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

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REPORT ON THE BIOASSAY OF TRIFLURALIN
FOR POSSIBLE CARCINOGENICITY

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

CONTRIBUTORS: This report presents the results of the bioassay of trifluralin conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This bioassay 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 Bioassay 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); the technical supervisor of animal treatment and observation was Ms. K. J. Petrovics (3).

Histopathologic examinations were performed by Dr. R. H. Habermann (3) and reviewed by Dr. R. W. Voelker (3) at the Hazleton Laboratories America, Inc., and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Dr. J. R. Joiner (6) and Mr. W. W. Belew (8), 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 (8) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (8), the task leader, Dr. M. R. Kornreich (8), and the senior biologist, Ms. P. Walker (8). The final report was reviewed by members of the participating organizations.

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SUMMARY

A bioassay for possible carcinogenicity of technical-grade trifluralin was conducted using Osborne-Mendel rats and B6C3F1 mice. Analysis of the technical product established the presence of 84 to 88 ppm dipropylnitrosoamine. The product was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Fifty animals of each sex were placed on test as controls for the rat bioassay, while 20 of each sex were utilized as controls for the mouse study. The time-weighted average high and low dietary concentrations of trifluralin were, respectively, 8000 and 4125 ppm for male rats, 7917 and 4125 ppm for female rats, 3744 and 2000 ppm for male mice, and 5192 and 2740 ppm for female mice. After a 78-week treatment period, there was an additional observation period of 33 weeks for rats and 12 weeks for mice.

For female mice the association between increased dosage and elevated incidence of hepatocellular carcinomas was significant (0/20, 12/47, and 21/44 of the control, low dose, and high dose, respectively) as was the relationship between dose and incidence of alveolar/bronchiolar adenomas. Significance of incidence for both types of tumors was supported by tests for significance at each dose level. Squamous-cell carcinomas of the stomach were observed in dosed female mice, but not in controls. Although incidences of these tumors were not statistically significant, they are unusual lesions in B6C3F1 mice and are considered to be treatment-related.

Neoplasms observed in treated rats were types that have occurred spontaneously in this strain and were apparently unrelated to trifluralin treatment.

Evaluation of the results of this bioassay indicates that technical-grade trifluralin is a carcinogen in female B6C3F1 mice, being associated in these animals with an elevated incidence of hepatocellular carcinomas, alveolar/bronchiolar adenomas and squamous-cell carcinomas of the forestomach. Sufficient evidence was not provided for the carcinogenicity or tumorigenicity of trifluralin in male B6C3F1 mice or in Osborne-Mendel rats of either sex.

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I. INTRODUCTION

Trifluralin (NCI No. C00442), a tertiary aromatic amine and dinitrotoluene derivative, is one of several widely used agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of adequate chronic toxicity data.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-benzenamine.* It may also be classified as a toluidine derivative (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) or as an aniline derivative (N,N-dipropyl-4-trifluoromethyl-2,6-dinitroaniline) and is commonly known as Treflan.

Introduced in 1960 as a selective preemergence herbicide (Spencer, 1973), trifluralin is presently used for weed control on a variety of field crops, fruits, vegetables and ornamentals. In 1971, the most recent year for which data are available, approximately 16.6 million acres of agricultural land were treated with 11.4 million pounds of the herbicide, with control of grasses in soybean and cotton fields accounting for 92 percent of total consumption (Andrilenas, 1974). Trifluralin was the single herbicide most frequently used on cotton in 1971 (Andrilenas, 1974) and was listed among those products identified by distributors as increasing most rapidly in use between 1971 and 1975 (Andrilenas and Eichers, 1976).

*The CAS registry number is 1582-09-8.

Recent production statistics for trifluralin are considered proprietary and are, therefore, not available; however, U.S. production in 1971 was estimated at 25 million pounds (Frost & Sullivan, Inc., 1977).

