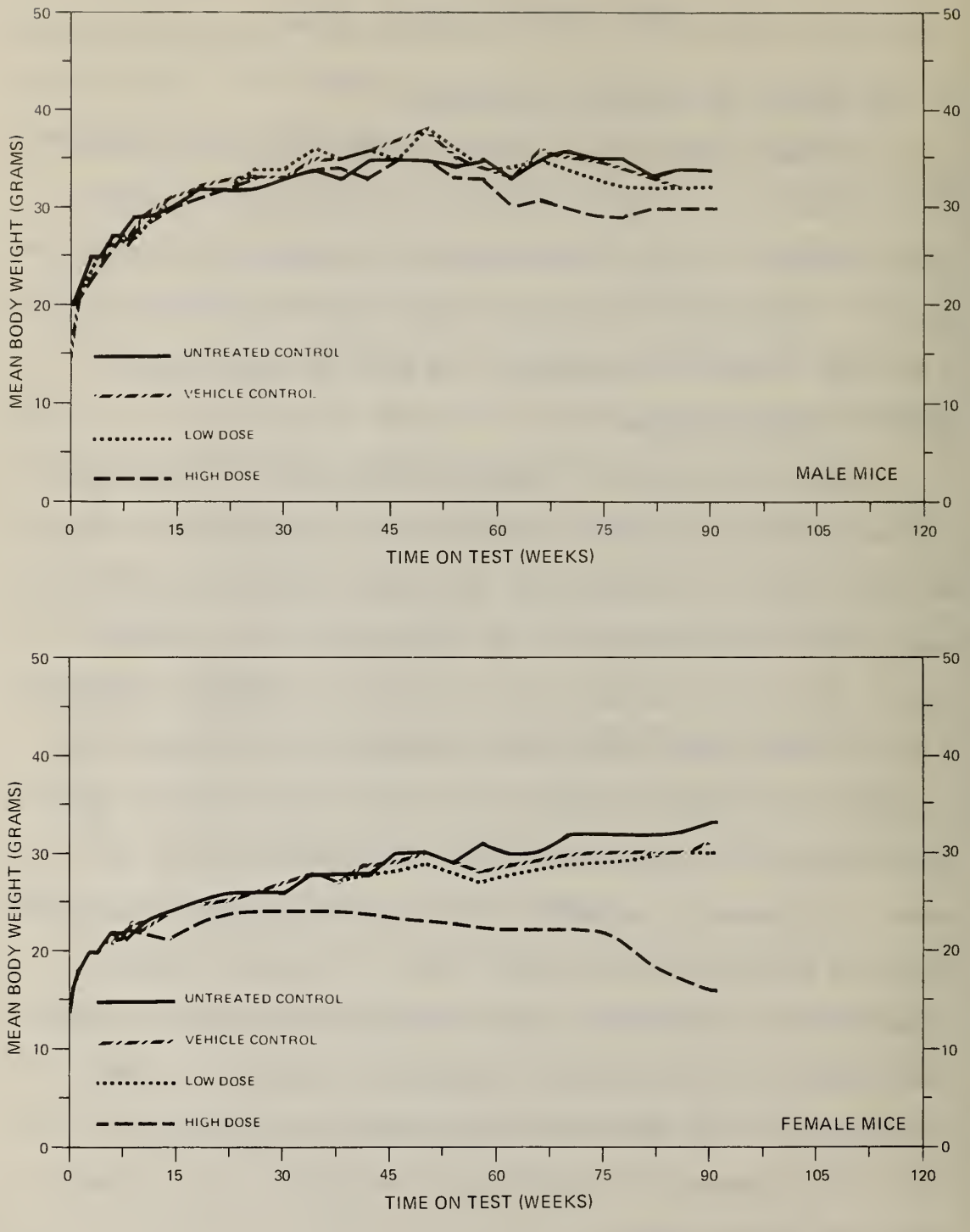


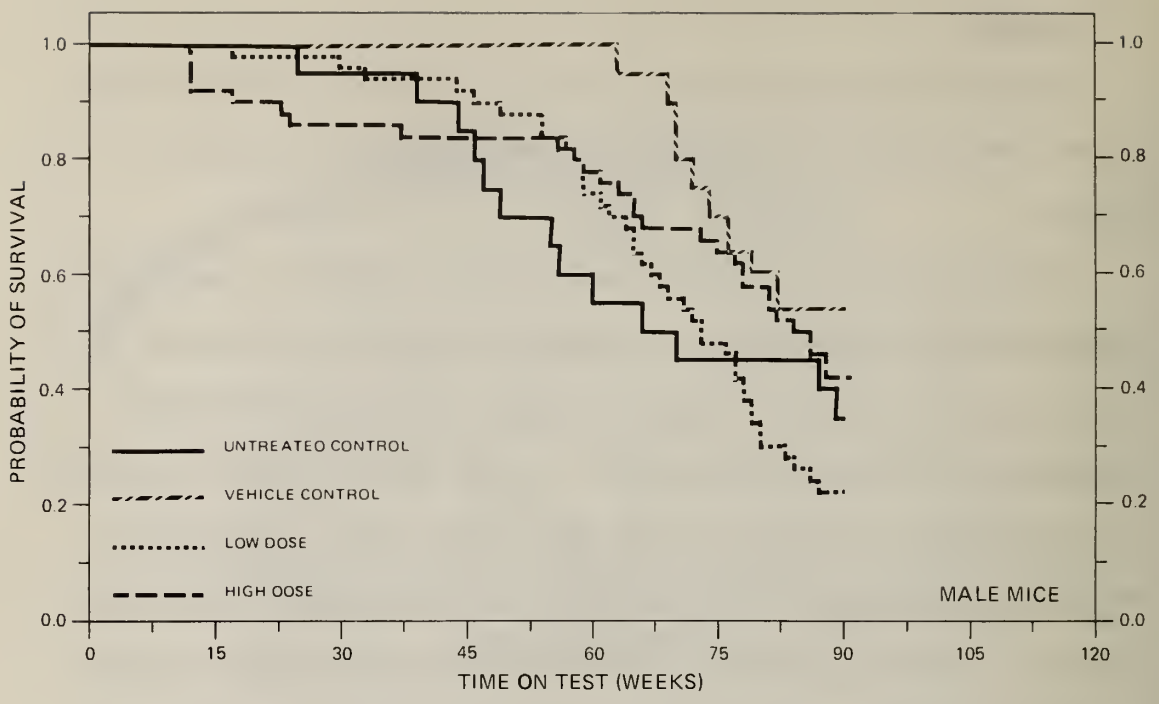
FIGURE 3
GROWTH CURVES FOR 1,2-DICHLOROETHANE CHRONIC STUDY MICE

B. Survival

The estimated probabilities of survival for male and female mice in the control and 1,2-dichloroethane-dosed groups are shown in Figure 4.

For male mice there was no statistically significant association between dosage and mortality. In the high dose group 50 percent (25/50) of the mice survived at least 84 weeks and 42 percent (21/50) survived until the end of the study. In the vehicle control group 55 percent (11/20) survived until the end of the study. In the other two groups, however, survival was low: 52 percent (26/50) of the low dose and 55 percent (11/20) of the untreated control groups survived less than 74 weeks.

For female mice the Tarone test showed a significant ($P < 0.001$) positive association between increased dosage and elevated mortality. The departure from linear trend was significant ($P < 0.001$) because of the severe mortality in the high dose group, where 72 percent (36/50) of the animals died between weeks 60 and 80; these deaths may have been tumor-related as 25/36 (69 percent) had one or more tumors. Survival was high in the other groups as 68 percent (34/50) of the low dose, 80 percent (16/20) of the vehicle control, and 80 percent (16/20) of the untreated control groups survived until the end of the study.



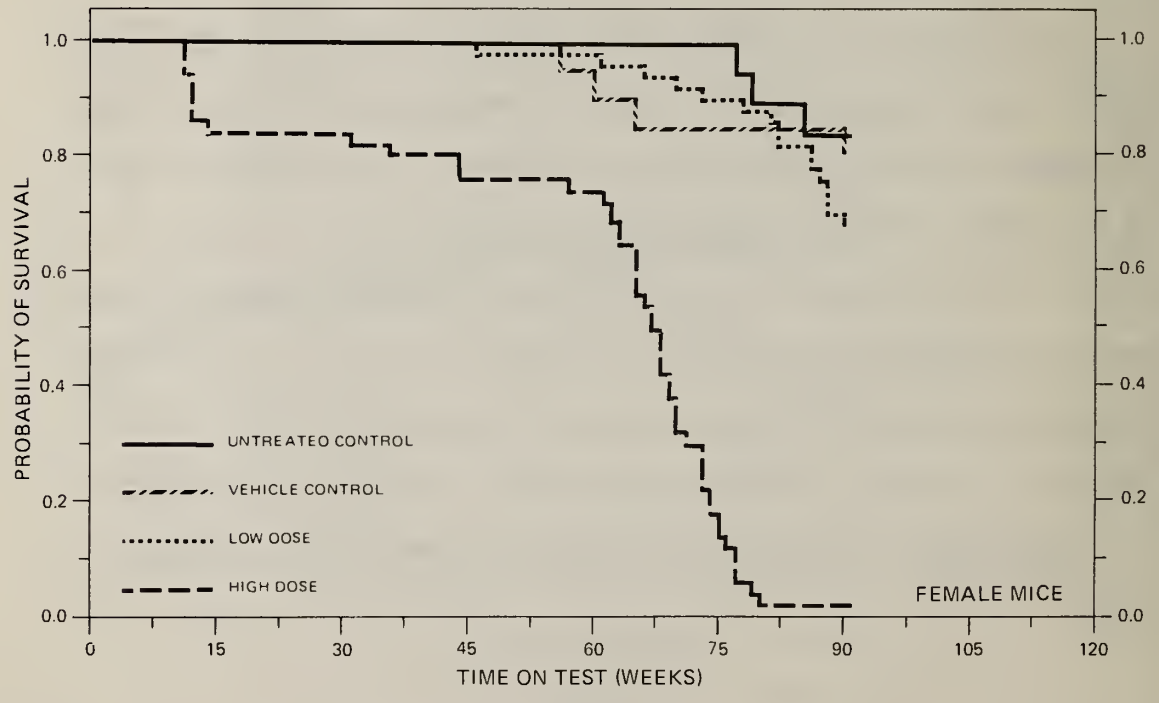


FIGURE 4
SURVIVAL COMPARISONS OF 1,2-DICHLOROETHANE CHRONIC STUDY MICE

C. Pathology

Histopathologic findings on neoplasms in mice are tabulated in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are tabulated in Appendix D (Tables D1 and D2).

