National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 106
1978

BIOASSAY OF TRICHLOROFLUOROMETHANE FOR POSSIBLE CARCINOGENICITY

CAS No. 75-69-4

NCI-CG-TR-106

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

BIOASSAY OF

TRICHLOROFLUOROMETHANE

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) 78-1356

REPORT ON THE BIOASSAY OF TRICHLOROFLUOROMETHANE
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 trichlorofluoromethane 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 chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly 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 trichlorofluoromethane 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. D. R. Patterson (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), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (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

The bioassay of technical-grade trichlorofluoromethane for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F1 mice. Trichlorofluoromethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, 5 days a week, over a period of 78 weeks. The time-weighted average high and low dosages of trichlorofluoromethane in the chronic bioassay were, respectively, 977 and 488 mg/kg/day for male rats, 1077 and 538 mg/kg/day for female rats, and 3925 and 1962 mg/kg/day for mice of both sexes. After the 78-week dosing period, rats were observed for an additional period of up to 33 weeks and mice were observed for an additional period of up to 13 weeks.

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 trichlorofluoromethane. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not gavaged.

A high rate of early deaths occurred among male and female rats in this bioassay. An insufficient number of rats of either sex survived long enough to be at risk from late-developing tumors. Survival of mice was adequate for meaningful statistical analysis of late-developing tumors.

Results of a time-adjusted statistical analysis of tumor incidence in rats indicated no significant positive associations between administration of trichlorofluoromethane and tumor incidence.

No groups of male or female mice dosed with trichlorofluoromethane had significantly increased tumor incidences relative to their respective control groups.

The results of the bioassay of trichlorofluoromethane in Osborne-Mendel rats for possible carcinogenicity are not conclusive because inadequate numbers of rats survived long enough to be at risk from late-developing tumors. Under the conditions of this bioassay, trichlorofluoromethane was not carcinogenic to male or female B6C3F1 mice.

TABLE OF CONTENTS

TABLE OF CONTENTS (Concluded)

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

Trichlorofluoromethane (NCI No. C04637), a widely used halocarbon aerosol propellant and refrigerant, was selected for bioassay by the National Cancer Institute because of the widespread exposure to this compound resulting from the indiscriminate use of aerosol sprays, and the well-documented hepatocarcinogenicity of the structurally analogous compound, carbon tetrachloride (International Agency for Research on Cancer, 1972).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is trichlorofluoromethane.* It is also called trichloromonofluoromethane; fluorotrichloromethane; fluorocarbon 11; propellant 11; Freon 11®; Arcton 11®; and Frigen 9®.

Trichlorofluoromethane is used as a low-pressure propellant and solvent in a wide variety of aerosol products, such as hair sprays, deodorants, and other cosmetic goods; external medicinals; house and garden pesticides; cleaners and/or disinfectants; spray paints; and floor and furniture polishes (Belej and Aviado, 1975; Gosselin et al., 1976). The propellant in such products usually constitutes 70 percent or more of the contents of the can (Crossland, 1974). As recently as 1976, trichlorofluoromethane was the most widely used aerosol propellant (Gosselin et al., 1976); however, the recent publication of theories which suggest that depletion of the Earth's stratospheric ozone

*The CAS registry number is 75-69-4.

layer may result from the photolytic dissociation of chlorofluoromethanes (e.g., Molina and Rowland, 1974) has aroused public sentiment against aerosol products which contain trichlorofluoromethane or its congeners, and sales of such products have drastically declined (Chemical and Engineering News, 1977).

Trichlorofluoromethane is also used to a substantial degree in refrigeration equipment requiring a refrigerant that is effective at negative pressures (Chemical Marketing Reporter, 1975; Windholz, 1976), and in smaller quantities as a blowing agent for polymeric foams, and as an active component of liquid-type fire extinguishers (Chemical Marketing Reporter, 1975; Gosselin et al., 1976).

In 1975, U.S. production and sales of trichlorofluoromethane were 269,611,000 and 253,756,000 pounds, respectively (U.S. International Trade Commission, 1977). The 1972 world production rate for this compound was about 0.3 megaton per year (Molina and Rowland, 1974). Proposed action by three federal agencies--the Environmental Protection Agency, Food and Drug Administration, and Consumer Product Safety Commission--to phase out all nonessential uses of chlorofluorocarbons as propellants by April 15, 1979 (Chemical Regulation Reporter, 1977) is expected to significantly reduce production and sales of trichlorofluoromethane.

The apparent global contamination of the biosphere by fluorocarbons (Molina and Rowland, 1974) has resulted in universal exposure to low levels of trichlorofluoromethane. Measurable concentrations of

the compound have been found throughout the troposphere: 124 to 403 parts per trillion (ppt) in Washington State (Grimsrud and Rasmussen, 1975); 80 to 2200 ppt in the Los Angeles Basin (Simmonds et al., 1974); 10 to 190 ppt in southwest Ireland (Lovelock, 1971); and 38 to 80 ppt over the Atlantic Ocean (from Antarctica to the United Kingdom) (Lovelock et al., 1973). In the latter study, trichlorofluoromethane was also detected in samples of seawater taken from corresponding locations in the Atlantic. Concentrations of the compound ranged from 20 to 70 ppt (Lovelock et al., 1973).

Users of aerosol sprays and workers at facilities which produce or use trichlorofluoromethane are exposed, by both inhalation and dermal contact, to concentrations of the compound that are considerably higher than ambient levels. Dermal contact is presumably unavoidable when using cosmetic or medicinal sprays. Vapor concentrations may reach 400 parts per million during normal use of aerosol sprays (Crossland, 1974) and vapor concentrations which are significantly higher than ambient trichlorofluoromethane levels are routinely found in homes and public buildings, and in or near factories at which the compound is used. Hester et al. (1974) measured fluorocarbon levels in samples of air taken from homes in the Los Angeles area and found concentrations of trichlorofluoromethane as high as 12 parts per billion (ppb) in two of ten homes. In one instance, this was 67 times higher than levels in outdoor samples collected at the same location at approximately the same time. Concentrations of trichlorofluoromethane

found in public buildings and around factories were also high: 12 ppb in a drug store; 10 ppb in a supermarket; 50 ppb in a beauty shop; 4.1 ppb in a hospital; 8.1 ppb near the storage area of a cosmetics plant (outdoors); and 42 ppb at a distance of 25 yards from a polyurethane plant (Hester et al., 1974). Elevated vapor levels in places other than factories are not necessarily solely attributable to aerosol use; a substantial portion may also be due to leakage from refrigeration equipment.

Deliberate inhalation of aerosol propellants for the purpose of inducing a euphoric mental state results in exposure to very high concentrations of trichlorofluoromethane (Polkis, 1975).

Regulatory action to phase out chlorofluorocarbon propellant uses would reduce the potential for human exposure to high concentrations of trichlorofluoromethane; however, the compound is relatively stable and atmospheric concentrations would only decline slowly over a period of 10 years or more (Chemical Regulation Reporter, 1977). Essential items excluded from the ban would include aerosol bronchiodilators for the treatment of asthma, contraceptive vaginal foams, cytology fixatives, a mine safety warning device, release agents for certain plastic molds, and some flying insect sprays used on airplanes and in commercial food-handling areas (Chemical Regulation Reporter, 1977).

The Freons® were originally considered to have a low degree of toxicity in humans; however, numerous recent accounts of spontaneous deaths among persons who deliberately inhaled aerosol propellants for their narcotic effect has prompted new concern over the toxic effects of the fluorochloromethanes (Crossland, 1974; Polkis, 1975). These deaths are thought to be an example of the "sudden death" syndrome which has been associated with many deaths among workers in the chemical industry since the turn of the century (Crossland, 1974). These compounds apparently sensitize the heart to epinephrine, resulting in severe cardiac arrhythmias and often death. Of the Freons®, trichlorofluoromethane has been shown to have the highest degree of cardiotoxicity in monkeys and other animals (Aviado, 1975; Gosselin et al., 1976); however, similar human studies are not feasible. An anesthetic or narcotic effect of Freons® in humans is reported to occur at levels of 4 percent by volume in air, and exposed workers have experienced fainting and dizziness (Crossland, 1974).

Trichlorofluoromethane showed no indication of carcinogenic or tumorigenic activity in 45 ICR/Ha Swiss mice of both sexes following neonatal injections of 0.1 ml on days 14 and 21 (Epstein et al., 1967).

II. MATERIALS AND METHODS

A. Chemicals

One batch of technical-grade trichlorofluoromethane (Figure 1) was purchased from Allied Chemical Company and analyzed by Hazleton Laboratories America, Inc., Vienna, Virginia. The purchased chemical was initially determined to be greater than 95 percent trichlorofluoromethane, using total-area analysis gas-liquid chromatography (GLC). Five small peaks were present in addition to the trichlorofluoromethane peak. When the material was assayed using the internal standard GLC method, purity was indicated to be approximately 97 percent.

A second purity determination was conducted approximately 2 years after the first to establish the stability of the trichlorofluoromethane. GLC results were similar to those obtained in the initial analysis, indicating little or no decomposition.

Throughout this report the term trichlorofluoromethane is used to represent this technical-grade material.

B. Dosage Preparation

Fresh solutions of trichlorofluoromethane in Duke's® corn oil (S. F. Sauer Company, Richmond, Virginia) were prepared weekly, sealed and stored in dark bottles at 1°C. Each time the solution was used, a slight excess was poured into a narrow-necked flask. The stock bottle was immediately resealed and returned to the refrigerator. During all transfers the flask was immersed in an ice bath. The concentrations

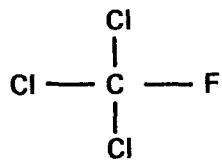


FIGURE 1
CHEMICAL STRUCTURE OF TRICHLORO FLUOROMETHANE

of trichlorofluoromethane in corn oil were 37.5 to 42.5 percent for rats and 20 to 40 percent for mice.

C. Animals

Two animals 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 with the Division of Cancer Treatment, National Cancer Institute. The Osborne-Mendel rats were procured from the Battelle Memorial Institute, Columbus, Ohio, 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 provided filtered air at a rate of 12 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. The 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 and sipper tubes were provided three times a week. Food (Wayne Lab-Blox® meal, Allied Mills, Inc., Chicago, Illinois) and water were available ad libitum.

The rats dosed with trichlorofluoromethane and the untreated controls were housed in the same room with other rats intubated with* methylchloroform (71-55-6) and chloropicrin (76-06-2). The vehicle control rats were housed with other rats intubated with 1,2-dichloroethane (107-06-2); 1,1-dichloroethane (75-34-3); and carbon disulfide (75-15-0).

All mice in the trichlorofluoromethane study, including controls, 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

*CAS registry numbers are given in parentheses.

(96-12-8); 1,2-dibromoethane (106-93-4); 1,2-dichloroethane (107-06-2); 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); carbon disulfide (75-15-0); hexachloroethane (67-72-1); 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 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 test solutions 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 estimate the maximum tolerated dosages of trichlorofluoromethane 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. Intubation was performed 5 days per week for 6 weeks, followed by a 2-week observation period to detect any delayed toxicity. Trichlorofluoromethane dissolved in corn oil was introduced by gavage to five of the six rat

groups and five of the six mouse groups at dosages of 1000, 1780, 3160, 5620, and 10,000 mg/kg/day. The sixth group of each species served as a vehicle control group, receiving only corn oil.