The risk of exposure to trifluralin is greatest for agricultural workers engaged in pesticide application; although workers at pesticide formulating plants may also experience significant contact with the herbicide. Trifluralin is generally considered to be three to four times more effective when incorporated into the soil (where it may persist for from 3 to 6 months) (Frost & Sullivan, Inc., 1977) than when applied as a surface spray, partially as a result of its relatively high vapor pressure (2.42×10^{-4} Torr at 30°C) and accompanying propensity to volatilize from the soil (Sonderquist et al., 1975). The presence of trifluralin in the atmosphere over treated fields may result in inhalational exposure of agricultural workers involved in planting and other nonpesticide-related activities. In addition, atmospheric transport of the chemical may enhance the probability of exposure of nonagricultural personnel residing in surrounding areas. The extent of this type of exposure may, however, be somewhat modified by the fact that trifluralin undergoes photodecomposition in the soil. Several photoproducts, as well as trifluralin itself, have been detected in the air above fields treated by either soil-incorporation or surface application (Sonderquist et al., 1975). Furthermore, vapor phase photolysis of trifluralin also

occurs (Sonderquist et al., 1975) and may represent one pathway for the removal of this contaminant from the atmosphere.

Although trifluralin is usually classified as nontoxic in mammals (Farm Chemicals Handbook, 1976), further investigations concerning the possibility of trifluralin-induced chromosomal aberrations and damage to the mitotic apparatus appear warranted from certain reports in the literature (Yoder et al., 1973; Hess and Bayer, 1974). Trifluralin has been found to disrupt the mitotic process in lateral root meristem cells of cotton (Hess and Bayer, 1974). The pesticide apparently attacks the spindle apparatus of dividing cells causing a reduction in the number of microtubules, arresting the mitotic process and, following nuclear envelope reformation, yielding cells that are polyploid, polymorphnucleate, binucleate or multinucleate.

Trifluralin was examined in a variety of microbial test systems designed to detect mutagenic activity. Trifluralin failed to induce reversion in eight histidine-requiring strains of Salmonella typhimurium (The Ames Test) and, in addition, failed to induce rII mutations in coliphage T_4 or to revert rII mutants to T_4 phenotype (Andersen et al., 1972).

Lymphocyte cultures from agricultural workers engaged in application of 14 herbicides, including trifluralin, were examined for chromosomal lesions during the peak spraying season and again in the wintertime (Yoder et al., 1973). These workers exhibited a fourfold increase in the mean number of chromatid gaps and a twenty-fivefold

increase in the mean number of breaks per person per 25 cells examined. No appreciable difference in the frequency of gaps or breaks at either sampling period was observed among nonexposed controls.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade trifluralin was purchased from Eli Lilly and Company and analyzed by Hazleton Laboratories America, Inc., Vienna, Virginia. The six-degree spread of the melting point (43° to 49°C) determined by the laboratory contrasted with the one-degree value reported by the manufacturer and by the FDA, suggesting the presence of impurities. Gas-liquid chromatographs, showing 13 minor peaks, confirmed the presence of contaminants, albeit of minimal concentration. Gas-liquid chromatography (GLC) indicated a purity greater than 90 percent. Analysis performed a year later showed no change in the melting point. Results of GLC analysis were comparable to those of the previous year and suggested no significant decomposition. Three years after completion of the bioassay, a sample of the compound was sent to the FDA for analysis of nitrosamines. Dipropylnitrosamine was shown to be present as a contaminant at a concentration of 84 to 88 ppm.

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

B. Dietary Preparation

The basal laboratory diet for both control and treated animals consisted of Wayne Lab-Blox® (Allied Mills, Inc.) plus 2 percent Duke's® corn oil (S. F. Sauer Company) by weight. Fresh mixtures of trifluralin in corn oil were prepared each week and stored in the

dark. The trifluralin mixtures were incorporated into the appropriate amount of laboratory diet in a twin-shell blender fitted with an accelerator bar.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. The Osborne-Mendel rat was selected on the basis of a comparative study of the tumorigenic responsiveness to carbon tetrachloride of five different strains of rats (Reuber and Glover, 1970). The B6C3F1 mouse was selected because it has been used by the NCI for carcinogenesis bioassays and has proven satisfactory in this capacity.

Rats and mice of both sexes were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. The Osborne-Mendel rats were procured from 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 treatment and control groups.

D. Animal Maintenance

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

lighting was provided on a 12-hour-daily cycle. The rats were individually housed in suspended galvanized-steel wire-mesh cages with perforated floors, while mice were housed by sex in groups of ten in solid-bottom polypropylene cages equipped with filter tops. Sanitized cages with fresh bedding (Sanichips®, Shurfire) 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, while fresh heat-sterilized glass water bottles were provided three times a week. Food and water were available ad libitum.