Hepatocellular carcinomas occurred in 2/17 (12 percent) untreated control males, 1/19 (5 percent) vehicle control males, 6/47 (13 percent) low dose males, 12/48 (25 percent) high dose males, 0/19 untreated control females, 1/20 (5 percent) vehicle control females, 0/50 low dose females, and 1/47 (2 percent) high dose females.

Microscopically, the hepatocellular carcinomas varied greatly in appearance. Some lesions contained well-differentiated hepatocytes with a relatively uniform arrangement of the cords, whereas others consisted of anaplastic cells with mitotic figures, large hyperchromatic nuclei, often with inclusion bodies and with vacuolated, pale cytoplasm. Arrangement of the neoplastic liver cells varied from short stubby cords to nests of hepatic cells and occasionally pseudoacinar formation. Some of the tumors were characterized by discrete areas of highly anaplastic cells. The hepatic neoplasms occurring in the control mice were not different in morphology from those noted in the treated mice.

Squamous-cell carcinomas of the forestomach occurred in 1/19 (5 percent) vehicle control males, 1/46 (2 percent) low dose males, 2/46 (4 percent) high dose males, 1/20 (5 percent) vehicle control females, 2/50 (4 percent) low dose females, and 5/48 (10 percent)

high dose females. The microscopic appearance of the squamous-cell carcinomas of the stomach in the mice was comparable to that described for the rats.

Uterine adenocarcinomas occurred in 3/49 (6 percent) low dose and 4/47 (9 percent) high dose female mice but in no controls. Microscopically, thickening of the endometrium and invasion of the anaplastic, hyperchromatic glandular epithelial cells into the myometrium were observed. The anaplastic cells formed irregular acini, cords, and sheets of cells supported by strands of fibrous connective tissue. Squamous metaplasia and mitotic figures were also seen.

Endometrial stromal sarcomas of the uterus occurred in 2/49 (4 percent) low dose and 3/47 (6 percent) high dose females but in no controls. Microscopically, the endometrial stromal sarcomas consisted of spindled to ovoid cells, some of which were anaplastic with hyperchromatic nuclei and scanty cytoplasm and invaded nearby tissue and organs. Endometrial stromal polyps were found in 3/49 (6 percent) low dose females and 2/47 (4 percent) high dose females but in no controls.

Mammary gland adenocarcinomas were present in 9/50 (18 percent) low dose females and 7/48 (15 percent) high dose females.

Hemangiosarcomas occurred in the spleen of 1/48 (2 percent) high dose males and 1/50 (2 percent) low dose females and in the mesenteric lymph node of 1/47 (2 percent) high dose males.

Alveolar/bronchiolar adenomas were present in none of the untreated or vehicle control males, 1/47 (2 percent) low dose males, 15/48 (31 percent) high dose males, 1/19 (5 percent) untreated control females, 1/20 (5 percent) vehicle control females, 7/50 (14 percent) low dose females, and 15/48 (31 percent) high dose females. An alveolar/bronchiolar carcinoma occurred only in 1/48 (2 percent) high dose females.

Other neoplasms that occurred were not considered unusual for aged B6C3F1 mice.

The inflammatory, degenerative, and proliferative lesions observed were similar in the control and treated animals.

An increased incidence of the following neoplasms, observed during the histopathologic examinations, provided evidence for the carcinogenicity of 1,2-dichloroethane in B6C3F1 mice: mammary adenocarcinomas, uterine adenocarcinomas, endometrial stromal neoplasms of the uterus, and squamous-cell carcinomas of the forestomach in females; and alveolar/bronchiolar adenomas in males and females.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis for every type of tumor that was observed in more than 5 percent of any of the 1,2-dichloroethane-dosed groups of either sex is included.

Two types of control groups were used for statistical analyses: the vehicle control group (designated in this section as the "matched"

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH 1,2-DICHLOROETHANE[a]

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibrosarcoma[b]	1/59(0.02)	0/19(0.00)	0/47(0.00)	4/48(0.08)
P Values[c]	N.S.	P = 0.041	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[d]	---	---	0.000	4.917
Lower Limit	---	---	0.000	0.507
Upper Limit	---	---	23.389	236.738
Relative Risk (Matched Vehicle Control)[d]	---	---	---	Infinite
Lower Limit	---	---	---	0.383
Upper Limit	---	---	---	Infinite
Weeks to First Observed Tumor	90	---	---	65
Lung: Alveolar/Bronchiolar Adenoma[b]	0/59(0.00)	0/19(0.00)	1/47(0.02)	15/48(0.31)
P Values[c]	P < 0.001	P < 0.001	N.S.	P < 0.001* P = 0.003**
Departure from Linear Trend[e]	P = 0.011	P = 0.046	---	---
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.067	5.766
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.022	1.952
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	83	81

TABLE 5 (Continued)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Malignant Lymphoma[b]	4/59(0.07)	2/19(0.10)	8/47(0.17)	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[d]	---	---	2.511	1.536
Lower Limit	---	---	0.718	0.349
Upper Limit	---	---	10.711	7.324
Relative Risk (Matched Vehicle Control)[d]	---	---	1.617	0.990
Lower Limit	---	---	0.370	0.184
Upper Limit	---	---	14.802	9.880
Weeks to First Observed Tumor	66	90	64	61
Liver: Hepatocellular Carcinoma[b]	4/59(0.07)	1/19(0.05)	6/47(0.13)	12/48(0.25)
P Values[c]	P = 0.006	P = 0.025	N.S.	P = 0.009*
Relative Risk (Pooled Vehicle Control)[d]	---	---	1.883	3.688
Lower Limit	---	---	0.474	1.204
Upper Limit	---	---	8.556	14.672
Relative Risk (Matched Vehicle Control)[d]	---	---	2.426	4.750
Lower Limit	---	---	0.331	0.800
Upper Limit	---	---	108.995	197.680
Weeks to First Observed Tumor	72	90	72	58

TABLE 5 (Concluded)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Stomach: Squamous-Cell Carcinoma[b]	1/59(0.02)	1/19(0.05)	1/46(0.02)	2/46(0.04)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[d]	---	---	1.283	2.565
Lower Limit	---	---	0.017	0.137
Upper Limit	---	---	98.529	147.995
Relative Risk (Matched Vehicle Control)[d]	---	---	0.413	0.826
Lower Limit	---	---	0.006	0.047
Upper Limit	---	---	31.749	47.694
Weeks to First Observed Tumor	---	82	90	77

[a]Treated groups received time-weighted average doses of 97 or 195 mg/kg by gavage.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the corresponding control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the pooled vehicle control group (*) or the matched vehicle control group (**) is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH 1,2-DICHLOROETHANE[a]

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	2/60(0.03)	1/20(0.05)	7/50(0.14)	15/48(0.31)
P Values[c]	P < 0.001	P = 0.005	P = 0.046*	P < 0.001* P = 0.016**
Relative Risk (Pooled Vehicle Control)[d]	---	---	4.200	9.375
Lower Limit	---	---	0.844	2.336
Upper Limit	---	---	39.891	80.509
Relative Risk (Matched Vehicle Control)[d]	---	---	2.800	6.250
Lower Limit	---	---	0.403	1.093
Upper Limit	---	---	123.408	255.479
Weeks to First Observed Tumor	90	90	91	62
Hematopoietic System: Malignant Lymphoma[b]	8/60(0.13)	4/20(0.20)	10/50(0.20)	2/48(0.04)
P Values[c]	N.S.	P = 0.024(N)	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.048	---	---	---
Relative Risk (Pooled Vehicle Control)[d]	---	---	1.500	0.313
Lower Limit	---	---	0.577	0.033
Upper Limit	---	---	4.029	1.473
Relative Risk (Matched Vehicle Control)[d]	---	---	1.000	0.208
Lower Limit	---	---	0.339	0.021
Upper Limit	---	---	3.981	1.349
Weeks to First Observed Tumor	56	56	70	68

TABLE 6 (Continued)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Stomach: Squamous-Cell Carcinoma[b]	1/60(0.02)	1/20(0.05)	2/50(0.04)	5/48(0.10)
P Values[c]	P = 0.035	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[d]	---	---	2.400	6.250
Lower Limit	---	---	0.129	0.732
Upper Limit	---	---	138.746	288.916
Relative Risk (Matched Vehicle Control)[d]	---	---	0.800	2.083
Lower Limit	---	---	0.045	0.259
Upper Limit	---	---	46.273	96.358
Weeks to First Observed Tumor	90	90	82	36
Mammary Gland: Adenocarcinoma NOS[b]	0/60(0.00)	0/20(0.00)	9/50(0.18)	7/48(0.15)
P Values[c]	P = 0.007	N.S.	P = 0.001* P = 0.039**	P = 0.003*
Departure from Linear Trend[e]	P = 0.037	---	---	---
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	3.139	2.416
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.096	0.843
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	88	63