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 least one death was recorded for all the male rat groups receiving 1780 mg/kg/day or greater and for all the female rat groups receiving 3160 mg/kg/day or greater. Mean group body weight depression was 26 percent in males receiving 1000 mg/kg/day while females receiving this same dosage gained 12 percent more weight than controls. At a level of 1780 mg/kg/day mean body weight depression was 11 percent in females. The initial high dosages selected for male and female rats in the chronic bioassay were 850 and 1500 mg/kg/day.

There was no mean group body weight depression when males receiving 5620 mg/kg/day or less or females receiving 3160 mg/kg/day or less were compared to controls. There were deaths in male groups treated with 5620 mg/kg/day or more and in female groups treated with 3160 mg/ kg/day or more. The initial high dosage selected for male and female mice in the chronic bioassay was 3160 mg/kg/day.

G. Experimental Design

The experimental design parameters for the chronic bioassay (species, sex, group size, dosages administered, duration of treated and

untreated observation periods, and time-weighted average dosages) are summarized in Tables 1 and 2.

The untreated control and treated rats were all approximately 6 weeks old when the bioassay was initiated and they all shared the same median date of birth. The vehicle control rats were approximately 7 weeks old when they were first intubated and that was approximately 7 months prior to initiation of the trichlorofluoromethane chronic bioassay. The initial dosages utilized for male rats were 850 and 425 mg/kg/day and for female rats were 1500 and 750 mg/kg/day. Throughout this report the male rats initially receiving 850 mg/kg/day and the female rats initially receiving 1500 mg/kg/day are referred to as the high dose groups, while the male rats initially receiving 425 mg/kg/day and the female rats initially receiving 750 mg/kg/day are referred to as the low dose groups. After 12 weeks the dosages for male rats were increased and for female rats were decreased to high and low levels of 1000 and 500 mg/kg/day, respectively. These dosages were maintained for the remainder of the 78-week intubation period, after which there was an additional observation period of up to 33 weeks.

The vehicle control and treated mice were all approximately 5 weeks old when the bioassay was initiated and they all shared the same median date of birth. The untreated control rats were approximately 3 weeks old when the other animals were first intubated. The initial dosages utilized for both male and female mice were 3160 and

TABLE 1

DESIGN SUMMARY FOR OSBORNE-MENDEL RATS
TRICHLOROFLUOROMETHANE GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	TRICHLORO-FLUOROMETHANE DOSAGE[a]	OBSERVATION PERIOD		TIME-WEIGHTED AVERAGE DOSAGE[b]
			TREATED (WEEKS)	UNTREATED (WEEKS)	
MALE					
UNTREATED CONTROL	20	0	0	99	0
VEHICLE CONTROL	20	0	78	32	0
LOW DOSE	50	425 500 0	12 66	 28	488
HIGH DOSE	50	850 1000 0	12 66	 33	977
FEMALE					
UNTREATED CONTROL	20	0	0	111	0
VEHICLE CONTROL	20	0	78	32	0
LOW DOSE	50	750 500 0	12 66	 33	538
HIGH DOSE	50	1500 1000 0	12 66	 33	1077

[a] Dosages, given in mg/kg body weight, were administered by gavage 5 consecutive days per week.

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

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
TRICHLOROFLUOROMETHANE GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	TRICHLORO-FLUOROMETHANE DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE DOSAGE[b]
MALE					
UNTREATED CONTROL	20	0	0	91	0
VEHICLE CONTROL	20	0	78	13	0
LOW DOSE	50	1580 2000 0	7 71	 13	1962
HIGH DOSE	49	3160 4000 0	7 71	 13	3925
FEMALE					
UNTREATED CONTROL	20	0	0	91	0
VEHICLE CONTROL	20	0	78	13	0
LOW DOSE	50	1580 2000 0	7 71	 13	1962
HIGH DOSE	50	3160 4000 0	7 71	 13	3925

[a] Dosages, given in mg/kg body weight, were administered by gavage 5 consecutive days per week.

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

1580 mg/kg/day. Throughout this report those mice initially receiving the former dosage are referred to as the high dose groups, while those mice initially receiving the latter dosage are referred to as the low dose groups. In week 8, high and low dosages were increased to 4000 and 2000 mg/kg/day, respectively. These dosages were maintained for the remainder of the 78-week intubation period, after which there was an additional observation period of 13 weeks.

The untreated controls received no trichlorofluoromethane or corn oil, while the vehicle controls were intubated with corn oil.

H. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. 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. From the first day, all animals were inspected daily for mortality. 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: brain, pituitary, adrenal, thyroid, parathyroid, trachea, esophagus, thymus, salivary gland, lymph nodes (mesenteric and cervical), heart, nasal passages, lung, spleen, liver, kidney, stomach, small intestine, large intestine, pancreas, urinary bladder, prostate or uterus, seminal vesicles and testis with epididymis or ovary, skin with mammary gland, muscle, nerve, bone marrow, and tissue masses.

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) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when 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 when appropriate. 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 compound-related mean body weight depression was apparent when trichlorofluoromethane-treated male and female rats were compared with untreated controls, but vehicle control rats consistently gained more weight than dosed rats (Figure 2). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

A decline in survival was observed in the treated groups during the first year of the study. Apparent compound-related deaths were noted as early as week 4 in the high dose females, increasing gradually in both sexes and at both dosages as the study progressed. Survival continued to decline during the second year, but at a comparable rate for all groups, including the controls.

Beginning with the first week of compound administration, a few rats at both dose levels started to show a hunched appearance and occasional labored respiration. These signs were observed with greater frequency in the treated groups than in the controls through week 30 but were noted at comparable rates in treated and control rats during the remainder of the bioassay. Chronic respiratory disease characterized by wheezing, nasal discharge and/or labored respiration were observed at a slight to moderate incidence during the first year and at a high incidence in all groups during the second year. Clinical

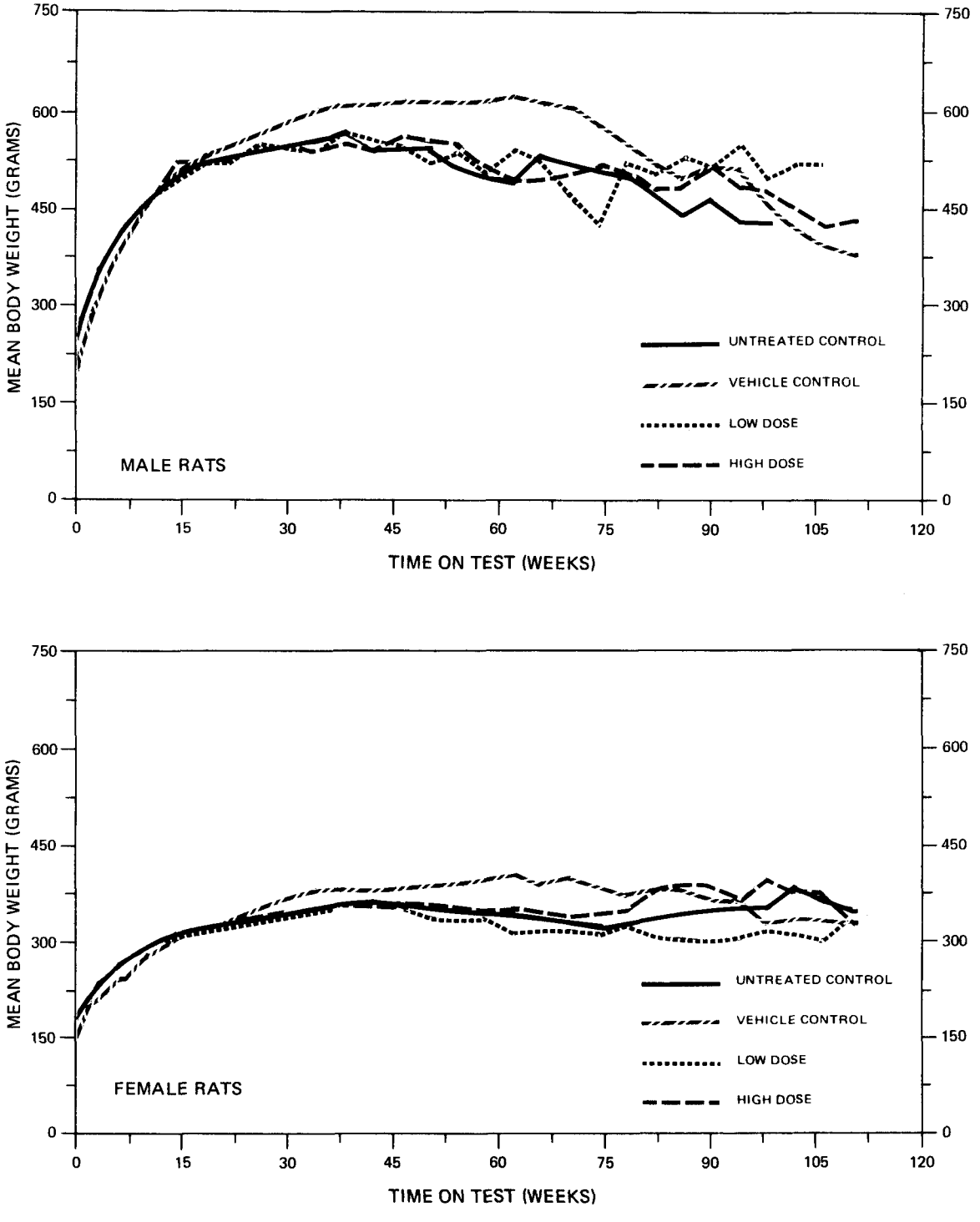


FIGURE 2
GROWTH CURVES FOR TRICHLOROFLUOROMETHANE CHRONIC STUDY RATS

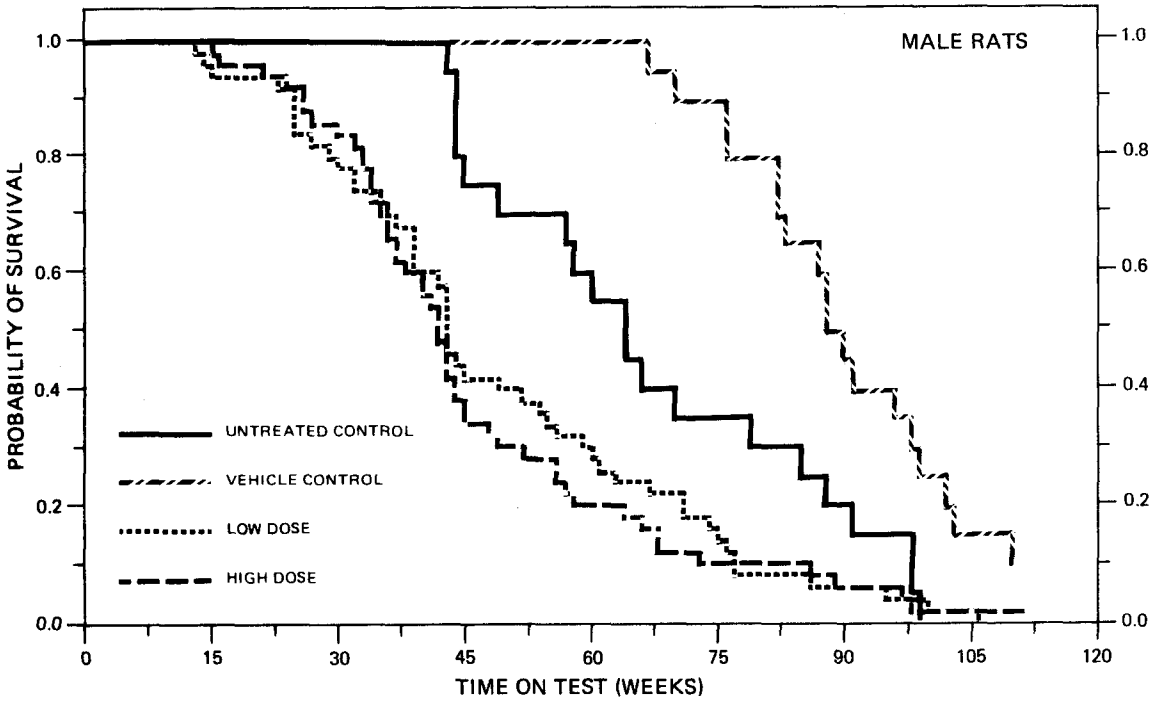
signs usually associated with aging were observed at a similar rate in the control and treated rats. These signs included alopecia, sores on the tail and other parts of the body, discolored fur, bloating, pale appearance, and palpable nodules.