The trifluralin-treated and control rats were housed in the same room with other rats receiving diets treated with* dioxathion (78-34-2), dicofol (115-32-2), nitrofen (1836-75-5), endosulfan (115-29-7), and mexacarbate (315-18-4). All mice, including controls, used in the trifluralin study were housed in the same room as other mice receiving diets treated with chlorobenzilate (510-15-6), dioxathion (78-34-2), sulfallate (95-06-7), p,p'-DDT (50-29-3), methoxychlor (72-43-5), p,p'-DDE (72-55-9), p,p'-TDE (72-54-8), dicofol (115-32-2), pentachloronitrobenzene (82-68-8), clonitralid (1420-04-8), nitrofen (1836-75-5), endosulfan (115-29-7), mexacarbate (315-18-4), amitrole (61-82-5), acetylaminofluorene (53-96-3), and safrole (94-59-7).

*CAS registry numbers are given in parentheses.

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of trifluralin 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. Trifluralin was premixed with a small amount of corn oil. This mixture was then incorporated into the laboratory diet and fed ad libitum to five of the six rat and five of the six mouse groups in concentrations of 3560, 6320, 11,240, 20,000 and 35,600 ppm. The sixth group of each species served as a control group, receiving only the mixture of corn oil and laboratory chow. The dosed dietary preparations were administered for a period of 6 weeks, followed by a 2-week observation period during which all animals were fed the basal diet.

A concentration inducing no mortality and resulting in a retardation in body weight gain of approximately 20 percent was to be selected as the initial high concentration for the chronic study. When weight gain criteria were not applicable, mortality data alone were utilized.

Mean body weight gain retardation for rats began at 6320 ppm for males, and at 11,240 ppm for females. The initial high concentration selected for male and female rats in the chronic bioassay was 13,000 ppm. For mice, mean body weight gain retardation began at 3560 ppm for both males and females. One male mouse from each group receiving

3560 and 6320 ppm died. The initial high concentrations selected for the chronic bioassay were 4000 ppm for male mice and 9000 ppm for female mice.

F. Experimental Design

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

The high dose, low dose, and control rats were all approximately 6 weeks old at the time they were placed on test. The high and low concentrations of trifluralin initially utilized for both male and female rats were 13,000 and 6500 ppm, respectively. In week 22 of the study, the high and low concentrations administered to the rats were decreased to 6500 and 3250 ppm, respectively, as the initial concentrations utilized induced signs of toxicity in the treated animals. In week 63 of the study, administration of trifluralin to the high dose female rats ceased for 1 week and was then followed by 4 weeks of feeding at the previous concentration of 6500 ppm. For the high dose male rats the same cyclic pattern of dose administration began in week 68. These cyclic feeding patterns were maintained for the remainder of the treatment period.

The control, high dose, and low dose mice were all approximately 6 weeks old on the day they were started on test. The high and low concentrations administered to male mice were 4000 and 2000 ppm,

TABLE 1

DESIGN SUMMARY FOR OSBORNE-MENDEL RATS
TRIFLURALIN FEEDING EXPERIMENT

	INITIAL GROUP SIZE	TRIFLURALIN CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[b]
MALE					
CONTROL	50	0		111	0
LOW DOSE	50	6,500	21		4,125
		3,250	57		
		0		33	
HIGH DOSE	50	13,000	21		8,000
		6,500	46		
		6,500[c]	8	3	
		0		33	
FEMALE					
CONTROL	50	0		111	0
LOW DOSE	50	6,500	21		4,125
		3,250	57		
		0		33	
HIGH DOSE	50	13,000	21		
		6,500	41		
		6,500[c]	12	4	7,917
		0		33	

[a] Concentrations given in parts per million.

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

[c] These concentrations were cyclically administered with a pattern of 1 dosage-free week followed by 4 weeks of dosage at the level indicated.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
TRIFLURALIN FEEDING EXPERIMENT

	INITIAL GROUP SIZE	TRIFLURALIN CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[b]
MALE					
CONTROL	20	0		90	0
LOW DOSE	50	2000	78		2000
		0		12	
HIGH DOSE	50	4000	56		3744
		4000[c]	17	5	
		0		12	
FEMALE					
CONTROL	20	0		90	0
LOW DOSE	50	4500	17		2740
		2250	61		
		0		12	
HIGH DOSE	50	9000	17		5192
		4500	39		
		4500[c]	17	5	
		0		12	

[a] Concentrations given in parts per million.