TABLE 6 (Continued)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Endometrium/Uterus: Adenocarcinoma NOS[b]	1/60(0.02)	0/20(0.00)	3/49(0.06)	4/47(0.09)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[d]	---	---	3.673	5.106
Lower Limit	---	---	0.305	0.527
Upper Limit	---	---	188.787	245.743
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.255	0.410
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	90	---	91	57
Uterus: Endometrial Stromal Polyp[b]	0/60(0.00)	0/20(0.00)	3/49(0.06)	2/47(0.04)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.733	0.376
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.255	0.130
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	91	63

TABLE 6 (Continued)

TOPOGRAPHY:MORPHOLOGY	POOLED VEHICLE CONTROL	MATCHED VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Sarcoma[b]	0/60(0.00)	0/20(0.00)	2/49(0.04)	3/47(0.06)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.360	0.765
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.125	0.266
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	81	71
Uterus: Endometrial Stromal Polyp or Endometrial Stromal Sarcoma[b]	0/60(0.00)	0/20(0.00)	5/49(0.10)	5/47(0.11)
P Values[c]	P = 0.017	N.S.	P = 0.016*	P = 0.014*
Relative Risk (Pooled Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.538	1.603
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Vehicle Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.536	0.559
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	81	63

TABLE 6 (Concluded)

[a]Treated groups received time-weighted average doses of 149 or 299 mg/kg by gavage.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the corresponding control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the pooled vehicle control group (*) or the matched vehicle control group (**) is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

vehicle control group) and a pooled vehicle control group, combining the vehicle controls from the studies of 1,2-dichloroethane, 1,1,2-trichloroethane, and trichloroethylene. The pooled control mice were of the same strain, were housed in the same room, were tested concurrently for at least one year, and were diagnosed by the same pathologists. The untreated control group was not used for the analyses of tumor incidence because the test conditions of the vehicle control more nearly approximated those of the dosed mice.

A significant number of hepatocellular carcinomas were observed in male mice. The Cochran-Armitage test indicated a positive dose-response association when comparing to either the matched ($P = 0.025$) or the pooled ($P = 0.006$) controls. The Fisher exact test supported this finding with a significant ($P = 0.009$) comparison of the high dose to the pooled control group. In historical vehicle control data collected by Hazleton Laboratories for the NCI Bioassay Program, 19/180 (11 percent) of the B6C3F1 male mice had a hepatocellular carcinoma and 20/180 (11 percent) had either a hepatocellular carcinoma, a hepatocellular adenoma, or a neoplastic nodule. Based upon these results the administration of 1,2-dichloroethane at the dose levels of this study was associated with the increased incidence of hepatocellular carcinomas in male B6C3F1 mice.

A large number of alveolar/bronchiolar adenomas were found in both male and female high dose mice. For both sexes the Cochran-Armitage test showed a significant ($P \leq 0.005$) positive dose-response

association when the dosed groups were compared to either control group. For males the departure from linear trend was significant, primarily because of the elevated incidence in the high dose group. For both males and females the Fisher exact test indicated that the high dose group had a significantly ($P \leq 0.016$) higher incidence rate than either of the controls. For female mice the Fisher exact test comparing the low dose group to the pooled control group was not significant under the Bonferroni criterion. In the historical vehicle controls, 6/180 (3 percent) of the male and 5/180 (3 percent) of the female B6C3F1 mice had this tumor. Based upon these results the administration of 1,2-dichloroethane was associated with the increased incidence of alveolar/bronchiolar adenomas in both male and female mice under the conditions of this study.

In female mice the Cochran-Armitage test also indicated a significant ($P = 0.007$) positive association between dosage and the incidence of adenocarcinomas of the mammary gland when compared to the pooled control. The departure from linear trend was significant ($P = 0.037$), partially because the incidence was higher in the low dose than in the high dose group. The Fisher exact test confirmed these results with significant ($P \leq 0.003$) comparisons of both high dose and low dose groups to the pooled vehicle control group. The comparison of low dose to matched vehicle control was not significant under the Bonferroni criterion. In the historical vehicle controls, none of the 180 females had this tumor.

To investigate endometrial tumors in females the proportion of mice with either an endometrial stromal polyp or an endometrial stromal sarcoma was considered. The Cochran-Armitage test indicated a significant ($P = 0.017$) positive association between dosage and the combined incidence. The Fisher exact tests confirmed these results in comparing either the high dose ($P = 0.014$) or the low dose ($P = 0.016$) groups to the pooled vehicle control group. In the historical controls none of the 180 mice had this tumor.

Based upon these results the administration of 1,2-dichloroethane was associated with an increased incidence of mammary adenocarcinomas and of endometrial tumors in female B6C3F1 mice under the conditions of this experiment.

For male mice the Cochran-Armitage test indicated a significant positive association between dosage and the incidence of fibrosarcomas of the subcutaneous tissue when comparing to the matched control ($P = 0.041$). The Fisher exact tests, however, were not significant.

For female mice the Cochran-Armitage test indicated a significant ($P = 0.035$) positive association between dosage and the incidence of squamous-cell carcinomas of the forestomach when comparing the dosed groups to the pooled vehicle control. The Fisher exact tests, however, were not significant. Additionally, a time-adjusted analysis, based upon those female mice which survived at least 36 weeks (the time of the first observed squamous-cell carcinoma), indicated similar findings. In the historical vehicle controls maintained by this

laboratory for the NCI Bioassay Program, only 1/180 female B6C3F1 mice had these neoplasms. Under the assumption of a binominal distribution with a spontaneous incidence rate of 1/180, the probability of observing 5 or more tumors out of 48 mice (as in the high dose group) was $P < 0.00001$, a significant result. The probability of observing 1 or more tumors out of 20 mice (as in the matched vehicle control) was $P = 0.105$, which was not a significant result.

For female mice the Cochran-Armitage test indicated a significant negative association between dosage and the incidence of malignant lymphomas. The Fisher exact tests, however, were not significant.

In summary, there were statistically significant incidences of hepatocellular carcinomas and of alveolar/bronchiolar adenomas in male mice and significant incidences of alveolar/bronchiolar adenomas, of mammary adenocarcinomas, of endometrial stromal polyps, and of squamous-cell carcinomas in female mice.

V. DISCUSSION

Under the conditions of this bioassay there was a statistically significant association between increased dosage and elevated mortality in both male and female rats and in female mice. During the second year of the study mortality rates in the male mice were higher for low dose and untreated control than for high dose mice. Despite this accelerated mortality, positive associations were established between 1,2-dichloroethane administration and the incidences of several neoplasms.

In rats squamous-cell carcinomas of the forestomach occurred in 3/50 (6 percent) low dose males, 9/50 (18 percent) high dose males, and 1/49 (2 percent) low dose females, but in no high dose females or male or female controls. Statistical analyses of these incidences revealed a significant positive association between dosage and tumor incidence in males but not in females.

Hemangiosarcomas occurred at a variety of body sites in all groups of treated rats. Statistical tests indicated a significant positive association between dosage and the incidence of hemangiosarcomas in male rats but not in females.

Adenocarcinomas of the mammary gland were observed in 0/20 vehicle control, 1/50 (2 percent) low dose, and 18/50 (36 percent) high dose female rats. These incidences provided significant positive associations between dosage and incidence. In addition, these malignant tumors were observed in female mice and the reported incidences (9/50

[18 percent] low dose, 7/48 [15 percent] high dose, none in controls) were also statistically significant.

In mice hepatocellular carcinomas occurred at incidences (i.e., 2/17 [12 percent] untreated control males, 1/19 [5 percent] vehicle control males, 6/47 [13 percent] low dose males, 12/48 [25 percent] high dose males, 1/20 [5 percent] vehicle control females, and 1/47 [2 percent] high dose females) that indicated a statistically significant positive dose-related association in males but not in females. Due to high incidence variability of hepatocellular neoplasms in historical control male mice, this statistical result is not considered to be convincing evidence that these tumors are attributable to administration of the chemical.

The increased incidence of endometrial stromal polyps in dosed female mice was not statistically significant. The same was true for endometrial stromal sarcomas in female mice. However, when the incidences of female mice having either of these endometrial tumors were evaluated, a significant positive association between chemical administration and tumor incidence was demonstrated.

Alveolar/bronchiolar adenomas in mice were observed in no control males, 1/47 (2 percent) low dose males, 15/48 (31 percent) high dose males, 1/19 (5 percent) untreated control females, 1/20 (5 percent) vehicle control females, 7/50 (14 percent) low dose females, and 15/48 (31 percent) high dose females. The statistical association between chemical administration and the development of alveolar/bronchiolar adenomas was significantly positive for both sexes.

Under the conditions of this study, 1,2-dichloroethane is carcinogenic to Osborne-Mendel rats, causing squamous-cell carcinomas of the stomach, hemangiosarcomas and subcutaneous fibromas in male rats and causing mammary adenocarcinomas in female rats. This compound was also found to be carcinogenic to B6C3F1 mice, causing mammary adenocarcinomas and endometrial tumors in female mice, and alveolar/bronchiolar adenomas in mice of both sexes.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.

Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.

Chemical Abstracts Service, The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.

Chemical Economics Newsletter, "Ethylene Dichloride Report Abstract." Chemical Information Services, Stanford Research Institute. Menlo Park, California. November-December 1975.

Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.

Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.

Ehrenberg, L., S. Osterman-Golkar and D. Singh, "On the Reaction Kinetics and Mutagenic Activity of Methylating and -halogeno-ethylating Gasoline Additives." Radiation Botany 15:185-194, 1974.

Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.

Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.

Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.

McCann, J., V. Simmon, D. Streitwieser and B.N. Ames, "Mutagenicity of Chloroacetaldehyde, a Possible Metabolic Product of 1,2-Dichloroethane (Ethylene Dichloride), Chloroethanol (Ethylene Chlorohydrin), Vinyl Chloride, and Cyclophosphamide." Proceedings of the National Academy of Sciences, U.S.A. 72:3190-3193, 1975.

Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.

Reuber, M.D., and E.L. Glover, "Cirrhosis and Carcinoma of the Liver in Male Rats Given Subcutaneous Carbon Tetrachloride." Journal of the National Cancer Institute 44:419-423, 1970.

Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.

Sykes, J.F., and A.K. Klein, "Chloro-organic Residues in Milk of Cows Orally Administered Ethylene Dichloride." Journal of the Association of Official Agricultural Chemists 40:203-209, 1957.

Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.

U.S. Department of Health, Education, and Welfare, Criteria for a Recomended Standard...Occupational Exposure to Ethylene Dichloride. Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health. DHEW Publication No. (NIOSH) 76-139, 1976.

U.S. Department of Health, Education, and Welfare, National Occupational Hazard Survey, Volume III. Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health. DHEW Publication No. (NIOSH) 78-114, 1977.

U.S. International Trade Commission, Synthetic Organic Chemicals. United States Production and Sales, 1974. USITC Publication 776, Washington, D.C., 1976.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 1,2-DICHLOROETHANE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 1,2-DICHLOROETHANE

	CONTROL(UNTR) 01-031M	CONTROL(VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
FIBROMA			5 (10%)	6 (12%)
FIBROSARCOMA	1 (5%)		1 (2%)	
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(20)	(20)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	1 (5%)			
#SPLEEN	(20)	(20)	(49)	(49)
SQUAMOUS CELL CARCINOMA, METASTA				1 (2%)
LEIOMYOSARCOMA, METASTATIC				1 (2%)
HEMANGIOSARCOMA			6 (12%)	2 (4%)
HEMANGIOSARCOMA, METASTATIC			1 (2%)	
#THYMUS	(13)	(12)	(40)	(24)
SQUAMOUS CELL CARCINOMA			1 (3%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(14)	(10)	(32)	(9)
CARCINOMA,NOS	1 (7%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
#LIVER	(20)	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA				1 (2%)
NEOPLASTIC NODULE				1 (2%)
HEPATOCELLULAR CARCINOMA				1 (2%)
HEMANGIOSARCOMA			1 (2%)	
#PANCREAS	(20)	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA, METASTA				1 (2%)
LEIOMYOSARCOMA, METASTATIC				1 (2%)
HEMANGIOSARCOMA			1 (2%)	1 (2%)
#STOMACH	(20)	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			3 (6%)	9 (18%)
LEIOMYOSARCOMA				1 (2%)
HEMANGIOSARCOMA			2 (4%)	
#SMALL INTESTINE	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS				1 (2%)
FIBROSARCOMA	1 (5%)			
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(50)
TUBULAR-CELL ADENOMA				1 (2%)
TUBULAR-CELL ADENOCARCINOMA				1 (2%)
LIPOMA				1 (2%)
MIXED TUMOR, MALIGNANT				1 (2%)
HAMARTOMA +			1 (2%)	2 (4%)
ENDOCRINE SYSTEM				
#PITUITARY	(20)	(20)	(50)	(49)
CHROMOPHOBE ADENOMA	2 (10%)	2 (10%)	1 (2%)	4 (8%)
#ADRENAL	(20)	(20)	(50)	(49)
CORTICAL ADENOMA				1 (2%)
CORTICAL CARCINOMA	2 (10%)			
HEMANGIOSARCOMA, METASTATIC				1 (2%)
#THYROID	(19)	(20)	(50)	(50)
FOLLICULAR-CELL ADENOMA			2 (4%)	1 (2%)
FOLLICULAR-CELL CARCINOMA	1 (5%)			
#PANCREATIC ISLETS	(20)	(20)	(50)	(48)
ISLET-CELL ADENOMA		1 (5%)	1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
+ THIS IS CONSIDERED TO BE A BENIGN FORM OF THE MALIGNANT MIXED TUMOR OF THE KIDNEY AND CONSISTS OF PROLIFERATIVE LIPOCYTES, TUBULAR STRUCTURES, FIBROBLASTS, AND VASCULAR SPACES IN VARYING PROPORTIONS.

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS	1 (5%)	1 (5%)	2 (4%)	
FIBROADENOMA	1 (5%)			
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(20)	(20)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)	1 (2%)
*TUNICA VAGINALIS	(20)	(20)	(50)	(50)
MESOTHELIOMA, NOS			3 (6%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC				1 (2%)
HEMANGIOSARCOMA				3 (6%)
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	11	16	48	50
MORIBUND SACRIFICE			1	
SCHEDULED SACRIFICE	5			
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	4	4	1	
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	4	20	20
TOTAL PRIMARY TUMORS	11	4	31	39
TOTAL ANIMALS WITH BENIGN TUMORS	2	3	7	10
TOTAL BENIGN TUMORS	3	3	10	17
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	1	15	16
TOTAL MALIGNANT TUMORS	8	1	18	21
TOTAL ANIMALS WITH SECONDARY TUMORS#			1	4
TOTAL SECONDARY TUMORS			1	7
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			3	1
TOTAL UNCERTAIN TUMORS			3	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 1,2-DICHLOROETHANE

	CONTROL(UNTR) 01-031F	CONTROL(VEH) 01-071F	LOW DOSE 01-074F	HIGH DOSE 01-075F
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
FIBROMA		1 (5%)	1 (2%) <-	2 (4%)
FIBROSARCOMA				2 (4%)
HEMANGIOSARCOMA			2 (4%)	1 (2%)
HEMANGIOPERICYTOMA, NOS				1 (2%)
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC				2 (4%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(20)	(50)	(50)
HEMANGIOSARCOMA			2 (4%)	1 (2%)
CIRCULATORY SYSTEM				
#HEART	(20)	(20)	(50)	(50)
MIXED TUMOR, METASTATIC	1 (5%)			
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(15)	(15)	(30)	(7)
SQUAMOUS CELL CARCINOMA			1 (3%)	
#LIVER	(20)	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA	1 (5%)		2 (4%)	
HEMANGIOSARCOMA				1 (2%)
#PANCREAS	(20)	(19)	(50)	(50)
HEMANGIOSARCOMA				1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
<- MULTIPLE OCCURRENCE OF MORPHOLOGY IN THE SAME ORGAN TISSUES IS COUNTED ONCE ONLY
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-071F	LOW DOSE 01-074F	HIGH DOSE 01-075F
#STOMACH	(20)	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)	
#LARGE INTESTINE	(19)	(20)	(48)	(50)
HEMANGIOSARCOMA			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(50)
TUBULAR-CELL ADENOMA				2 (4%)
TUBULAR-CELL ADENOCARCINOMA				1 (2%)
MIXED TUMOR, MALIGNANT	1 (5%)			
HAMARTOMA +	2 (10%)			1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(19)	(20)	(50)	(49)
CHROMOPHOBE ADENOMA	6 (32%)	7 (35%)	7 (14%)	5 (10%)
#ADRENAL	(20)	(20)	(50)	(50)
CORTICAL CARCINOMA			1 (2%)	1 (2%)
#THYROID	(20)	(20)	(50)	(50)
FOLLICULAR-CELL ADENOMA			3 (6%)	
C-CELL ADENOMA	2 (10%)			
C-CELL CARCINOMA	2 (10%)			
#PANCREATIC ISLETS	(20)	(19)	(50)	(50)
ISLET-CELL ADENOMA	1 (5%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS	2 (10%)		1 (2%)	18 (36%)
FIBROADENOMA	2 (10%)		14 (28%)	8 (16%)
#UTERUS	(20)	(20)	(48)	(49)
ENDOMETRIAL STROMAL POLYP			2 (4%)	1 (2%)
HEMANGIOMA	1 (5%)			
#OVARY	(20)	(20)	(47)	(47)
CYSTADENOCARCINOMA, NOS	1 (5%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
+ THIS IS CONSIDERED TO BE A BENIGN FORM OF THE MALIGNANT MIXED TUMOR OF THE KIDNEY AND CONSISTS OF PROLIFERATIVE LIPOCYTES, TUBULAR STRUCTURES, FIBROBLASTS, AND VASCULAR SPACES IN VARYING PROPORTIONS.

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-071F	LOW DOSE 01-074F	HIGH DOSE 01-075F
GRANULOSA-CELL TUMOR		1 (5%)		1 (2%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	2	12	45	47
MORIBUND SACRIFICE			4	3
SCHEDULED SACRIFICE	5			
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	13	8	1	
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 01-031F	CONTROL(VEH) 01-071F	LOW DOSE 01-074F	HIGH DOSE 01-075F
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	7	24	33
TOTAL PRIMARY TUMORS	21	9	39	47
TOTAL ANIMALS WITH BENIGN TUMORS	12	7	20	18
TOTAL BENIGN TUMORS	14	8	28	19
TOTAL ANIMALS WITH MALIGNANT TUMORS	6		8	25
TOTAL MALIGNANT TUMORS	7		11	26
TOTAL ANIMALS WITH SECONDARY TUMORS#	1			2
TOTAL SECONDARY TUMORS	1			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1		2
TOTAL UNCERTAIN TUMORS		1		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 1,2-DICHLOROETHANE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 1,2-DICHLOROETHANE