B. Survival

The estimated probabilities of survival for male and female rats in the control and trichlorofluoromethane-dosed groups are shown in Figure 3.

For male rats the Tarone test for positive association between increased dosage and accelerated mortality was significant ($P < 0.001$) when the dosed groups were compared to the vehicle control group. Accelerated mortality was observed in both the treated and control groups with less than 10 percent of the animals in any group surviving on test until the end of the study. By week 52 only 30 percent (15/50) of the high dose and 40 percent (20/50) of the low dose rats were alive, while all 20 of the vehicle control and 70 percent (14/20) of the untreated control rats were still alive on test. The number of male rats surviving long enough to be at risk from late-developing tumors was inadequate.

For female rats the Tarone test also showed a significant ($P < 0.001$) positive association between increased dosage and accelerated mortality when the dosed groups were compared to the vehicle control. As with the males, early mortality was high as only 34 percent (17/50) of the high dose and 62 percent (31/50) of the low dose compared to



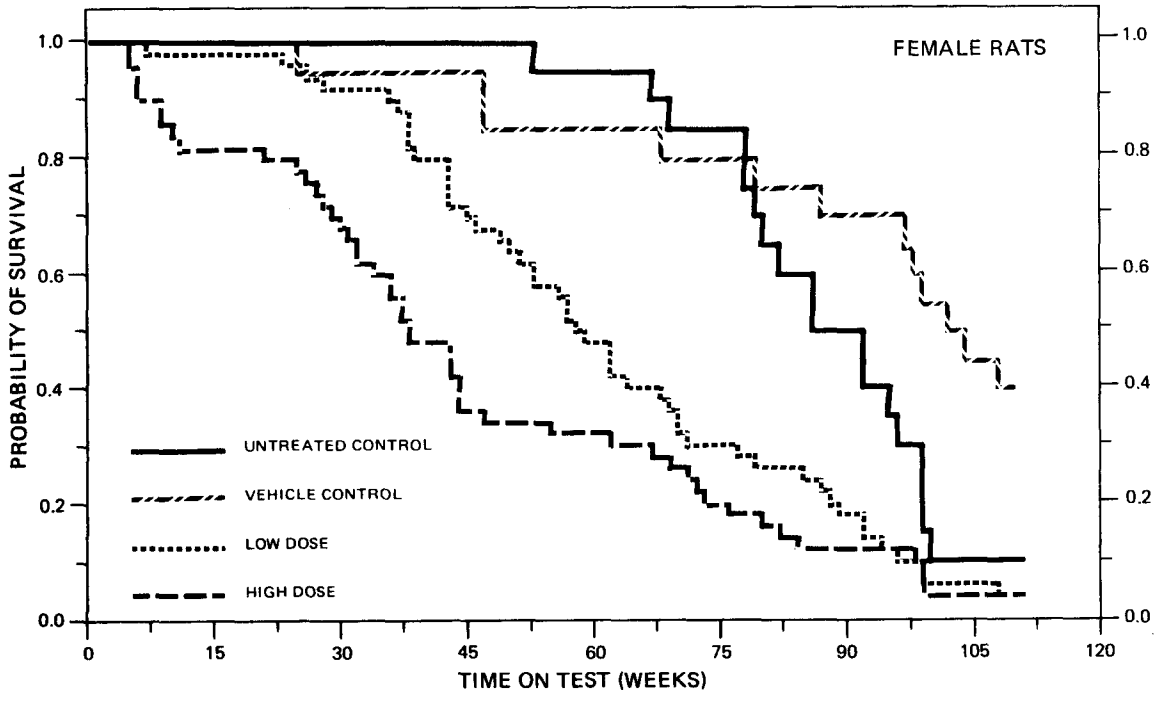


FIGURE 3
SURVIVAL COMPARISONS OF TRICHLOROFLUOROMETHANE CHRONIC STUDY RATS

85 percent (17/20) of the vehicle control and all 20 of the untreated control females were still alive on test at week 52. Survival of female rats was not adequate to perform meaningful analyses of late-developing tumors.

C. Pathology

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

Neoplasms present in the vehicle control and treated groups were similar histologically to those present in untreated control rats or those which have been observed previously in rats of similar strain, age, and sex. No appreciable difference in the incidence of these neoplasms was noted between the control and treated rats in this study.

Inflammatory, degenerative, and proliferative lesions as seen in the vehicle control and treated animals were usually similar, as to number and kind, to those naturally occurring lesions found in aged rats. Chronic murine pneumonia occurred in 88 to 100 percent of the rats and appeared to be a factor in early mortality. In addition, pleuritis and pericarditis were seen, primarily in dosed groups.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. Due to the high early mortality in treated rats of both sexes, many may have died before

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH TRICHLOROFLUOROMETHANE
WHICH SURVIVED AT LEAST 52 WEEKS[a]

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	2/13(0.15)	0/20(0.00)	2/19(0.11)	0/14(0.00)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.324	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	64	---	74	---

[a]Treated groups received time-weighted average doses of 488 or 977 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 vehicle control group is given beneath the incidence of tumors in the vehicle control 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.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH TRICHLOROFLUOROMETHANE
WHICH SURVIVED AT LEAST 52 WEEKS[a]

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	3/20(0.15)	4/17(0.24)	1/29(0.03)	3/16(0.19)
P Values[c]	---	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	---	P = 0.030	---	---
Relative Risk (Vehicle Control)[d]	---	---	0.147	0.797
Lower Limit	---	---	0.003	0.137
Upper Limit	---	---	1.260	3.940
Weeks to First Observed Tumor	92	68	111	98
Mammary Gland: Fibroadenoma[b]	3/20(0.15)	3/17(0.18)	1/31(0.03)	2/17(0.12)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.183	0.667
Lower Limit	---	---	0.004	0.062
Upper Limit	---	---	1.977	5.067
Weeks to First Observed Tumor	99	97	64	99
Mammary Gland: Fibroadenoma or Adenocarcinoma NOS[b]	3/20(0.15)	4/17(0.24)	2/31(0.06)	2/17(0.12)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.274	0.500
Lower Limit	---	---	0.028	0.051
Upper Limit	---	---	1.727	2.985
Weeks to First Observed Tumor	99	97	64	99

TABLE 4 (CONCLUDED)

---

[a]Treated groups received time-weighted average doses of 538 or 1077 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 vehicle control group is given beneath the incidence of tumors in the vehicle control 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 $P < 0.05$.

they were at risk from late-developing tumors. To compensate for this, the analyses performed were based solely upon those rats that survived at least 52 weeks. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or trichlorofluoromethane-dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between the administration of trichlorofluoromethane and tumor incidence. It must be noted, however, that the high early mortality in both sexes precluded meaningful analyses of late-developing tumors.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In all of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that all of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by trichlorofluoromethane that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

No distinct compound-related mean body weight depression was apparent when trichlorofluoromethane-treated male and female mice were compared with controls (Figure 4). Fluctuations in the growth curve 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 appearance and behavior of the treated mice were generally comparable with the vehicle controls. A slight decline in survival was evident for the high dose groups beginning in week 18 of the study and continuing through the duration of compound administration. Clinical signs usually associated with group-housing, particularly in the males, were observed at essentially comparable frequencies in all groups and included sores on the body or extremities, generalized or localized alopecia, rough or stained fur, external genital irritation, abdominal distension or bloating, and swollen areas. Palpable nodules and/or tissue masses were observed at a slightly greater frequency in the treated male mice than in the remaining groups. Isolated, apparently incidental, observations included head tilt or circling in one to three high dose female mice during the second year of the study.

B. Survival

The estimated probabilities of survival for male and female mice in the control and trichlorofluoromethane-dosed groups are shown in Figure 5.