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

[c] These concentrations were cyclically administered with a pattern of 1 dosage-free week followed by 4 weeks of dosage at the level indicated.

respectively. Female mice initially received high and low concentrations of 9000 and 4500 ppm, respectively. In week 18 of the study, the high and low concentrations administered to the female mice were decreased to 4500 and 2250 ppm, respectively, as signs of toxicity had been observed at the initial dosage levels. In week 57 of the study, dietary administration of the compound to the high dose males and females ceased for 1 week and was followed by 4 weeks of dietary administration at the previous concentrations. This pattern of cyclic administration continued for the remainder of the treatment period.

The control animals were maintained and observed in the same manner as the treated animals.

G. Clinical and Histopathologic Examinations

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

During the course of this bioassay several pathology protocols were in effect, each for different periods of time. The majority of the necropsies were conducted using a protocol which specified that,

if possible, certain tissues were to be taken and examined histopathologically from at least 10 grossly normal males and 10 grossly normal females from each treated group, from 10 male and 10 female control mice, and from all control rats. These same tissues were to be taken and examined histopatologically from all tumor-bearing animals. In addition, an attempt was made to microscopically examine the liver, lung, and stomach from all mice and the liver from all rats. Subsequently, the remaining tissues and organs from all control mice were examined. Therefore, all tissues from all animals placed on test were not examined histopathologically and this is reflected in Appendices A through D.

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

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice) and bile duct, pancreas, esophagus, stomach, small intestine, large intestine, rectum, kidney, urinary bladder, pituitary, adrenal,

thyroid, parathyroid, pancreatic islets, testis, prostate, seminal vesicle, brain, muscle, nerves, uterus, mammary gland, and ovary.

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

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and used Tarone's (1975) extensions of Cox's methods for testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

lymphomas), the denominators consist of the numbers of animals necropsied.

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

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

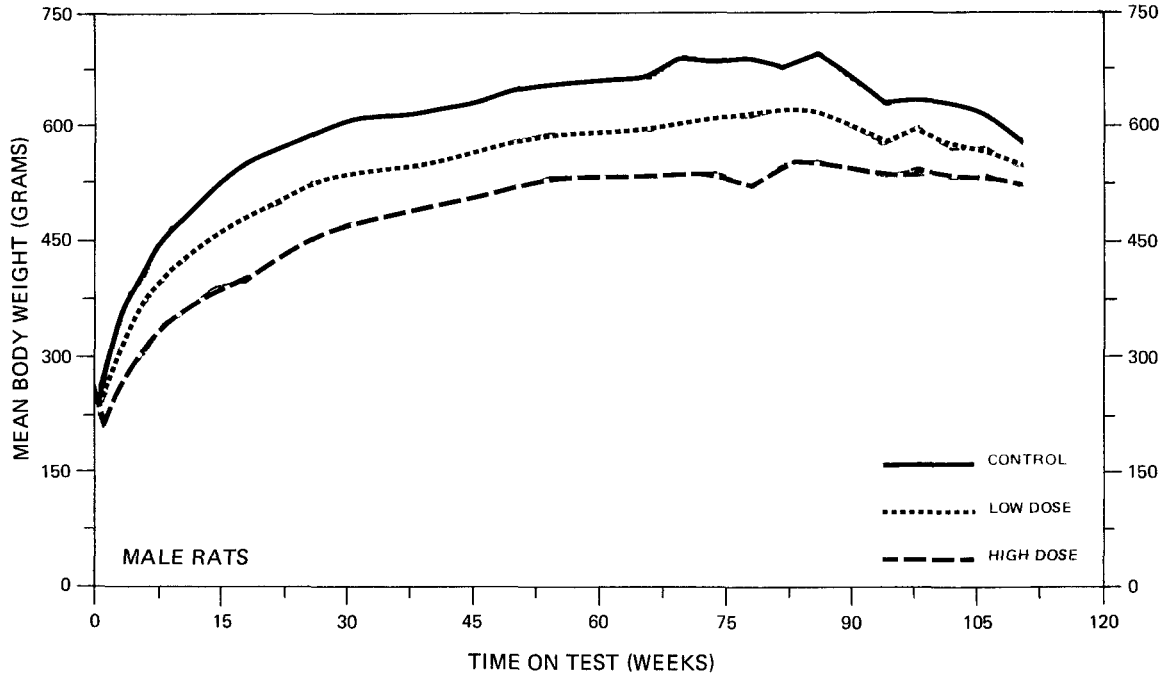
III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Distinct, dose-related body weight gain retardation was evident throughout the bioassay in both male and female treated rats (Figure 1).