	CONTROL(UNTR) 02-M081	CONTROL(VEH) 02-M071	LOW DOSE 02-M072	HIGH DOSE 02-M073
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	18	19	47	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	17	19	46	47
INTEGUMENTARY SYSTEM				
*SKIN	(18)	(19)	(47)	(48)
SQUAMOUS CELL CARCINOMA			1 (2%)	
FIBROSARCOMA	1 (6%)			
*SUBCUT TISSUE	(18)	(19)	(47)	(48)
FIBROSARCOMA				4 (8%)
RESPIRATORY SYSTEM				
*LUNG	(17)	(19)	(47)	(48)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)	15 (31%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(18)	(19)	(47)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (5%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			4 (9%)	4 (8%)
#SPLEEN	(17)	(18)	(47)	(48)
HEMANGIOSARCOMA				1 (2%)
#MESENTERIC L. NODE	(17)	(19)	(46)	(47)
HEMANGIOSARCOMA				1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (4%)	1 (2%)
#RENAL LYMPH NODE	(17)	(19)	(46)	(47)
SQUAMOUS CELL CARCINOMA, METASTA		1 (5%)		
#KIDNEY	(17)	(18)	(47)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (6%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (4%)	
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 02-M081	CONTROL (VEH) 02-M071	LOW DOSE 02-M072	HIGH DOSE 02-M073
DIGESTIVE SYSTEM				
#LIVER	(17)	(19)	(47)	(48)
HEPATOCELLULAR CARCINOMA	2 (12%)	1 (5%)	6 (13%)	12 (25%)
#STOMACH	(17)	(19)	(46)	(46)
SQUAMOUS CELL CARCINOMA		1 (5%)	1 (2%)	2 (4%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL(UNTR) 02-M081	CONTROL(VEH) 02-M071	LOW DOSE 02-M072	HIGH DOSE 02-M073
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	12	9	39	29
MORIBUND SACRIFICE	1			
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	7	11	11	21
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	4	15	28
TOTAL PRIMARY TUMORS	3	4	17	40
TOTAL ANIMALS WITH BENIGN TUMORS			1	15
TOTAL BENIGN TUMORS			1	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	4	15	22
TOTAL MALIGNANT TUMORS	3	4	16	25
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1	1
TOTAL SECONDARY TUMORS		1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 1,2-DICHLOROETHANE

	CONTROL(UNTR) 02-F081	CONTROL(VEH) 02-F071	LOW DOSE 02-F074	HIGH DOSE 02-F075
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	19	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	20	50	48
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(19)	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA			1 (2%)	1 (2%)
ADENOMA, NOS			1 (2%)	
OSTEOSARCOMA			1 (2%)	
RESPIRATORY SYSTEM				
#LUNG	(19)	(20)	(50)	(48)
ADENOCARCINOMA, NOS, METASTATIC			3 (6%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (5%)	7 (14%)	15 (31%)
ALVEOLAR/BRONCHIOLAR CARCINOMA				1 (2%)
OSTEOSARCOMA, METASTATIC			1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(19)	(20)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (11%)	2 (10%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	2 (10%)	8 (16%)	1 (2%)
#SPLEEN	(19)	(20)	(50)	(46)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)	
ADENOCARCINOMA, NOS, METASTATIC				1 (2%)
ENDOMETRIAL STROMAL SARCOMA, MET				1 (2%)
HEMANGIOSARCOMA			1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	1 (2%)
#MESENTERIC L. NODE	(19)	(20)	(50)	(39)
SQUAMOUS CELL CARCINOMA, METASTA			2 (4%)	
#RENAL LYMPH NODE	(19)	(20)	(50)	(39)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 02-F081	CONTROL (VEH) 02-F071	LOW DOSE 02-F074	HIGH DOSE 02-F075
DIGESTIVE SYSTEM				
#LIVER	(19)	(20)	(50)	(47)
HEPATOCELLULAR CARCINOMA		1 (5%)		1 (2%)
#PANCREAS	(19)	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)	
ADENOCARCINOMA, NOS, METASTATIC				1 (2%)
ENDOMETRIAL STROMAL SARCOMA, MET				1 (2%)
#ESOPHAGUS	(19)	(20)	(50)	(46)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)	
#STOMACH	(19)	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA		1 (5%)	2 (4%)	5 (10%)
#SMALL INTESTINE	(19)	(20)	(50)	(46)
SQUAMOUS CELL CARCINOMA, METASTA			2 (4%)	
#LARGE INTESTINE	(19)	(19)	(50)	(47)
SQUAMOUS CELL CARCINOMA, METASTA			2 (4%)	
ENDOMETRIAL STROMAL SARCOMA, MET				2 (4%)
#COLON	(19)	(19)	(50)	(47)
ADENOCARCINOMA, NOS			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(19)	(20)	(50)	(47)
TUBULAR-CELL ADENOCARCINOMA				1 (2%)
ENDOMETRIAL STROMAL SARCOMA, MET				1 (2%)
#URINARY BLADDER	(19)	(19)	(49)	(45)
ENDOMETRIAL STROMAL SARCOMA, MET				1 (2%)
ENDOCRINE SYSTEM				
#ADRENAL	(19)	(20)	(50)	(47)
ENDOMETRIAL STROMAL SARCOMA, MET				1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(19)	(20)	(50)	(48)
ADENOCARCINOMA, NOS			9 (18%)	7 (15%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 02-F081	CONTROL (VEH) 02-F071	LOW DOSE 02-F074	HIGH DOSE 02-F075
*VAGINA	(19)	(20)	(50)	(48)
ADENOCARCINOMA, NOS, METASTATIC				1 (2%)
#UTERUS	(19)	(20)	(49)	(47)
ADENOCARCINOMA, NOS			3 (6%)	4 (9%)
ENDOMETRIAL STROMAL POLYP			3 (6%)	2 (4%)
ENDOMETRIAL STROMAL SARCOMA			2 (4%)	3 (6%)
*OVARY	(19)	(20)	(49)	(46)
ADENOCARCINOMA, NOS, METASTATIC				1 (2%)
GRANULOSA-CELL TUMOR			1 (2%)	1 (2%)
ENDOMETRIAL STROMAL SARCOMA, MET				1 (2%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(19)	(20)	(50)	(48)
ADENOMA, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL(UNTR) 02-F081	CONTROL(VEH) 02-F071	LOW DOSE 02-F074	HIGH DOSE 02-F075
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	3	4	15	49
MORIBUND SACRIFICE			1	
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED	1			
TERMINAL SACRIFICE	16	16	34	1
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	6	33	29
TOTAL PRIMARY TUMORS	4	7	43	43
TOTAL ANIMALS WITH BENIGN TUMORS	1	1	12	16
TOTAL BENIGN TUMORS	1	1	12	17
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	5	26	21
TOTAL MALIGNANT TUMORS	3	6	30	25
TOTAL ANIMALS WITH SECONDARY TUMORS#			6	6
TOTAL SECONDARY TUMORS			13	13
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1	1
TOTAL UNCERTAIN TUMORS			1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 1,2-DICHLOROETHANE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 1,2-DICHLOROETHANE

	CONTROL(UNTR) 01-031M	CONTROL(VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)			1 (2%)
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
ABSCESS, NOS		2 (10%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM				
#TRACHEA	(15)	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	
#LUNG/BRONCHUS	(20)	(20)	(50)	(50)
ABSCESS, NOS			1 (2%)	
#LUNG	(20)	(20)	(50)	(50)
PNEUMONIA, CHRONIC MURINE	16 (80%)	15 (75%)	47 (94%)	30 (60%)
CALCIUM DEPOSIT	1 (5%)			
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(3)	(20)	(48)	(48)
METAMORPHOSIS FATTY			1 (2%)	3 (6%)
#SPLEEN	(20)	(20)	(49)	(49)
HEMORRHAGE				1 (2%)
HEMATOPOIESIS	1 (5%)	1 (5%)	5 (10%)	2 (4%)
#CERVICAL LYMPH NODE	(19)	(16)	(50)	(44)
INFLAMMATION, NOS	1 (5%)		1 (2%)	
#TRACHEAL LYMPH NODE	(19)	(16)	(50)	(44)
ANGIECTASIS			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
#MESENTERIC L. NODE	(19)	(16)	(50)	(44)
ANGIECTASIS			1 (2%)	
#THYMUS	(13)	(12)	(40)	(24)
ANGIECTASIS				1 (4%)
CIRCULATORY SYSTEM				
#HEART	(20)	(20)	(50)	(50)
THROMBUS, ORGANIZED				4 (8%)
CALCIUM DEPOSIT	1 (5%)		1 (2%)	
#MYOCARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	2 (10%)			
FIBROSIS				1 (2%)
DEGENERATION, NOS	1 (5%)		3 (6%)	2 (4%)
#ENDOCARDIUM	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)			
*AORTA	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION	2 (10%)		1 (2%)	1 (2%)
*MESENTERIC ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION	1 (5%)			
DIGESTIVE SYSTEM				
#LIVER	(20)	(20)	(50)	(50)
CYST, NOS				1 (2%)
THROMBUS, ORGANIZED				1 (2%)
INFLAMMATION, NOS	1 (5%)		1 (2%)	1 (2%)
PERIARTERITIS			1 (2%)	
METAMORPHOSIS FATTY	2 (10%)	2 (10%)	6 (12%)	
ANGIECTASIS	3 (15%)		2 (4%)	4 (8%)
*BILE DUCT	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS	4 (20%)	1 (5%)	1 (2%)	1 (2%)
#PANCREAS	(20)	(20)	(50)	(48)
PERIARTERITIS	4 (20%)	1 (5%)	1 (2%)	
#ESOPHAGUS	(15)	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
#STOMACH	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	
ULCER, FOCAL			3 (6%)	3 (6%)
CALCIUM DEPOSIT	2 (10%)	1 (5%)	1 (2%)	1 (2%)
HYPERKERATOSIS		1 (5%)	2 (4%)	2 (4%)
ACANTHOSIS		1 (5%)	3 (6%)	1 (2%)
#COLON	(19)	(20)	(50)	(47)
NEMATODIASIS	1 (5%)			
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(50)
HYDRONEPHROSIS				1 (2%)
CYST, NOS				1 (2%)
PYELONEPHRITIS, NOS	1 (5%)		3 (6%)	2 (4%)
INFLAMMATION, CHRONIC	15 (75%)	6 (30%)	29 (58%)	8 (16%)
CALCIUM DEPOSIT	1 (5%)			1 (2%)
#URINARY BLADDER	(19)	(18)	(49)	(49)
INFLAMMATION, NOS	1 (5%)		1 (2%)	2 (4%)
HYPERPLASIA, EPITHELIAL				1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(20)	(20)	(50)	(49)
ANGIECTASIS	1 (5%)			
#ADRENAL	(20)	(20)	(50)	(49)
ANGIECTASIS			2 (4%)	2 (4%)
#ADRENAL CORTEX	(20)	(20)	(50)	(49)
DEGENERATION, NOS			2 (4%)	2 (4%)
ANGIECTASIS	1 (5%)			
#THYROID	(19)	(20)	(50)	(50)
ULTIMOBRANCHIAL CYST	2 (11%)			
CYST, NOS			1 (2%)	1 (2%)
HYPERPLASIA, C-CELL	1 (5%)			
HYPERPLASIA, FOLLICULAR-CELL	1 (5%)			1 (2%)
#PARATHYROID	(3)	(20)	(50)	(50)
HYPERPLASIA, NOS	2 (67%)		2 (4%)	1 (2%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
REPRODUCTIVE SYSTEM				
#PROSTATE	(20)	(13)	(45)	(28)
INFLAMMATION, NOS	5 (25%)		2 (4%)	3 (11%)
CALCIUM DEPOSIT			1 (2%)	
*SEMINAL VESICLE	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)			1 (2%)
#TESTIS	(20)	(20)	(50)	(50)
GRANULOMA, SPERMATIC	1 (5%)			
ATROPHY, NOS	11 (55%)	7 (35%)	28 (56%)	13 (26%)
*EPIDIDYMIS	(20)	(20)	(50)	(50)
NECROSIS, FAT	1 (5%)	1 (5%)		
ATROPHY, NOS	3 (15%)			
NERVOUS SYSTEM				
#BRAIN/MENINGES	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			2 (4%)	
SPECIAL SENSE ORGANS				
*EYE/LACRIMAL GLAND	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)			
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(20)	(20)	(50)	(50)
HEMORRHAGE				1 (2%)
INFLAMMATION, NOS	1 (5%)			
*PLEURA	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	
*PERICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	2 (10%)		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-071M	LOW DOSE 01-072M	HIGH DOSE 01-073M
*MESENTERY	(20)	(20)	(50)	(50)
PERIARTERITIS	4 (20%)	1 (5%)	1 (2%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED		2		7