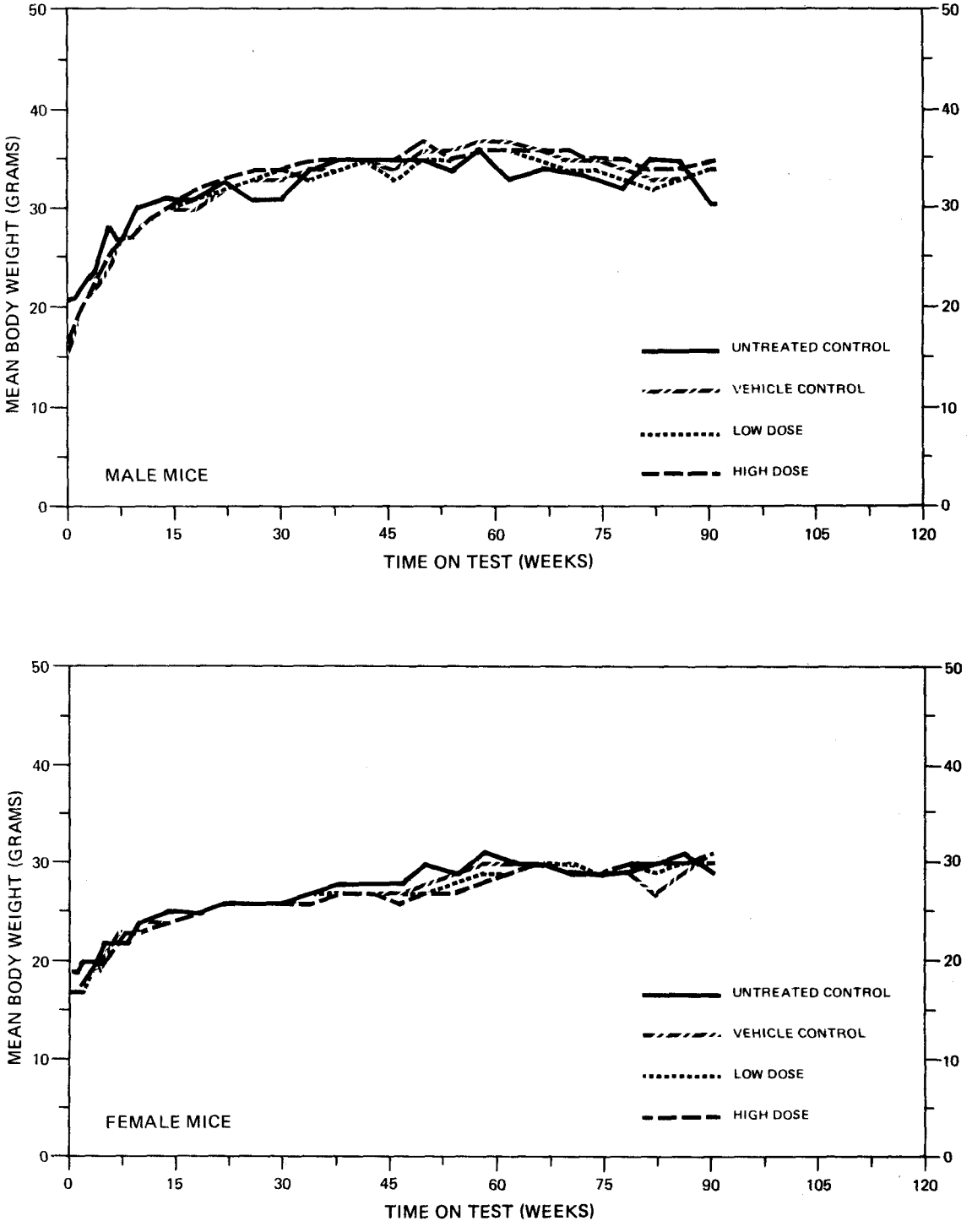
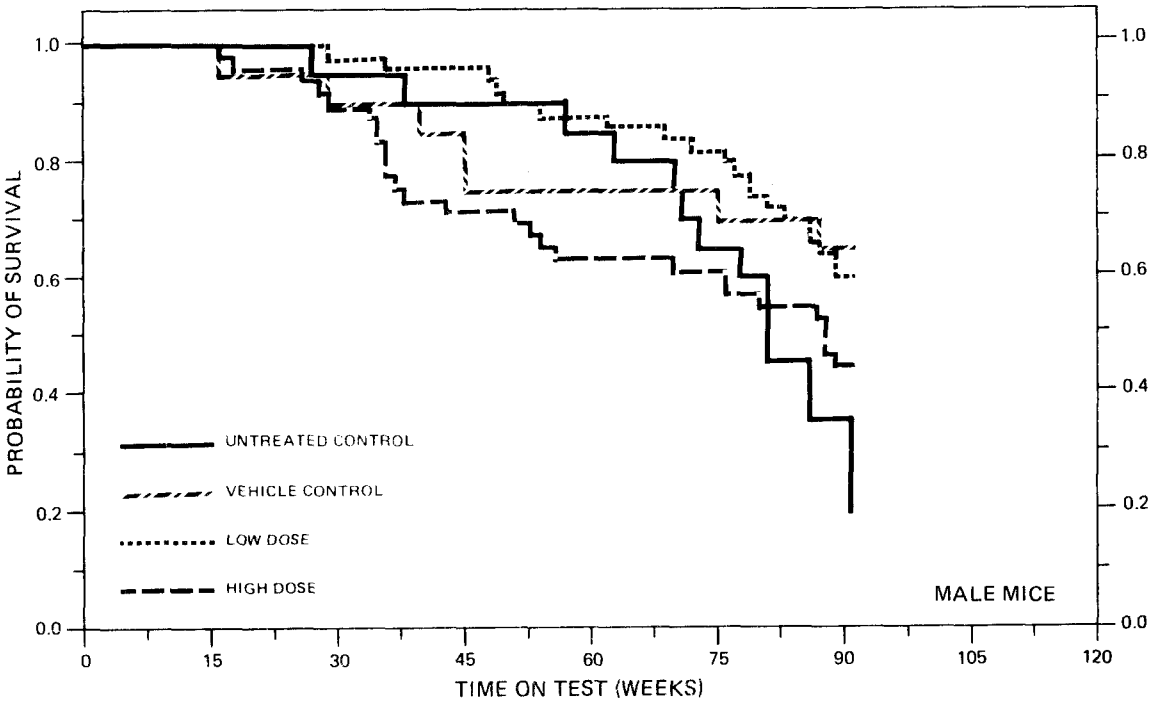


FIGURE 4
GROWTH CURVES FOR TRICHLOROFLUOROMETHANE CHRONIC STUDY MICE



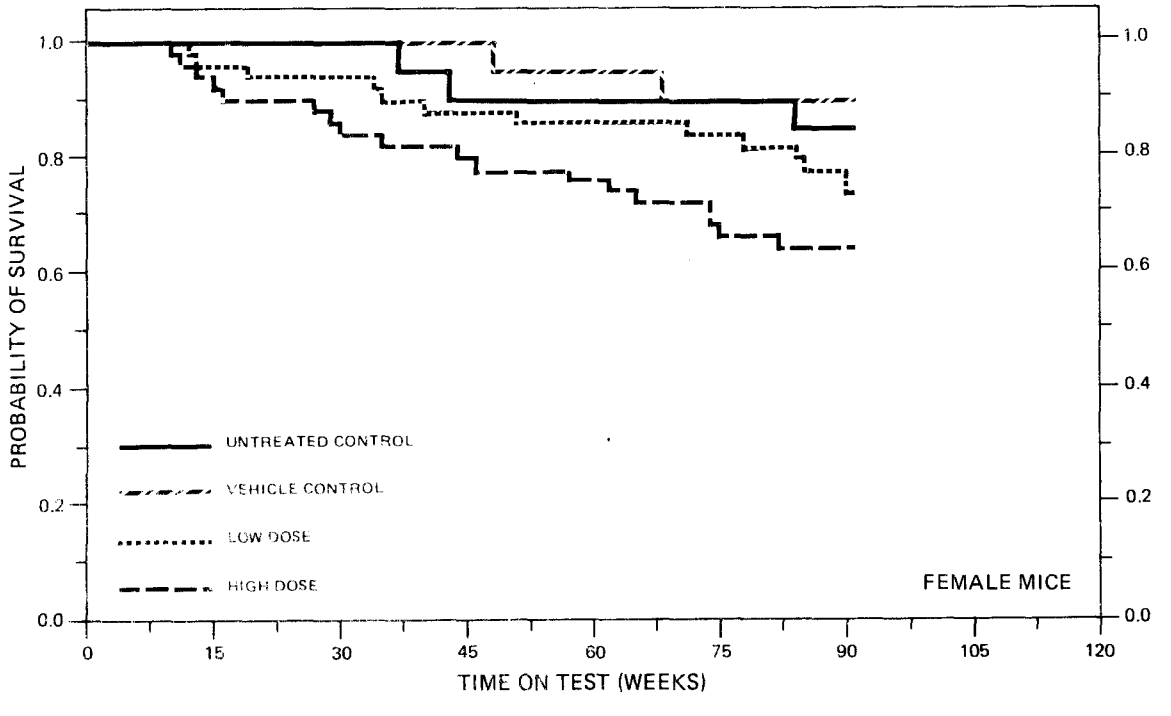


FIGURE 5
SURVIVAL COMPARISONS OF TRICHLOROFLUOROMETHANE CHRONIC STUDY MICE

For male mice the Tarone test for positive association between increased dosage and accelerated mortality was not significant when dosed groups were compared to the vehicle control. Adequate numbers of male mice survived to be at risk from late-developing tumors, with 58 percent (29/50) of the high dose, 82 percent (41/50) of the low dose, 75 percent (15/20) of the vehicle control, and 65 percent (13/20) of the untreated control mice surviving on test at least 75 weeks.

For female mice the Tarone test showed a significant ($P = 0.009$) positive association between increased dosage and accelerated mortality when dosed groups were compared to the vehicle control. Survival was adequate, however, as 64 percent (32/50) of the high dose, 74 percent (37/50) of the low dose, 90 percent (18/20) of the vehicle control, and 85 percent (17/20) of the untreated control mice were alive on test at the termination of the study.

C. Pathology

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

Hepatocellular carcinoma was the most commonly observed neoplasm and was diagnosed in 5/19 (26 percent) vehicle control males, 12/50 (24 percent) low dose males, 10/47 (21 percent) high dose males, 1/19 (5 percent) vehicle control females, 4/50 (8 percent) low dose females, and 2/49 (4 percent) high dose females. The hepatic neoplasms

occurring in the vehicle control mice were not different in appearance or incidence from those noted in the trichlorofluoromethane-treated mice. No appreciable difference was noted in the incidence of other neoplasms between the control and treated groups.

Inflammatory, degenerative, and proliferative lesions as seen in the control and treated animals were similar in number and kind to those naturally occurring lesions found in aging B6C3F1 mice.

This histopathologic examination did not provide evidence for the carcinogenicity of trichlorofluoromethane in B6C3F1 mice under the conditions of this bioassay.

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 is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or trichlorofluoromethane dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests for any site in mice of either sex indicated a significant positive association between the administration of trichlorofluoromethane and tumor incidence. Thus, at the dose levels used in this experiment there was no convincing evidence that trichlorofluoromethane was a carcinogen in B6C3F1 mice.

To provide additional insight into the carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH TRICHLOROFLUOROMETHANE[a]

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Malignant Lymphoma[b]	0/19(0.00)	1/19(0.05)	4/50(0.08)	4/47(0.09)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	1.520	1.617
Lower Limit	---	---	0.168	0.178
Upper Limit	---	---	73.309	77.876
Weeks to First Observed Tumor	---	75	72	88
Liver: Hepatocellular Carcinoma[b]	3/19(0.16)	5/19(0.26)	12/50(0.24)	10/47(0.21)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.912	0.809
Lower Limit	---	---	0.359	0.302
Upper Limit	---	---	2.959	2.699
Weeks to First Observed Tumor	71	87	79	88
Liver: Neoplastic Nodule or Hepatocellular Carcinoma[b]	3/19(0.16)	5/19(0.26)	15/50(0.30)	10/47(0.21)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	1.140	0.809
Lower Limit	---	---	0.476	0.302
Upper Limit	---	---	3.571	2.699
Weeks to First Observed Tumor	71	87	79	88

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	0/19(0.00)	2/19(0.11)	0/50(0.00)	1/47(0.02)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.000	0.202
Lower Limit	---	---	0.000	0.004
Upper Limit	---	---	1.278	3.710
Weeks to First Observed Tumor	---	91	---	80

[a]Treated groups received time-weighted average doses of 1962 or 3925 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 vehicle control group is given beneath the incidence of tumors in the vehicle control 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.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH TRICHLOROFLUOROMETHANE[a]

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	1/19(0.05)	1/19(0.05)	4/50(0.08)	2/49(0.04)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	1.520	0.776
Lower Limit	---	---	0.168	0.044
Upper Limit	---	---	73.309	44.838
Weeks to First Observed Tumor	91	91	84	74

[a]Treated groups received time-weighted average doses of 1962 or 3925 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 vehicle control group is given beneath the incidence of tumors in the vehicle control 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.

been estimated and entered in the tables based upon the observed tumor incidence rates. In all of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that all of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by trichlorofluoromethane that could not be established under the conditions of this test.

V. DISCUSSION

Due to a high rate of early deaths among rats of both sexes, the number of rats at risk from late-developing tumors was not adequate for conclusions to be based on results of this bioassay. Survival of mice was adequate for meaningful statistical analysis of tumor incidence.