Beginning in week 1, a hunched appearance and urine staining of the abdominal area were observed in trifluralin-treated female rats. Throughout the study, these symptoms were observed with greater frequency in the high dose females than in the remaining groups. As the bioassay progressed, increasing numbers of treated female, and to a lesser extent, treated male rats, showed a hunched appearance and abdominal urine stains. After week 70, these symptoms were observed in control rats with a frequency similar to that of low dose females and males at both dose levels.

Respiratory signs characterized by labored respiration, wheezing, and/or nasal discharge were observed at a low incidence in all groups, including the controls, during the first 102 weeks; however, the frequency with which these signs were observed increased gradually during the final weeks of observation. Other signs associated with aging in the laboratory rat were noted in comparable numbers of treated and control rats during the second year. These signs included: alopecia; sores on the body or extremities; rough fur; reddish discharge or crust around the eyes or nose; squinted, cloudy, protruding or lacrimating eyes; and palpable nodules, swollen areas, and/or tissue



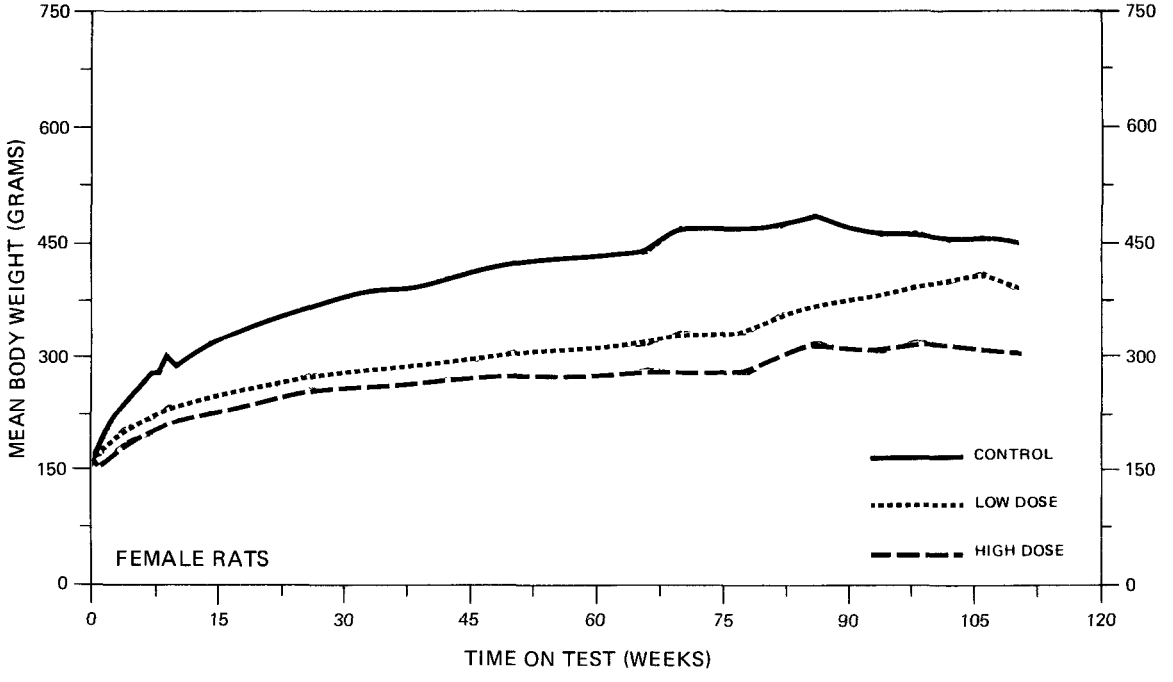


FIGURE 1
GROWTH CURVES FOR TRIFLURALIN CHRONIC STUDY RATS

masses. Observations noted sporadically in a few animals included penile irritation or anal prolapse, vaginal discharge, apparent hind-limb paralysis, circling or head tilt, and small-appearing testes.