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 1,2-DICHLOROETHANE

	CONTROL(UNTR) 01-031F	CONTROL(VEH) 01-071F	LOW DOSE 01-074F	HIGH DOSE 01-075F
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	
HYPERKERATOSIS			1 (2%)	
ACANTHOSIS			1 (2%)	
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(50)
PNEUMONIA, CHRONIC MURINE	18 (90%)	19 (95%)	44 (88%)	35 (70%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(1)	(20)	(49)	(50)
METAMORPHOSIS FATTY			4 (8%)	
#SPLEEN	(20)	(20)	(50)	(50)
HEMORRHAGE				3 (6%)
HEMATOPOIESIS		1 (5%)		16 (32%)
#CERVICAL LYMPH NODE	(20)	(19)	(49)	(45)
INFLAMMATION, NOS		1 (5%)	2 (4%)	1 (2%)
CIRCULATORY SYSTEM				
#HEART	(20)	(20)	(50)	(50)
THROMBUS, ORGANIZED				4 (8%)
#MYOCARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			3 (6%)	2 (4%)
FIBROSIS	1 (5%)			
*AORTA	(20)	(20)	(50)	(50)
THROMBUS, ORGANIZED				1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL(UNTR) 01-031F	CONTROL(VEH) 01-071F	LOW DOSE 01-074F	HIGH DOSE 01-075F
MEDIAL CALCIFICATION	1 (5%)		1 (2%)	
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(15)	(15)	(30)	(7)
INFLAMMATION, NOS				1 (14%)
FIBROSIS				1 (14%)
ATROPHY, NOS				1 (14%)
#LIVER	(20)	(20)	(50)	(50)
INFLAMMATION, NOS		1 (5%)		6 (12%)
ABSCESS, NOS			1 (2%)	
METAMORPHOSIS FATTY	1 (5%)		1 (2%)	
ANGIECTASIS			1 (2%)	1 (2%)
#LIVER/CENTRILOBULAR	(20)	(20)	(50)	(50)
DEGENERATION, NOS			1 (2%)	
NECROSIS, NOS	1 (5%)			1 (2%)
*BILE DUCT	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS				1 (2%)
#PANCREAS	(20)	(19)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	
PERIARTERITIS			1 (2%)	
#ESOPHAGUS	(15)	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	
#STOMACH	(20)	(20)	(49)	(50)
INFLAMMATION, NOS	1 (5%)		2 (4%)	1 (2%)
ULCER, FOCAL			5 (10%)	3 (6%)
HYPERKERATOSIS	1 (5%)	1 (5%)	6 (12%)	7 (14%)
ACANTHOSIS	1 (5%)	1 (5%)	8 (16%)	7 (14%)
PARAKERATOSIS			2 (4%)	
#GASTRIC SUBMUCOSA	(20)	(20)	(49)	(50)
ULCER, NOS			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(50)
PYELONEPHRITIS, NOS			2 (4%)	
INFLAMMATION, CHRONIC	9 (45%)	3 (15%)	14 (28%)	6 (12%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-071F	LOW DOSE 01-074F	HIGH DOSE 01-075F
CALCIUM DEPOSIT	1 (5%)			
ENDOCRINE SYSTEM				
#ADRENAL	(20)	(20)	(50)	(50)
ANGIECTASIS		2 (10%)	10 (20%)	5 (10%)
#ADRENAL CORTEX	(20)	(20)	(50)	(50)
DEGENERATION, NOS		2 (10%)	4 (8%)	4 (8%)
ANGIECTASIS	3 (15%)			
#THYROID	(20)	(20)	(50)	(50)
HYPERPLASIA, C-CELL	4 (20%)			
#PARATHYROID	(1)	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (100%)			
REPRODUCTIVE SYSTEM				
*VAGINA	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			3 (6%)	2 (4%)
#UTERUS	(20)	(20)	(48)	(49)
HYDROMETRA	4 (20%)		1 (2%)	1 (2%)
INFLAMMATION, NOS		1 (5%)	7 (15%)	3 (6%)
ABSCESS, NOS			1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(20)	(48)	(49)
INFLAMMATION, NOS	1 (5%)			
HYPERPLASIA, CYSTIC	1 (5%)			
#OVARY	(20)	(20)	(47)	(47)
CYST, NOS		1 (5%)		2 (4%)
INFLAMMATION, NOS			1 (2%)	
NERVOUS SYSTEM				
#BRAIN/MENINGES	(20)	(20)	(50)	(50)
INFLAMMATION, NOS		1 (5%)	1 (2%)	
SPECIAL SENSE ORGANS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 01-031F	CONTROL(VEH) 01-071F	LOW DOSE 01-074F	HIGH DOSE 01-075F
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PLEURA	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	1 (2%)
*PERICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			6 (12%)	3 (6%)
*MESENTERY	(20)	(20)	(50)	(50)
PERIARTERITIS			1 (2%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED		1	1	5

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 1,2-DICHLOROETHANE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 1,2-DICHLOROETHANE