In an attempt to compensate for the large number of early deaths in the rat bioassay, statistical analyses of tumor incidence in rats were based on those animals surviving at least 52 weeks. None of these statistical tests indicated a significant positive association between administration of trichlorofluoromethane and tumor incidence. No unusual tumors were observed during histopathologic examinations of dosed or control rats in this bioassay. The fact that vehicle control rats were placed on test approximately seven months before dosed rats had no apparent effect on the results of this study.

No significant increase in tumor incidence occurred among mice dosed with trichlorofluoromethane. No unusual tumors were observed during the histopathologic examinations of mice in this bioassay.

Negative results for carcinogenesis were obtained by Epstein et al. (1967) in a one-year bioassay of Swiss mice (ICR/Ha) in which trichlorofluoromethane solutions (10 percent by volume in tricaprylin) were injected subcutaneously into the neck of neonatal mice (0.1 ml into 1- and 7-day old mice, then 0.2 ml into 14- and 21-day old mice).

The results of the bioassay of trichlorofluoromethane in Osborne-Mendel rats for possible carcinogenicity are not conclusive because inadequate numbers of rats survived long enough to be at risk from late-developing tumors. Under the conditions of this bioassay, trichlorofluoromethane was not carcinogenic to B6C3F1 mice.

VI. BIBLIOGRAPHY

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

Aviado, D.M., "Toxicity of Aerosol Propellants in the Respiratory and Circulatory Systems. IX. Summary of the Most Toxic: Trichlorofluoromethane (FC 11)." Toxicology 3:311-319, 1975.

Belej, M.A. and D.M. Aviado, "Cardiopulmonary Toxicity of Propellants for Aerosols." Journal of Clinical Pharmacology 15(1):105-115, 1975.

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 and Engineering News, "Aerosol Producers Have Another Bad Year." 55(21):6, 1977.

Chemical Marketing Reporter, "Chemical Profile: Fluorocarbons." 208(10), 1975.

Chemical Regulation Reporter, "Federal Agencies to Phase Out Non-essential Propellant Use By 1979." 1(9):298-299, 1977.

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.

Crossland, J., "Aerosols." Environment 16(6):16-20,25,26, 1974.

Epstein, S.S., S. Joshi, J. Andrea, P. Clapp, H. Falk, and N. Mantel, "Synergistic Toxicity and Carcinogenicity of 'Freons' and Piperonyl Butoxide." Nature 214:526-528, 1967.

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.

Gosselin, R.E., H.C. Hodge, R.P. Smith, and M.N. Gleason, Clinical Toxicology of Commercial Products, 4th edition. The Williams and Wilkins Company, Baltimore, Maryland, 1976.

Grimsrud, E.P. and R.A. Rasmussen, "The Analysis of Chlorofluoromethanes in the Troposphere by Gas Chromatography-Mass Spectrometry." Atmospheric Environment 9:1010-1013, 1975.

Hester, N.E., E.R. Stephens, and O.C. Taylor, "Fluorocarbons in the Los Angeles Basin." Journal of the Air Pollution Control Association 24(6):591-595, 1974.

International Agency for Research on Cancer, "IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Volume 1. IARC, Lyon, France, 1972.

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.

Lovelock, J.E., "Atmospheric Fluorine Compounds as Indicators of Air Movements." Nature 230:379, 1971.

Lovelock, J.E., R.J. Maggs, and R.J. Wade, "Halogenated Hydrocarbons In and Over the Atlantic." Nature 241:194-196, 1973.

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

Molina, M.J. and F.S. Rowland, "Stratospheric Sink for Chlorofluoromethanes: Chlorine Atom-Catalysed Destruction of Ozone." Nature 249:810-812, 1974.

Polkis, A., "Determination of Flurocarbon 11 and Fluorocarbon 12 in Post-Mortem Tissues: A Case Report." Forensic Science 5:53-59, 1975.

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.

Simmonds, P.G., S.L., Kerrin, J.E. Lovelock, and F.H. Shair, "Distribution of Atmospheric Halocarbons in the Air over the Los Angeles Basin." Atmospheric Environment 8:209-216, 1974.

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

U.S. International Trade Commission, Synthetic Organic Chemicals: United States Production and Sales, 1975. USITC Publication 804, Washington, D.C., 1977.

Windholz, M., editor, The Merck Index: An Encyclopedia of Chemicals and Drugs, Ninth edition. Merck and Co., Inc., Rahway, New Jersey, 1976.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH TRICHLOROFLUOROMETHANE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED
WITH TRICHLOROFLUOROMETHANE

	CONTROL (UNTR) 01-171M	CONTROL (VEH) 01-091M	LOW DOSE 01-172M	HIGH DOSE 01-173M
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)
SEBACEOUS ADENOMA			1 (2%)	
FIBROMA			1 (2%)	
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(50)
ADENOSQUAMOUS CARCINOMA		1 (5%)		
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(20)	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)			
#SPLEEN	(20)	)	(49)	(50)
HEMANGIOSARCOMA	1 (5%)	1 (5%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
#CERVICAL LYMPH NODE	(19)	(20)	(46)	(49)
ADENOSQUAMOUS CARCINOMA, METASTA		1 (5%)		
#LUNG	(20)	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-171M	CONTROL (VEH) 01-091M	LOW DOSE 01-172M	HIGH DOSE 01-173M
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(50)
ADENOSQUAMOUS CARCINOMA, METASTA		1 (5%)		
MIXED TUMOR, MALIGNANT		2 (10%)		
HAMARTOMA +		1 (5%)		
ENDOCRINE SYSTEM				
#PITUITARY	(19)	(20)	(45)	(49)
CHROMOPHOBE ADENOMA	2 (11%)		2 (4%)	
#ADRENAL	(20)	(20)	(50)	(50)
PHEOCHROMOCYTOMA	1 (5%)			
#THYROID	(20)	(20)	(48)	(50)
FOLLICULAR-CELL ADENOMA		1 (5%)	2 (4%)	
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
#BRAIN	(20)	(20)	(49)	(50)
NEUROFIBROSARCOMA				1 (2%)
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(20)	(20)	(50)	(50)
SPINDLE/GIANT-CELL CARCINOMA		1 (5%)		
ALL OTHER SYSTEMS				
NONE				

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 (CONCLUDED)

	CONTROL (UNTR) 01-171M	CONTROL (VEH) 01-091M	LOW DOSE 01-172M	HIGH DOSE 01-173M
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	19	18	49	49
MORIBUND SACRIFICE	1		1	
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE		2		1
ANIMAL MISSING				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	5	4	1
TOTAL PRIMARY TUMORS	6	7	7	1
TOTAL ANIMALS WITH BENIGN TUMORS	3	2	4	
TOTAL BENIGN TUMORS	3	2	6	
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	5	1	1
TOTAL MALIGNANT TUMORS	3	5	1	1
TOTAL ANIMALS WITH SECONDARY TUMORS#		1		
TOTAL SECONDARY TUMORS		2		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

@ INCLUDES AUTOLYZED ANIMALS

* 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 TRICHLOROFLUOROMETHANE

	CONTROL (UNTR) 01-171F	CONTROL (VEH) 01-091F	LOW DOSE 01-174F	HIGH DOSE 01-175F
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	20	49	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA				1 (2%)
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
FIBROSARCOMA				1 (2%)
RESPIRATORY SYSTEM				
#TRACHEA	(20)	(16)	(49)	(48)
HEMANGIOSARCOMA, METASTATIC			1 (2%)	
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(20)	(49)	(50)
HEMANGIOSARCOMA	1 (5%)			
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (5%)		
#LUNG	(20)	(20)	(49)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#ESOPHAGUS	(20)	(17)	(49)	(46)
HEMANGIOSARCOMA, METASTATIC			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(49)	(50)
HAMARTOMA +				1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
+ 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) 01-171F	CONTROL (VEH) 01-091F	LOW DOSE 01-174F	HIGH DOSE 01-175F
ENDOCRINE SYSTEM				
#PITUITARY	(20)	(20)	(48)	(45)
CHROMOPHOBE ADENOMA	3 (15%)	4 (20%)	1 (2%)	3 (7%)
#ADRENAL	(20)	(20)	(49)	(49)
CORTICAL CARCINOMA				1 (2%)
PHEOCHROMOCYTOMA	2 (10%)			
#THYROID	(20)	(20)	(48)	(46)
FOLLICULAR-CELL CARCINOMA	2 (10%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS		1 (5%)	1 (2%)	
FIBROADENOMA	3 (15%)	3 (15%)	1 (2%)	2 (4%)
#UTERUS	(19)	(19)	(49)	(49)
ADENOCARCINOMA, NOS			1 (2%)	
ENDOMETRIAL STROMAL POLYP				1 (2%)
#OVARY	(20)	(20)	(49)	(49)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
GRANULOSA-CELL TUMOR	1 (5%)			
GRANULOSA-CELL CARCINOMA		1 (5%)		
#OVARY/FOLLICLE	(20)	(20)	(49)	(49)
CYSTADENOMA, NOS				1 (2%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*MUSCLE OF THORAX	(20)	(20)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)	

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 01-171F	CONTROL (VEH) 01-091F	LOW DOSE 01-174F	HIGH DOSE 01-175F
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	18	12	46	45
MORIBUND SACRIFICE			2	3
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	2	8	2	2
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	7	4	7
TOTAL PRIMARY TUMORS	13	10	5	11
TOTAL ANIMALS WITH BENIGN TUMORS	7	6	2	6
TOTAL BENIGN TUMORS	8	7	2	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	2	3	3
TOTAL MALIGNANT TUMORS	4	3	3	3
TOTAL ANIMALS WITH SECONDARY TUMORS#			2	
TOTAL SECONDARY TUMORS			3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1			
TOTAL UNCERTAIN TUMORS	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 B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH TRICHLOROFLUOROMETHANE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED
WITH TRICHLOROFLUOROMETHANE

	CONTROL (UNTR) 02-M181	CONTROL (VEH) 02-M171	LOW DOSE 02-M172	HIGH DOSE 02-M173
ANIMALS INITIALLY IN STUDY	20	20	50	49
ANIMALS MISSING				1
ANIMALS NECROPSIED	19	19	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	18	19	50	47
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(19)	(19)	(50)	(47)
FIBROSARCOMA	1 (5%)		1 (2%)	2 (4%)
FIBROUS HISTIOCYTOMA, MALIGNANT			1 (2%)	
RESPIRATORY SYSTEM				
#LUNG	(19)	(19)	(50)	(47)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (5%)		1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (5%)		
HEMATOPOIETIC SYSTEM				
*NERVE TRACT	(19)	(19)	(50)	(47)
MALIG.LYMPHOMA, UNDIFFER-TYPE				1 (2%)
*MULTIPLE ORGANS	(19)	(19)	(50)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			3 (6%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (5%)		2 (4%)
*SUBCUT TISSUE	(19)	(19)	(50)	(47)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
#MESENTERIC L. NODE	(17)	(19)	(47)	(47)
HEMANGIOMA			1 (2%)	
#KIDNEY	(19)	(19)	(49)	(47)
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 B1 (CONTINUED)