B. Survival

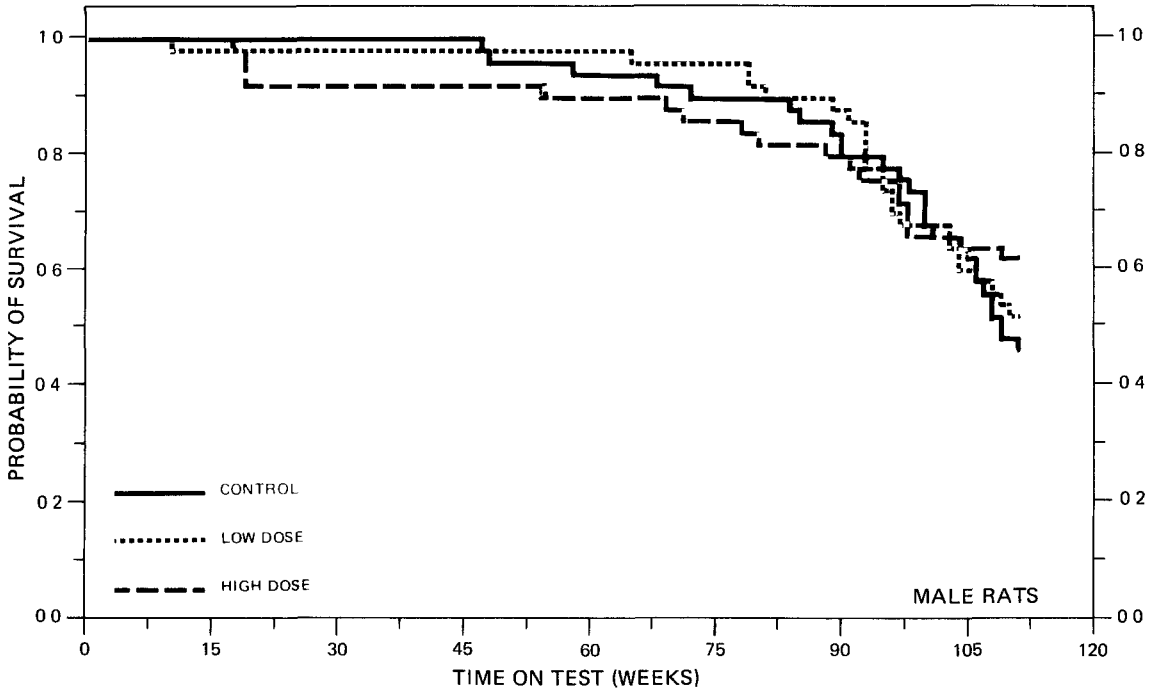
The estimated probabilities of survival for male and female rats in the control and trifluralin-treated groups are shown in Figure 2. For both males and females the Tarone test did not indicate a statistically significant association between increased dosage and elevated mortality.

For males the actual survival was adequate as 62 percent of the high dose, 52 percent of the low dose, and 46 percent of the control rats survived until the end of the study. For females the actual survival was also adequate as 62 percent of the high dose, 76 percent of the low dose, and 70 percent of the control rats survived until the end of the study.

C. Pathology

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

A variety of neoplasms were present in both the chemically treated and control rats. Each of the tumor types represented has been encountered previously as a spontaneous lesion in the Osborne-Mendel rat and was apparently unrelated to the administration of trifluralin.