	CONTROL(UNTR) 02-M081	CONTROL(VEH) 02-M071	LOW DOSE 02-M072	HIGH DOSE 02-M073
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	18	19	47	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	17	19	46	47
INTEGUMENTARY SYSTEM				
*SKIN	(18)	(19)	(47)	(48)
EPIDERMAL INCLUSION CYST			2 (4%)	
INFLAMMATION, NOS	1 (6%)	1 (5%)		
ACARIASIS				1 (2%)
ACANTHOSIS				1 (2%)
*SUBCUT TISSUE	(18)	(19)	(47)	(48)
ABSCESS, NOS	1 (6%)	1 (5%)	4 (9%)	
RESPIRATORY SYSTEM				
#TRACHEA	(17)	(19)	(47)	(48)
INFLAMMATION, NOS			3 (6%)	
#LUNG	(17)	(19)	(47)	(48)
PNEUMONIA, CHRONIC MURINE			5 (11%)	11 (23%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(17)	(18)	(47)	(48)
AMYLOIDOSIS	4 (24%)	7 (39%)	23 (49%)	11 (23%)
HEMATOPOIESIS		1 (6%)		3 (6%)
#MESENTERIC L. NODE	(17)	(19)	(46)	(47)
INFLAMMATION, NOS		2 (11%)	1 (2%)	
ANGIECTASIS		2 (11%)		2 (4%)
CIRCULATORY SYSTEM				
#HEART	(17)	(19)	(46)	(48)
THROMBUS, ORGANIZED		1 (5%)	2 (4%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 02-M081	CONTROL(VEH) 02-M071	LOW DOSE 02-M072	HIGH DOSE 02-M073
CALCIUM DEPOSIT			1 (2%)	2 (4%)
#MYOCARDIUM	(17)	(19)	(46)	(48)
INFLAMMATION, NOS		1 (5%)	1 (2%)	
#ENDOCARDIUM	(17)	(19)	(46)	(48)
INFLAMMATION, NOS		1 (5%)	2 (4%)	
DIGESTIVE SYSTEM				
#LIVER	(17)	(19)	(47)	(48)
AMYLOIDOSIS		3 (16%)	5 (11%)	1 (2%)
HYPERPLASIA, NODULAR				1 (2%)
#PANCREAS	(17)	(18)	(47)	(48)
INFLAMMATION, NOS		1 (6%)		
#STOMACH	(17)	(19)	(46)	(46)
CALCIUM DEPOSIT				1 (2%)
HYPERKERATOSIS		1 (5%)	4 (9%)	5 (11%)
ACANTHOSIS		1 (5%)	3 (7%)	4 (9%)
#LARGE INTESTINE	(17)	(19)	(47)	(48)
NEMATODIASIS			4 (9%)	4 (8%)
#COLON	(17)	(19)	(47)	(48)
NEMATODIASIS		3 (16%)		
URINARY SYSTEM				
#KIDNEY	(17)	(18)	(47)	(48)
CYST, NOS			2 (4%)	
PYELONEPHRITIS, NOS	3 (18%)	1 (6%)	2 (4%)	
INFLAMMATION, CHRONIC	5 (29%)	12 (67%)	24 (51%)	16 (33%)
AMYLOIDOSIS	4 (24%)	6 (33%)	20 (43%)	9 (19%)
CALCIUM DEPOSIT			1 (2%)	1 (2%)
#URINARY BLADDER	(17)	(19)	(47)	(48)
CALCULUS, NOS				1 (2%)
INFLAMMATION, NOS				2 (4%)
CALCIUM DEPOSIT				2 (4%)
ENDOCRINE SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 02-M081	CONTROL (VEH) 02-M071	LOW DOSE 02-M072	HIGH DOSE 02-M073
REPRODUCTIVE SYSTEM				
*PENIS	(18)	(19)	(47)	(48)
EPIDERMAL INCLUSION CYST		1 (5%)		
#PROSTATE	(17)	(19)	(46)	(45)
INFLAMMATION, NOS		1 (5%)		1 (2%)
*SEMINAL VESICLE	(18)	(19)	(47)	(48)
INFLAMMATION, NOS			1 (2%)	
#TESTIS	(17)	(19)	(47)	(48)
CALCIUM DEPOSIT			1 (2%)	2 (4%)
ATROPHY, NOS			3 (6%)	4 (8%)
*EPIDIDYMIS	(18)	(19)	(47)	(48)
GRANULOMA, SPERMATIC				2 (4%)
NERVOUS SYSTEM				
#BRAIN/MENINGES	(17)	(19)	(48)	(48)
INFLAMMATION, NOS		1 (5%)	1 (2%)	
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERICARDIUM	(18)	(19)	(47)	(48)
INFLAMMATION, NOS				1 (2%)
ALL OTHER SYSTEMS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 02-M081	CONTROL (VEH) 02-M071	LOW DOSE 02-M072	HIGH DOSE 02-M073
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	6	1	3	7
AUTO/NECROPSY/NO HISTO	1		1	1
AUTOLYSIS/NO NECROPSY	2	1	3	2

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 1,2-DICHLOROETHANE

	CONTROL(UNTR) 02-F081	CONTROL(VEH) 02-F071	LOW DOSE C2-F074	HIGH DOSE 02-F075
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	19	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	20	50	48
INTEGUMENTARY SYSTEM				
*SKIN	(19)	(20)	(50)	(48)
EPIDERMAL INCLUSION CYST			1 (2%)	2 (4%)
INFLAMMATION, NOS			3 (6%)	
ACARIASIS			1 (2%)	1 (2%)
HYPERKERATOSIS			1 (2%)	
ACANTHOSIS			1 (2%)	
RESPIRATORY SYSTEM				
#TRACHEA	(19)	(20)	(48)	(45)
INFLAMMATION, NOS				1 (2%)
#LUNG	(19)	(20)	(50)	(48)
THROMBUS, ORGANIZED				1 (2%)
PNEUMONIA, CHRONIC MURINE			1 (2%)	6 (13%)
CALCIUM DEPOSIT				1 (2%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(19)	(20)	(50)	(46)
AMYLOIDOSIS			2 (4%)	
HEMATOPOIESIS		1 (5%)	5 (10%)	
#CERVICAL LYMPH NODE	(19)	(20)	(50)	(39)
INFLAMMATION, NOS			1 (2%)	
#MESENTERIC L. NODE	(19)	(20)	(50)	(39)
INFLAMMATION, NOS			1 (2%)	
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 02-F081	CONTROL (VEH) 02-F071	LOW DOSE 02-F074	HIGH DOSE 02-F075
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(19)	(19)	(50)	(37)
CYST, NOS			1 (2%)	
#LIVER	(19)	(20)	(50)	(47)
THROMBUS, ORGANIZED				1 (2%)
INFLAMMATION, NOS			1 (2%)	
AMYLOIDOSIS			1 (2%)	
ANGIECTASIS			1 (2%)	
#PANCREAS	(19)	(20)	(50)	(48)
INFLAMMATION, NOS	1 (5%)			
#STOMACH	(19)	(20)	(50)	(48)
CALCIUM DEPOSIT				1 (2%)
HYPERKERATOSIS		1 (5%)	2 (4%)	
ACANTHOSIS		1 (5%)	1 (2%)	
#LARGE INTESTINE	(19)	(19)	(50)	(47)
NEMATODIASIS			5 (10%)	
#COLON	(19)	(19)	(50)	(47)
NEMATODIASIS		1 (5%)		
URINARY SYSTEM				
#KIDNEY	(19)	(20)	(50)	(47)
HYDRONEPHROSIS				1 (2%)
PYELONEPHRITIS, NOS			2 (4%)	
AMYLOIDOSIS			1 (2%)	
#URINARY BLADDER	(19)	(19)	(49)	(45)
INFLAMMATION, NOS		1 (5%)		
CALCIUM DEPOSIT				1 (2%)
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#UTERUS	(19)	(20)	(49)	(47)
HYDROMETRA	3 (16%)	4 (20%)	5 (10%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 02-F081	CONTROL (VEH) 02-F071	LOW DOSE 02-F074	HIGH DOSE 02-F075
INFLAMMATION, NOS			10 (20%)	9 (19%)
#UTERUS/ENDOMETRIUM	(19)	(20)	(49)	(47)
INFLAMMATION, NOS	2 (11%)	2 (10%)		
HYPERPLASIA, NOS			1 (2%)	
HYPERPLASIA, CYSTIC	7 (37%)	11 (55%)	17 (35%)	
#OVARY	(19)	(20)	(49)	(46)
CYST, NOS	10 (53%)	10 (50%)	17 (35%)	5 (11%)
INFLAMMATION, NOS	2 (11%)	3 (15%)	10 (20%)	1 (2%)
NERVOUS SYSTEM				
#BRAIN/MENINGES	(19)	(20)	(50)	(46)
INFLAMMATION, NOS				1 (2%)
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(19)	(20)	(50)	(48)
INFLAMMATION, NOS			1 (2%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	3	1	3	10
ACCIDENTAL DEATH	1			
AUTOLYSIS/NO NECROPSY				2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of 1,2-Dichloroethane* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

March 6, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 1,2-Dichloroethane for carcinogenicity.

The primary reviewer said that the chemical induced squamous-cell carcinomas of the forestomach, hemangiosarcomas, and subcutaneous fibromas in male rats; mammary adenocarcinomas in female rats; mammary adenocarcinomas and endometrial tumors in female mice; and alveolar/bronchiolar adenomas in mice of both sexes. Although there were shortcomings in the experimental design and conduct of the study, he said they were not significant enough to invalidate the findings. Given the carcinogenicity of 1,2-Dichloroethane in both sexes of rats and mice, he concluded that the chemical poses a potential carcinogenic risk to humans.

The secondary reviewer pointed out that 1,2-Dichloroethane was one of the few chlorinated hydrocarbons which induced mammary cancer in both mice and rats. He suggested that the corn oil vehicle, in which 1,2-Dichloroethane was administered, may have localized in the mammary gland resulting in a concentration of the chemical at that site.

A carcinogenic response was elicited in the mammary gland, as opposed to other fatty tissues, due to its sensitivity and epithelial component.

A motion was made that the report on the bioassay of 1,2-Dichloroethane be accepted as written. It was seconded and approved unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold Brown, Mayo Clinic
Lawrence Garfinkel, American Cancer Society
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Sheldon Samuels, Industrial Union Department, AFL-CIO
Michael Shimkin, University of California at San Diego
John Weisburger, American Health Foundation
Sidney Wolfe, Health Research Group

---

* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

☆U.S. GOVERNMENT PRINTING OFFICE: 1978-260-899/3153

TECHNICAL BACKGROUND INFORMATION

REPORT ON CARCINOGENESIS BIOASSAY OF 1,2-DICHLOROETHANE (EDC)

U.S. Department of Health, Education, and Welfare
National Institutes of Health
National Cancer Institute
Bethesda, Maryland 20014

FOR RELEASE IN A.M. PAPERS
Tuesday, September 26, 1978

Further Information:
MELVA WEBER
(301) 496-6641

Bioassay Results in Brief: In a carcinogenesis bioassay of the halogenated solvent 1,2-dichloroethane (ethylene dichloride, or EDC), oral administration of the compound produced cancers in rats and mice. In male rats, dosage with EDC caused forestomach cancers, hemangiosarcomas (vascularized cancers) of multiple organs, and subcutaneous fibromas (cancers beneath the skin). Female rats exposed to EDC developed mammary (breast) cancers--some in high-dose animals as early as the 20th week of the study. The chemical also caused breast cancers as well as uterine cancers in female mice, and respiratory tract cancers in both male and female mice.

Reasons for Bioassay: EDC is one of several chlorinated hydrocarbons, or halogenated solvents, selected for bioassay by the National Cancer Institute (NCI). The chemical is produced in large quantities, the major portion for use in manufacturing vinyl chloride monomer, which in turn is used in making polyvinyl chloride (PVC) plastic. There is considerable potential for human exposure in the workplace. Other industrial workers who may be exposed to EDC are those who make

(more)

insecticides or antiknock agents, and who make or use metal degreasing compounds.