	CONTROL (UNTR) 02-M181	CONTROL (VEH) 02-M171	LOW DOSE 02-M172	HIGH DOSE 02-M173
DIGESTIVE SYSTEM				
#LIVER	(19)	(19)	(50)	(47)
NEOPLASTIC NODULE			3 (6%)	
HEPATOCELLULAR CARCINOMA	3 (16%)	5 (26%)	12 (24%)	10 (21%)
HEMANGIOSARCOMA				1 (2%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(16)	(16)	(45)	(47)
CHROMOPHOBE ADENOMA				1 (2%)
REPRODUCTIVE SYSTEM				
#TESTIS	(19)	(19)	(50)	(47)
INTERSTITIAL-CELL TUMOR			1 (2%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(19)	(19)	(50)	(47)
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 B1 (CONCLUDED)

	CONTROL (UNTR) 02-M181	CONTROL (VEH) 02-M171	LOW DOSE 02-M172	HIGH DOSE 02-M173
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	49
NATURAL DEATH@	14	7	20	27
MORIBUND SACRIFICE	2			
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	4	13	30	21
ANIMAL MISSING				1
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	7	22	16
TOTAL PRIMARY TUMORS	4	8	24	19
TOTAL ANIMALS WITH BENIGN TUMORS		1	3	2
TOTAL BENIGN TUMORS		1	3	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	6	17	15
TOTAL MALIGNANT TUMORS	4	7	18	17
TOTAL ANIMALS WITH SECONDARY TUMORS#			1	1
TOTAL SECONDARY TUMORS			1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			3	
TOTAL UNCERTAIN TUMORS			3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

@ INCLUDES AUTOLYZED ANIMALS

* 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 TRICHLOROFLUOROMETHANE

	CONTROL (UNTR) 02-F181	CONTROL (VEH) 02-F171	LOW DOSE 02-F174	HIGH DOSE 02-F175
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	19	19	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	18	19	50	49
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(19)	(19)	(50)	(49)
ALVECLAR/BRONCHIOLAR ADENOMA		1 (5%)		2 (4%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(19)	(19)	(50)	(49)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (2%)
#SPLEEN	(19)	(19)	(50)	(49)
HEMANGIOSARCOMA			1 (2%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(19)	(19)	(50)	(49)
HEPATOCELLULAR CARCINOMA	1 (5%)	1 (5%)	4 (8%)	2 (4%)
#STOMACH	(19)	(19)	(50)	(49)
SQUAMOUS CELL PAPILLOMA			1 (2%)	1 (2%)
*RECTUM	(19)	(19)	(50)	(49)
LEIOMYOSARCOMA			1 (2%)	

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 02-F181	CONTROL (VEH) 02-F171	LOW DOSE 02-F174	HIGH DOSE 02-F175
URINARY SYSTEM				
*URETER	(19)	(19)	(50)	(49)
LEIOMYOSARCOMA, METASTATIC			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(16)	(18)	(50)	(49)
CHROMOPHOBE ADENOMA	1 (6%)		1 (2%)	
#ADRENAL	(18)	(19)	(50)	(49)
PHEOCHROMOCYTOMA				1 (2%)
#THYROID	(15)	(15)	(50)	(49)
FOLLICULAR-CELL ADENOMA				1 (2%)
REPRODUCTIVE SYSTEM				
*VAGINA	(19)	(19)	(50)	(49)
LEIOMYOSARCOMA, METASTATIC			1 (2%)	
#UTERUS	(17)	(19)	(50)	(49)
LEIOMYOSARCOMA, METASTATIC			1 (2%)	
ENDOMETRIAL STROMAL POLYP	1 (6%)		1 (2%)	
#OVARY	(18)	(19)	(50)	(49)
GRANULOSA-CELL TUMOR		1 (5%)		
NERVOUS SYSTEM				
*CRANIAL NERVE	(19)	(19)	(50)	(49)
NEUROFIBROMA				1 (2%)
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(19)	(19)	(50)	(49)
ADENOMA, NOS			1 (2%)	2 (4%)
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 02-F181	CONTROL (VEH) 02-F171	LOW DOSE 02-F174	HIGH DOSE 02-F175
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	3	2	12	17
MORIBUND SACRIFICE			1	1
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	17	18	37	32
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	3	11	9
TOTAL PRIMARY TUMORS	3	3	12	11
TOTAL ANIMALS WITH BENIGN TUMORS	2	1	4	7
TOTAL BENIGN TUMORS	2	1	4	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	1	7	3
TOTAL MALIGNANT TUMORS	1	1	8	3
TOTAL ANIMALS WITH SECONDARY TUMORS#			1	
TOTAL SECONDARY TUMORS			3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1		
TOTAL UNCERTAIN TUMORS		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 TRICHLOROFLUOROMETHANE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH TRICHLOROFLUOROMETHANE

	CONTROL (UNTR) 01-171M	CONTROL (VEH) 01-091M	LOW DOSE 01-172M	HIGH DOSE 01-173M
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)
EPIDERMAL INCLUSION CYST				1 (2%)
INFLAMMATION, NOS		1 (5%)		
ULCER, ACUTE			1 (2%)	1 (2%)
HYPERKERATOSIS		1 (5%)		
ACANTHOSIS		1 (5%)		
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
ABSCESS, NOS		1 (5%)		
NECROSIS, FOCAL			1 (2%)	
RESPIRATORY SYSTEM				
#TRACHEA	(20)	(20)	(49)	(50)
INFLAMMATION, CHRONIC			1 (2%)	
#LUNG	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
PNEUMONIA, CHRONIC MURINE	20 (100%)	19 (95%)	48 (96%)	49 (98%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(20)	(49)	(50)
HEMOSIDEROSIS				3 (6%)
HEMATOPOIESIS		2 (10%)	2 (4%)	
#CERVICAL LYMPH NODE	(19)	(20)	(46)	(49)
INFLAMMATION, NOS			1 (2%)	1 (2%)
#MEDIASTINAL L.NODE	(19)	(20)	(46)	(49)
HYPERPLASIA, LYMPHOID				1 (2%)
#MESENTERIC L. NODE	(19)	(20)	(46)	(49)
PERIARTERITIS		1 (5%)		

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-171M	CONTROL (VEH) 01-091M	LOW DOSE 01-172M	HIGH DOSE 01-173M
#THYMUS	(18)	(17)	(46)	(45)
INFLAMMATION, NOS		1 (6%)		
CIRCULATORY SYSTEM				
#MYOCARDIUM	(20)	(20)	(50)	(50)
MINERALIZATION				1 (2%)
INFLAMMATION, SUPPURATIVE				1 (2%)
INFLAMMATION, CHRONIC				1 (2%)
FIBROSIS			1 (2%)	2 (4%)
DEGENERATION, NOS			1 (2%)	1 (2%)
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(17)	(17)	(18)	(22)
INFLAMMATION, NOS		1 (6%)		
INFLAMMATION, ACUTE/CHRONIC				1 (5%)
#LIVER	(20)	(19)	(50)	(50)
HEMORRHAGE				1 (2%)
INFLAMMATION, CHRONIC FOCAL				1 (2%)
GRANULOMA, NOS			1 (2%)	
PERIARTERITIS				1 (2%)
PELIOSIS HEPATIS	1 (5%)			
NECROSIS, FOCAL				1 (2%)
METAMORPHOSIS FATTY		1 (5%)		
#HEPATIC LOBULE	(20)	(19)	(50)	(50)
CYTOLOGIC DEGENERATION			1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(19)	(50)	(50)
NECROSIS, NOS	1 (5%)		2 (4%)	
NECROSIS, COAGULATIVE				1 (2%)
METAMORPHOSIS FATTY	1 (5%)			
*BILE DUCT	(20)	(20)	(50)	(50)
INFLAMMATION, FOCAL				1 (2%)
#PANCREAS	(20)	(19)	(49)	(50)
PERIARTERITIS		1 (5%)		1 (2%)
#ESOPHAGUS	(20)	(19)	(49)	(47)
FRACTURE, NOS			1 (2%)	3 (6%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-171M	CONTROL (VEH) 01-091M	LOW DOSE 01-172M	HIGH DOSE 01-173M
ULCER, NOS			1 (2%)	
INFLAMMATION, SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE				1 (2%)
#STOMACH	(20)	(19)	(49)	(50)
ULCER, NOS	1 (5%)		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)	
ULCER, FOCAL	1 (5%)	1 (5%)	4 (8%)	6 (12%)
ULCER, ACUTE	1 (5%)			
INFLAMMATION, CHRONIC FOCAL			1 (2%)	1 (2%)
CALCIUM DEPOSIT		1 (5%)		
HYPERKERATOSIS	1 (5%)			
ACANTHOSIS	1 (5%)			
#LARGE INTESTINE	(20)	(18)	(48)	(50)
NEMATODIASIS			3 (6%)	3 (6%)
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(50)
MINERALIZATION			1 (2%)	1 (2%)
HEMORRHAGE			1 (2%)	
PYELONEPHRITIS, NOS				1 (2%)
PYELONEPHRITIS, ACUTE	2 (10%)		1 (2%)	1 (2%)
ABSCESS, NOS		1 (5%)		
INFLAMMATION, CHRONIC	8 (40%)	7 (35%)	12 (24%)	11 (22%)
#RENAL PAPILLA	(20)	(20)	(50)	(50)
MINERALIZATION			2 (4%)	
HYPERPLASIA, EPITHELIAL				1 (2%)
#KIDNEY/PELVIS	(20)	(20)	(50)	(50)
MINERALIZATION	1 (5%)			
INFLAMMATION, NOS			1 (2%)	
#URINARY BLADDER	(19)	(19)	(48)	(50)
CALCULUS, NOS	1 (5%)		1 (2%)	1 (2%)
ULCER, NOS				1 (2%)
INFLAMMATION, ACUTE				1 (2%)
ULCER, ACUTE			1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)	1 (2%)
HYPERPLASIA, EPITHELIAL	1 (5%)			
ENDOCRINE SYSTEM				
#PITUITARY	(19)	(20)	(45)	(49)
INFLAMMATION, NOS		1 (5%)		

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-171M	CONTROL (VEH) 01-091M	LOW DOSE 01-172M	HIGH DOSE 01-173M
HYPERPLASIA, CHROMOPHOBE-CELL	1 (5%)		2 (4%)	1 (2%)
#ADRENAL CORTEX	(20)	(20)	(50)	(50)
DEGENERATION, NOS	9 (45%)		11 (22%)	3 (6%)
HYPERPLASIA, FOCAL	1 (5%)			
ANGIECTASIS	3 (15%)		3 (6%)	2 (4%)
#ADRENAL MEDULLA	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)		1 (2%)	
HYPERPLASIA, FOCAL	1 (5%)			
#THYROID	(20)	(20)	(48)	(50)
CYST, NOS		1 (5%)		
INFLAMMATION, NOS				1 (2%)
REPRODUCTIVE SYSTEM				
#PROSTATE	(19)	(2)	(44)	(44)
INFLAMMATION, NOS		1 (50%)		
INFLAMMATION, ACUTE	1 (5%)		7 (16%)	4 (9%)
INFLAMMATION, ACUTE FOCAL			1 (2%)	5 (11%)
INFLAMMATION, CHRONIC				2 (5%)
*SEMINAL VESICLE	(20)	(20)	(50)	(50)
INFLAMMATION, NOS		1 (5%)		
INFLAMMATION, ACUTE			1 (2%)	
#TESTIS	(20)	(20)	(50)	(50)
MINERALIZATION				1 (2%)
ATROPHY, NOS	5 (25%)	3 (15%)	5 (10%)	3 (6%)
HYPOSPERMATOGENESIS	6 (30%)		7 (14%)	2 (4%)
*EPIDIDYMIS	(20)	(20)	(50)	(50)
STEATITIS				1 (2%)
NECROSIS, FAT		1 (5%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-171M	CONTROL (VEH) 01-091M	LOW DOSE 01-172M	HIGH DOSE 01-173M
BODY CAVITIES				
*PLEURA	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)	2 (4%)
INFLAMMATION, ACUTE/CHRONIC				1 (2%)
INFLAMMATION, PYOGRANULOMATOUS				3 (6%)
*PERICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC				1 (2%)
INFLAMMATION, CHRONIC				1 (2%)
INFLAMMATION, PYOGRANULOMATOUS				1 (2%)
*EPICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)	2 (4%)
INFLAMMATION, ACUTE/CHRONIC				1 (2%)
INFLAMMATION, CHRONIC				2 (4%)
INFLAMMATION, PYOGRANULOMATOUS				1 (2%)
*MESENTERY	(20)	(20)	(50)	(50)
PERIARTERITIS		1 (5%)		1 (2%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NONE				

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 TRICHLOROFLUOROMETHANE

	CONTROL (UNTR) 01-171F	CONTROL (VEH) 01-091F	LOW DOSE 01-174F	HIGH DOSE 01-175F
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	20	49	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
ULCER, NOS			1 (2%)	
ULCER, CHRONIC	1 (5%)			
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(49)	(50)
PNEUMONIA, CHRONIC MURINE	20 (100%)	20 (100%)	49 (100%)	44 (88%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(20)	(20)	(49)	(48)
METAMORPHOSIS FATTY		6 (30%)		
#SPLEEN	(20)	(20)	(49)	(50)
HEMOSIDEROSIS	1 (5%)		1 (2%)	
HEMATOPOIESIS	1 (5%)	1 (5%)	4 (8%)	1 (2%)
#CERVICAL LYMPH NODE	(19)	(20)	(49)	(47)
INFLAMMATION, NOS			1 (2%)	
INFLAMMATION, ACUTE	1 (5%)			
HYPERPLASIA, LYMPHOID			1 (2%)	
#MEDIASTINAL L.NODE	(19)	(20)	(49)	(47)
PIGMENTATION, NOS			1 (2%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(20)	(16)	(35)	(17)
EDEMA, NOS			1 (3%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-171F	CONTROL (VEH) 01-091F	LOW DOSE 01-174F	HIGH DOSE 01-175F
INFLAMMATION, NOS			1 (3%)	
#LIVER	(20)	(19)	(49)	(50)
INFLAMMATION, NOS		1 (5%)		
GRANULOMA, NOS	1 (5%)			
METAMORPHOSIS FATTY		1 (5%)		
ANGIECTASIS			1 (2%)	1 (2%)
#LIVER/CENTRILOBULAR	(20)	(19)	(49)	(50)
NECROSIS, ISCHEMIC			1 (2%)	
#PANCREAS	(20)	(18)	(49)	(50)
CYST, NOS		1 (6%)		
INFLAMMATION, ACUTE NECROTIZING			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
PERIARTERITIS			1 (2%)	
ATROPHY, NOS		1 (6%)		
#ESOPHAGUS	(20)	(17)	(49)	(46)
FRACTURE, NOS			2 (4%)	
#STOMACH	(20)	(20)	(48)	(50)
ULCER, NOS	1 (5%)			
INFLAMMATION, FOCAL	4 (20%)			
ULCER, FOCAL	2 (10%)	1 (5%)	7 (15%)	
INFLAMMATION, CHRONIC				2 (4%)
HYPERKERATOSIS			1 (2%)	
ACANTHOSIS			1 (2%)	
#GASTRIC SUBMUCOSA	(20)	(20)	(48)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)	
#SMALL INTESTINE	(20)	(19)	(48)	(50)
ULCER, ACUTE			1 (2%)	
#LARGE INTESTINE	(20)	(20)	(49)	(49)
NEMATODIASIS			3 (6%)	6 (12%)
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(49)	(50)
MINERALIZATION	1 (5%)		4 (8%)	
CYST, NOS		1 (5%)		
PYELONEPHRITIS, ACUTE			1 (2%)	
INFLAMMATION, ACUTE FOCAL				1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-171F	CONTROL (VEH) 01-091F	LOW DOSE 01-174F	HIGH DOSE 01-175F
INFLAMMATION, CHRONIC	4 (20%)	5 (25%)	5 (10%)	1 (2%)
INFARCT, FOCAL			1 (2%)	
#KIDNEY/CORTEX	(20)	(20)	(49)	(50)
CYST, NOS			1 (2%)	
#RENAL PAPILLA	(20)	(20)	(49)	(50)
HYPERPLASIA, EPITHELIAL			8 (16%)	
HYPERPLASIA, FOCAL			1 (2%)	
#KIDNEY/PELVIS	(20)	(20)	(49)	(50)
MINERALIZATION			8 (16%)	9 (18%)
#URINARY BLADDER	(20)	(18)	(47)	(45)
INFLAMMATION, ACUTE			1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(20)	(20)	(48)	(45)
CYST, NOS			1 (2%)	
HYPERPLASIA, CHROMOPHOBE-CELL	1 (5%)			
#ADRENAL CORTEX	(20)	(20)	(49)	(49)
DEGENERATION, NOS	2 (10%)		3 (6%)	4 (8%)
ANGIECTASIS	8 (40%)	2 (10%)	9 (18%)	13 (27%)
#ADRENAL MEDULLA	(20)	(20)	(49)	(49)
HYPERPLASIA, FOCAL			1 (2%)	
#THYROID	(20)	(20)	(48)	(46)
INFLAMMATION, GRANULOMATOUS			1 (2%)	
HYPERPLASIA, C-CELL				1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)	
#UTERUS	(19)	(19)	(49)	(49)
HYDROMETRA			1 (2%)	4 (8%)
#UTERUS/ENDOMETRIUM	(19)	(19)	(49)	(49)
CYST, NOS	1 (5%)			

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-171F	CONTROL (VEH) 01-091F	LOW DOSE 01-174F	HIGH DOSE 01-175F
INFLAMMATION, ACUTE	2 (11%)		2 (4%)	
*OVARY	(20)	(20)	(49)	(49)
MULTIPLE CYSTS	1 (5%)			
INFLAMMATION, ACUTE			2 (4%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PLEURA	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)	1 (2%)
INFLAMMATION, ACUTE/CHRONIC				1 (2%)
INFLAMMATION, PYOGRANULOMATOUS			1 (2%)	
*PERICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
ABSCESS, NOS			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC				2 (4%)
INFLAMMATION, CHRONIC				3 (6%)
*MESENTERY	(20)	(20)	(50)	(50)
PERIARTERITIS				1 (2%)
ALL OTHER SYSTEMS				
DIAPHRAGM				
NECROSIS, FAT				1
ADIPOSE TISSUE				
INFLAMMATION, CHRONIC FOCAL				1

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

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 01-171F	CONTROL(VEH) 01-091F	LOW DOSE 01-174F	HIGH DOSE C1-175F
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED				1
AUTC/NECROPSY/NO HISTO			1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH TRICHLOROFLUOROMETHANE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH TIRCHLOROFLUOROMETHANE

	CONTROL (UNTR) 02-M181	CONTROL (VEH) 02-M171	LOW DOSE 02-M172	HIGH DOSE 02-M173
ANIMALS INITIALLY IN STUDY	20	20	50	49
ANIMALS MISSING				1
ANIMALS NECROPSIED	19	19	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	18	19	50	47
INTEGUMENTARY SYSTEM				
*SKIN	(19)	(19)	(50)	(47)
EPIDERMAL INCLUSION CYST		1 (5%)		
INFLAMMATION, ACUTE				1 (2%)
PARASITISM		9 (47%)	33 (66%)	24 (51%)
*SUBCUT TISSUE	(19)	(19)	(50)	(47)
EDEMA, NOS			1 (2%)	
ABSCESS, NOS	1 (5%)		1 (2%)	
RESPIRATORY SYSTEM				
#LUNG	(19)	(19)	(50)	(47)
INFLAMMATION, ACUTE			2 (4%)	
INFLAMMATION, ACUTE FOCAL				1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
NECROSIS, NOS			1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (5%)		
HEMATOPOIETIC SYSTEM				
#SPLEEN	(19)	(19)	(50)	(47)
AMYLOIDOSIS	7 (37%)		7 (14%)	6 (13%)
HEMATOPOIESIS			4 (8%)	1 (2%)
#HEMOLYMPH NODES	(19)	(19)	(50)	(47)
ANGIECTASIS			1 (2%)	
HYPERPLASIA, LYMPHOID			1 (2%)	
#LYMPH NODE	(17)	(19)	(47)	(47)
HYPERPLASIA, LYMPHOID		1 (5%)		1 (2%)
#CERVICAL LYMPH NODE	(17)	(19)	(47)	(47)
HYPERPLASIA, LYMPHOID		1 (5%)	8 (17%)	1 (2%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 02-M181	CONTROL (VEH) 02-M171	LOW DOSE 02-M172	HIGH DOSE 02-M173
#MESENTERIC L. NODE	(17)	(19)	(47)	(47)
CONGESTION, NOS		1 (5%)		
INFLAMMATION, NOS	1 (6%)			
INFLAMMATION, ACUTE			2 (4%)	1 (2%)
INFLAMMATION, GRANULOMATOUS			1 (2%)	
ANGIECTASIS			2 (4%)	9 (19%)
ERYTHROPHAGOCYTOSIS		1 (5%)		
HYPERPLASIA, LYMPHOID			10 (21%)	3 (6%)
CIRCULATORY SYSTEM				
#HEART	(18)	(19)	(50)	(47)
INFLAMMATION, CHRONIC FOCAL				1 (2%)
AMYLOIDOSIS				1 (2%)
CALCIUM DEPOSIT	1 (6%)			
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(19)	(19)	(49)	(46)
ABSCESS, NOS			2 (4%)	
INFLAMMATION, CHRONIC FOCAL		8 (42%)	14 (29%)	7 (15%)
#LIVER	(19)	(19)	(50)	(47)
THROMBOSIS, NOS				1 (2%)
INFLAMMATION, NOS	1 (5%)			
INFLAMMATION, FOCAL	1 (5%)			
INFLAMMATION, ACUTE			1 (2%)	
NECROSIS, ISCHEMIC		1 (5%)	1 (2%)	
AMYLOIDOSIS	2 (11%)		4 (8%)	3 (6%)
HYPERPLASTIC NODULE			1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)	
#LIVER/PERIPORTAL	(19)	(19)	(50)	(47)
DEGENERATION, NOS			1 (2%)	
NECROSIS, NOS			1 (2%)	
*BILE DUCT	(19)	(19)	(50)	(47)
INFLAMMATION, CHRONIC		2 (11%)	1 (2%)	
HYPERPLASIA, NOS				1 (2%)
#PANCREAS	(18)	(18)	(50)	(47)
INFLAMMATION, NOS	1 (6%)			
AMYLOIDOSIS			1 (2%)	2 (4%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 02-M181	CONTROL (VEH) 02-M171	LOW DOSE 02-M172	HIGH DOSE 02-M173
#STOMACH	(19)	(18)	(50)	(46)
MINERALIZATION	1 (5%)			
ULCER, NOS			1 (2%)	
INFLAMMATION, CHRONIC FOCAL				1 (2%)
PARASITISM	1 (5%)			
#SMALL INTESTINE	(18)	(19)	(50)	(47)
AMYLOIDOSIS				1 (2%)
#LARGE INTESTINE	(15)	(19)	(46)	(41)
PARASITISM			7 (15%)	4 (10%)
#COLON	(15)	(19)	(46)	(41)
NEMATODIASIS	1 (7%)			
URINARY SYSTEM				
#KIDNEY	(19)	(19)	(49)	(47)
MINERALIZATION	2 (11%)		1 (2%)	1 (2%)
HYDRONEPHROSIS	2 (11%)			
MULTIPLE CYSTS			1 (2%)	
INFLAMMATION, ACUTE			2 (4%)	
INFLAMMATION, CHRONIC	9 (47%)	1 (5%)	6 (12%)	9 (19%)
INFLAMMATION, CHRONIC FOCAL		13 (68%)	27 (55%)	19 (40%)
INFLAMMATION, CHRONIC DIFFUSE			3 (6%)	
AMYLOIDOSIS	4 (21%)		1 (2%)	1 (2%)
#RENAL PAPILLA	(19)	(19)	(49)	(47)
INFLAMMATION, NECROTIZING			1 (2%)	
ENDOCRINE SYSTEM				
#ADRENAL	(17)	(19)	(50)	(47)
AMYLOIDOSIS			2 (4%)	1 (2%)
#THYROID	(15)	(17)	(44)	(44)
FOLLICULAR CYST, NOS		3 (18%)		
INFLAMMATION, CHRONIC		1 (6%)		
INFLAMMATION, CHRONIC FOCAL		1 (6%)		
REPRODUCTIVE SYSTEM				
#PROSTATE	(16)	(19)	(50)	(47)
INFLAMMATION, ACUTE				1 (2%)