EDC also has been found as a contaminant in water and air. Because EDC is used as a soil and crop fumigant, agricultural workers are exposed to it. The general population may be exposed to EDC as a grain and food contaminant, through inhaling gasoline or exhaust fumes, and through contaminated water supplies. EDC also is used as an ingredient in cosmetics and as a food additive. It is highly toxic and potentially lethal when inhaled in large doses.

In the NCI effort to identify cancer-causing chemicals in the environment, approximately 300 chemicals are currently in long-term studies with laboratory animals, chiefly mice, rats and hamsters. The compounds under test include pesticides, pharmaceuticals, industrial chemicals, food additives, and naturally occurring substances. These studies provide data for use by Federal regulatory agencies, NCI research programs, other scientific and academic institutions, and for the information of the public.

The test series is directed by the Carcinogenesis Testing Program. The bioassay of 1,2-dichloroethane was conducted at Hazelton Laboratories America, Inc., Vienna, Virginia, initially under direct contract with NCI and later through the bioassay prime contractor, Tracor Jitco, Inc., Rockville, Maryland.

The Compound: EDC is a colorless, oily liquid with a chloroform-like odor. It is a source chemical from which other chemicals are made.

(more)

These include vinyl chloride, methyl chloroform, vinylidine chloride, perchloroethylene, trichloroethylene (TCE), carbon tetrachloride, and the chlorofluorocarbons (Freon compounds). Formerly EDC was in extensive use as a commercial solvent and extraction solvent, but many of these applications have been taken over by methyl chloroform, TCE, and perchloroethylene--all made from EDC. EDC is used in making gasoline antiknock additives, although this use is declining as leaded gasolines are phased out. Other uses include metal degreasing and some textile drycleaning, for making adhesives, fumigating grain, and in paint removers. EDC may appear as a food additive as a result of its use to extract spices such as annatto, paprika and turmeric. Its use in cosmetics is reported to be limited to nail lacquers.

EDC was rated in the Condensed Chemical Dictionary as the sixteenth highest-volume chemical produced in 1975. Production is now estimated at about 10 billion pounds annually in the United States and, according to the Environmental Protection Agency (EPA), is projected to increase by about 4 percent annually through 1979. According to the National Occupational Hazard Survey conducted by the National Institute for Occupational Safety and Health (NIOSH), about 2 million workers are exposed to EDC annually in the United States, and of these, 33,675 receive full-time occupational exposure.

The Environmental Protection Agency estimates about 163 million pounds of EDC were lost into the environment through emissions in 1974.

In an EPA-sponsored study of surface waters near heavily industrialized areas, EDC was detected in 26 percent of the samples taken

(53 of 204). The concentrations ranged from the detection limit of one part per billion (ppb) to a high of 90 ppb, found at a Pennsylvania site in the Delaware River Basin.

Toxicity in Animals and Humans: EDC can be taken into the body by ingestion, inhalation or skin absorption. By any of these means it can be highly toxic. Acute poisoning may cause headache, dizziness, feelings of drunkenness, loss of consciousness, internal bleeding, and death. Repeated exposures can bring on nausea, vomiting, stomach pain, irritated mucous membranes, loss of appetite, liver and kidney failure, and possible death. Numerous cases of EDC poisoning, both fatal and nonfatal, have been documented by the National Institute for Occupational Safety and Health. When EDC is ingested, the predominant characteristic is blood disorders, including clotting problems. With skin absorption or inhalation, the first effects are headache, weakness, eye irritation, cyanosis (blackening of skin), and nausea. EDC has been found in human milk and in the exhaled breath of nursing mothers who were exposed to the chemical.

Animal studies have demonstrated EDC effects similar to those in humans, including drowsiness, breathing difficulty, clotting disorders, and damage to liver, kidneys and adrenal glands.

Animals, Test Procedures, and Dosages: Osborne-Mendel rats and B6C3F1 mice were used for the study. Groups of 50 male and 50 female animals of each species were used for each of two dose levels of 1,2-dichloroethane mixed in corn oil and given orally through a stomach tube (gavage) five days per week.

Groups of 20 mice and rats of each sex were placed on test as vehicle controls, and given corn oil alone in the same way and at the same times as dosed animals were given corn oil with EDC. In addition, 20 animals of each sex and each species were put on test as untreated controls, and were given neither corn oil nor EDC.

Short-term studies were made in order to establish the proper dose levels of EDC. The maximum tolerated dose sought was one that would demonstrate any cancer-causing potential of the chemical, but would not curtail the animals' lifespan or growth. This dose level would become the high dose for the test, while half this dose became the low dose.

EDC was administered to rats and mice for 78 weeks. Dosages were set at 100/mg/kg/day as high doses for both male and female rats, 50 mg/kg/day as the low dose. The initial high doses selected for mice were 150 mg/kg/day for males, 250 mg/kg/day for female mice. Low doses were 75 mg/kg/day for male mice, 125 mg/kg/day for female mice.

During the study, however, dosage adjustments were found necessary. At weeks seven and eight, dosages were increased for all animals, and later were reduced again for all groups except male mice. After the dosage periods, surviving animals were observed untreated until the termination date. Low-dose rats of both sexes were observed for 32 weeks. The last high-dose male rat died after 23 weeks of observation, and the last high-dose female rat after 15 weeks. All mice at both doses were observed for 12 or 13 weeks.

(more)

Because of changes in EDC dosages, the net intakes of the test chemical were later calculated as time-weighted average doses. Thus, for high-dose male and female rats the time-weighted average (twa) dosage over the 78-week period was 95 mg/kg/day, and the low dose was 47 mg/kg/day. In male mice, the twa high dosage was 195 mg/kg/day, the low dose 97 mg/kg/day. Time-weighted average high dose in female mice was 299 mg/kg/day, low dose was 148 mg/kg/day.

During the test, animals were housed in temperature- and humidity-controlled, air-conditioned quarters. High standards of sanitation were maintained in housing, bedding, and feeding facilities. When placed on test, mice were about five weeks old, and rats were placed on test at approximately nine weeks of age.

The following tissues were taken from sacrificed mice and rats, and where possible from those found dead: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (in mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, tunica vaginalis, uterus, mammary gland, and ovary.

Data Recording and Statistical Analysis: Data were recorded in the Carcinogenesis Bioassay Data System (CBDS), a computerized data system. Data Elements were those recommended by the International Union Against Cancer, and included details on the chemical, the animals, the design of the experiment, clinical observations, survival figures, animal

weights, and individual pathology results for each animal. Data tables were prepared for statistical analysis. Final data are tabulated in a Carcinogenesis Technical Report.

Clinical Effects: As in other tests, doses of the test chemical were purposely set high, and just within toxic range. The purpose is to give maximum opportunity for development of cancer within the animal's lifespan, if the test chemical is capable of producing cancer.

Body weights of EDC-dosed rats were not significantly depressed when compared with vehicle control animals. Untreated controls, however, weighed more than other rat groups after the first six months of the study.

Body weights were not distinctly depressed in treated male mice or in low-dose females, when compared with both untreated and vehicle control animals. High-dose female mice began to show weight depression by the fifteenth week of the study.

Higher death rates could be related to EDC dosage in rats early in the study, and the difference in survival between high and low-dose rats was substantial after the first year. In mice, treated animals had earlier death rates only during the second year.

Pathology and Tumor Incidences: In rats, the types of cancer providing evidence for carcinogenicity of EDC were: squamous-cell carcinomas of the forestomach in 9 out of 50 (18 percent) high-dose males and in 3 of 50 (6 percent) low-dose males; hemangiosarcomas of the spleen in 6 of 49 (12 percent) low-dose male rats and in 2 of 49 (4 percent) of high-dose males; adenocarcinomas of the mammary gland in 18 of 50

(36) percent) high-dose female rats; and subcutaneous fibromas in 6 of 50 (12 percent) high-dose male rats. High-dose female rats developed mammary gland cancers as early as the 20th week of dosage. Hemangiosarcomas occurred at lower rates in a variety of other body tissues in dosed animals, but no hemangiosarcomas at all were found in either male or female control animals. Forestomach cancers also were not found in control rats.

In mice, the cancers providing evidence for carcinogenicity of EDC were: mammary gland adenocarcinomas in 9 of 50 (18 percent) low-dose females and in 7 of 48 (15 percent) high-dose females, but in none of the control females; uterine adenocarcinomas in 4 of 47 (9 percent) high-dose female mice and in 3 of 49 (6 percent) low-dose females but in no controls; endometrial stromal sarcomas of the uterus in 3 of 47 (6 percent) high-dose and in 2 of 49 (4 percent) low-dose females but in no controls; and squamous cell carcinomas of the forestomach in 5 of 48 (10 percent) high-dose females, 2 of 50 (4 percent) low-dose females, but in only one control animal. In both male and female mice, alveolar-bronchiolar cancers were found at significant levels of 15 in 48 (31 percent) high-dose males and females alike, and in 7 of 50 (14 percent) low-dose females, but only in 1 in 47 (2 percent) low-dose males.

Other kinds of tumors of various organs and tissues occurred among all animal groups, with no distinct difference between the EDC-dosed and the untreated animals.

Conclusions: Thorough analysis and evaluation of all data from the bioassay of 1,2-dichloroethane led NCI scientists to these conclusions: Under the test conditions, 1,2-dichloroethane is carcinogenic to rats and mice; causing cancers of stomach, spleen and other organs, and subcutaneous cancers in male rats, mammary cancers in female rats and mice, uterine cancers in female mice, and lung cancers in both male and female mice.

Copies of the report, Bioassay of 1,2-Dichloroethane for Possible Carcinogenicity, are available from the Office of Cancer Communications, National Cancer Institute, Bethesda, Maryland 20014.

# # # #

☆ U. S. GOVERNMENT PRINTING OFFICE: 1978—260-899/3208

DATE DUE

GAYLORD			PRINTED IN U.S.A.