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 02-M181	CONTROL (VEH) 02-M171	LOW DOSE 02-M172	HIGH DOSE 02-M173
INFLAMMATION, CHRONIC		1 (5%)	1 (2%)	
INFLAMMATION, CHRONIC FOCAL		1 (5%)		
#TESTIS	(19)	(19)	(50)	(47)
MINERALIZATION			1 (2%)	
ATROPHY, NOS			2 (4%)	
*EPIDIDYMIS	(19)	(19)	(50)	(47)
SPERMATOCELE			1 (2%)	
NECROSIS, FAT		1 (5%)		
*SCROTUM	(19)	(19)	(50)	(47)
FOREIGN BODY, NOS		1 (5%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
NECROSIS, FOCAL			1	
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	3	2	1	6
ANIMAL MISSING/NO NECROPSY				1
AUTO/NECROPSY/HISTO PERF			2	
AUTO/NECROPSY/NO HISTO	1			
AUTOLYSIS/NO NECROPSY	1	1		1

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH TRICHLOROFLUOROMETHANE

	CONTROL (UNTR) 02-F181	CONTROL (VEH) 02-F171	LOW DOSE 02-F174	HIGH DOSE 02-F175
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	19	19	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	18	19	50	49
INTEGUMENTARY SYSTEM				
*SKIN	(19)	(19)	(50)	(49)
EPIDERMAL INCLUSION CYST	1 (5%)			
ULCER, FOCAL		1 (5%)		
PARASITISM		11 (58%)	38 (76%)	31 (63%)
*SUBCUT TISSUE	(19)	(19)	(50)	(49)
STEATITIS				2 (4%)
RESPIRATORY SYSTEM				
#LUNG	(19)	(19)	(50)	(49)
INFLAMMATION, ACUTE				1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
PNEUMONIA, CHRONIC MURINE	2 (11%)	2 (11%)	6 (12%)	4 (8%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM				1 (2%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(19)	(19)	(50)	(49)
AMYLOIDOSIS		1 (5%)	1 (2%)	
HYPERPLASIA, LYMPHOID		1 (5%)	1 (2%)	
HEMATOPOIESIS			1 (2%)	4 (8%)
#LYMPH NODE	(19)	(19)	(50)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)	
#CERVICAL LYMPH NODE	(19)	(19)	(50)	(49)
HYPERPLASIA, LYMPHOID		1 (5%)	2 (4%)	4 (8%)
#MESENTERIC L. NODE	(19)	(19)	(50)	(49)
LYMPHANGIECTASIS			2 (4%)	1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 02-F181	CONTROL (VEH) 02-F171	LOW DOSE 02-F174	HIGH DOSE 02-F175
INFLAMMATION, NOS	2 (11%)			
INFLAMMATION, ACUTE				1 (2%)
HYPERPLASIA, LYMPHOID		3 (16%)	1 (2%)	3 (6%)
CIRCULATORY SYSTEM				
*MYOCARDIUM	(19)	(19)	(50)	(49)
MINERALIZATION			1 (2%)	
DIGESTIVE SYSTEM				
*SALIVARY GLAND	(17)	(19)	(50)	(49)
INFLAMMATION, CHRONIC FOCAL		13 (68%)	22 (44%)	14 (29%)
*LIVER	(19)	(19)	(50)	(49)
INFLAMMATION, ACUTE				1 (2%)
INFLAMMATION, ACUTE FOCAL			1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (5%)		
NECROSIS, FOCAL			3 (6%)	
NECROSIS, ISCHEMIC		1 (5%)		
*BILE DUCT	(19)	(19)	(50)	(49)
INFLAMMATION, CHRONIC		10 (53%)	9 (18%)	4 (8%)
*PANCREAS	(19)	(19)	(50)	(49)
DILATATION/DUCTS				2 (4%)
INFLAMMATION, NOS	2 (11%)			
ATROPHY, NOS			1 (2%)	
*ESOPHAGUS	(19)	(17)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
HYPERKERATOSIS				2 (4%)
*STOMACH	(19)	(19)	(50)	(49)
ULCER, FOCAL				2 (4%)
HYPERKERATOSIS				2 (4%)
*LARGE INTESTINE	(18)	(19)	(50)	(49)
PARASITISM			2 (4%)	8 (16%)
URINARY SYSTEM				
*KIDNEY	(19)	(19)	(50)	(49)
CYST, NOS			1 (2%)	

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 02-F181	CONTROL (VEH) 02-F171	LOW DOSE 02-F174	HIGH DOSE 02-F175
PYELONEPHRITIS, NOS	1 (5%)			1 (2%)
INFLAMMATION, CHRONIC		1 (5%)	1 (2%)	
INFLAMMATION, CHRONIC FOCAL		12 (63%)	28 (56%)	24 (49%)
ENDOCRINE SYSTEM				
#ADRENAL	(18)	(19)	(50)	(49)
ANGIECTASIS				1 (2%)
#ADRENAL CORTEX	(18)	(19)	(50)	(49)
CYST, NOS			1 (2%)	
#THYROID	(15)	(15)	(50)	(49)
FOLLICULAR CYST, NOS		1 (7%)		
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)	
REPRODUCTIVE SYSTEM				
#UTERUS	(17)	(19)	(50)	(49)
HYDROMETRA	1 (6%)			
INFLAMMATION, NOS	2 (12%)			
INFLAMMATION, ACUTE			1 (2%)	
#UTERUS/ENDOMETRIUM	(17)	(19)	(50)	(49)
CYST, NOS			1 (2%)	
HYPERPLASIA, CYSTIC	5 (29%)	17 (89%)	31 (62%)	28 (57%)
#OVARY/OVIDUCT	(17)	(19)	(50)	(49)
RETENTION FLUID		1 (5%)		
INFLAMMATION, NOS	1 (6%)			
#OVARY	(18)	(19)	(50)	(49)
CYST, NOS	3 (17%)		8 (16%)	8 (16%)
FOLLICULAR CYST, NOS		1 (5%)		1 (2%)
PAROVARIAN CYST		1 (5%)		
HEMORRHAGIC CYST			2 (4%)	3 (6%)
INFLAMMATION, NOS	3 (17%)			
NERVOUS SYSTEM				
#BRAIN	(19)	(19)	(50)	(49)
COMPRESSION			1 (2%)	

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

TABLE D2 (CONCLUDED)

	CONTROL(UNTR) 02-F181	CONTROL(VEH) 02-F171	LOW DOSE 02-F174	HIGH DOSE 02-F175
SPECIAL SENSE ORGANS				
*EYE	(19)	(19)	(50)	(49)
MICROPHTHALMIA				1 (2%)
ATROPHY, NOS	1 (5%)			
PHTHISIS BULBI	2 (11%)			
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(19)	(19)	(50)	(49)
INFLAMMATION, ACUTE FOCAL				2 (4%)
BODY CAVITIES				
*PERITONEUM	(19)	(19)	(50)	(49)
INFLAMMATION, NOS	1 (5%)			
*EPICARDIUM	(19)	(19)	(50)	(49)
INFLAMMATION, CHRONIC			1 (2%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	5		2	6
AUTO/NECROPSY/HISTO PERF			2	
AUTO/NECROPSY/NO HISTO	1			
AUTOLYSIS/NO NECROPSY	1	1		1

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

Review of the Bioassay of Trichlorofluoromethane*
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 Trichlorofluoromethane for carcinogenicity.

The primary reviewer agreed that the rat portion of the study was too inadequate, due to the high early mortality, to evaluate the carcinogenicity of Trichlorofluoromethane in this species. Under the conditions of test, he said that Trichlorofluoromethane was not carcinogenic in either sex of treated mice. After a brief description of the experimental design, the primary reviewer added that no unusual tumors or increases in overall tumor incidence were observed among the treated mice. In his critique, he said that a number of other chemicals were studied in the same room during the bioassay of Trichlorofluoromethane and that the vehicle control group was initiated several months prior to the treated animals being placed on test.

The secondary reviewer noted that occupational exposure to Trichlorofluoromethane could be in the same range as the low dosages administered in the bioassay. He pointed out the high incidence of lymphoid hyperplasia among the low dose male mice and said that these lesions may be under-diagnosed. He recommended that they be reevaluated to

determine if some are malignant lymphomas. He criticized the report for not sufficiently noting the lymphoid hyperplasias in the treated male mice. A Program pathologist said the slides of the lymphoid hyperplasias will be reevaluated in view of the secondary reviewer's comments. Several members agreed that it was more likely that the lymphoid hyperplasias were over-diagnosed rather than under-diagnosed. It was noted that abnormal lymph nodes are commonly found among older animals. A staff pathologist pointed out that the mice had amyloidosis, which may have contributed to the lymphoid hyperplasia. It was agreed that the Subgroup would be notified if the results of the reevaluation differed from the diagnoses given in the report.

The primary reviewer moved that the report on the bioassay of Trichlorofluoromethane be accepted as written. The motion was seconded. A Subgroup member offered an amendment, which was accepted, that the report be represented to the Subgroup if the conclusion is changed based on the reevaluation of the lymphoid hyperplasia. A vote on the amended motion was 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 260-899/3169 1-3

DHEW Publication No. (NIH) 78-1356