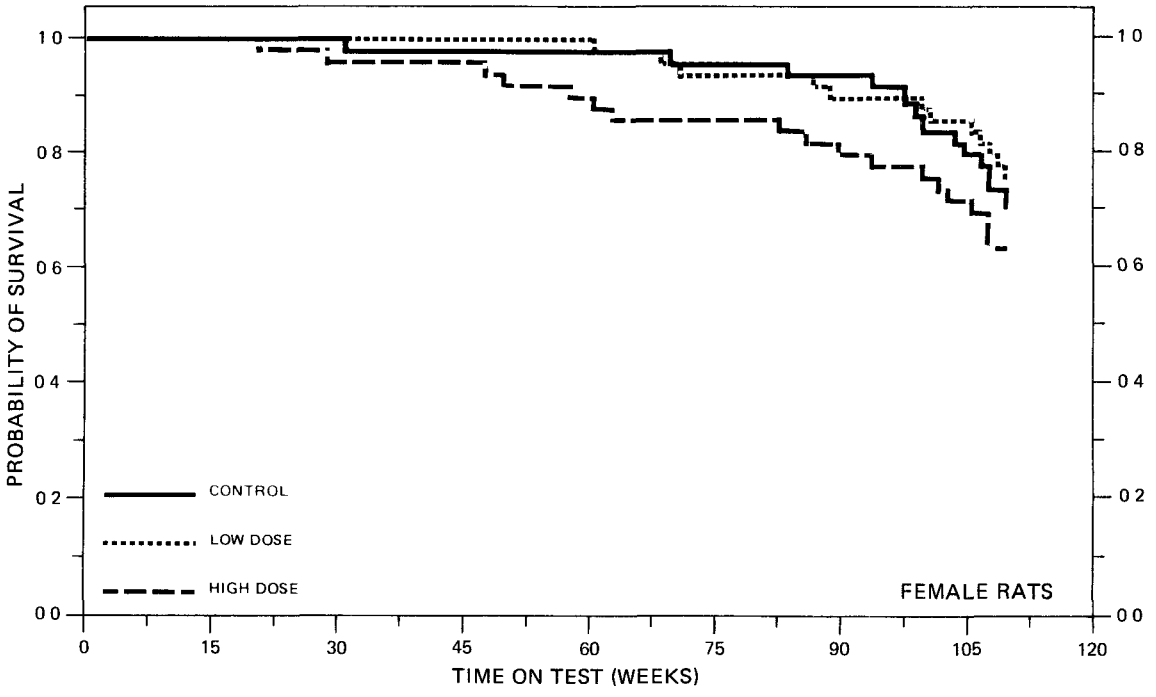


FIGURE 2
SURVIVAL COMPARISONS OF TRIFLURALIN CHRONIC STUDY RATS

Inflammatory, degenerative, and proliferative lesions as seen in control and treated animals were similar in number and kind to those naturally occurring lesions found in aged Osborne-Mendel rats.

In this study trifluralin was not toxic or carcinogenic in Osborne-Mendel rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis for every type of tumor that was observed in more than 5 percent of any of the trifluralin-dosed groups of either sex is included.

In female rats, for the comparison of low dose to control, the Fisher exact test showed a probability level of $P = 0.028$ for the combined incidence of follicular-cell adenomas and carcinomas of the thyroid, a marginal result which was not considered significant under the Bonferroni criteria. Similarly, the incidence of hemangiosarcomas in the low dose females was not considered significant.

No other statistical tests for rats of either sex indicated a significant positive association between the administration of trifluralin and increased tumor incidence. Thus, at the dose levels used in this experiment there was inadequate evidence to demonstrate that trifluralin was a carcinogen in Osborne-Mendel rats.

To provide additional insight, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables

TABLE 3

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Malignant Lymphoma[b]	1/49(0.02)	4/49(0.08)	0/50(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.024	---	---
Relative Risk (Control)[e]	---	4.000	0.000
Lower Limit	---	0.413	0.000
Upper Limit	---	192.765	18.285
Weeks to First Observed Tumor	107	79	---
Kidney: Tubular-Cell Adenoma[b]	0/47(0.00)	0/11(0.00)	1/12(0.08)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	---	Infinite
Lower Limit	---	---	0.210
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	111
Urinary Bladder: Papilloma,NOS[b]	3/46(0.07)	1/13(0.08)	1/11(0.09)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.179	1.394
Lower Limit	---	0.023	0.027
Upper Limit	---	12.738	14.641
Weeks to First Observed Tumor	100	103	111

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma[b]	0/46(0.00)	1/11(0.09)	0/10(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.031	---	---
Relative Risk (Control)[e]	---	Infinite	---
Lower Limit	---	0.224	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	111	---
Pancreatic Islets: Islet-Cell Adenoma[b]	1/46(0.02)	1/11(0.09)	0/12(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	4.182	0.000
Lower Limit	---	0.055	0.000
Upper Limit	---	296.317	67.175
Weeks to First Observed Tumor	111	111	---
Pituitary: Chromophobe Adenoma[b]	4/41(0.10)	3/11(0.27)	0/11(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.039	---	---
Relative Risk (Control)[e]	---	2.795	0.000
Lower Limit	---	0.451	0.000
Upper Limit	---	12.949	3.652
Weeks to First Observed Tumor	108	65	---

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Adenoma[b]	3/48(0.06)	4/49(0.08)	5/48(0.10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.306	1.667
Lower Limit	---	0.235	0.346
Upper Limit	---	8.495	10.203
Weeks to First Observed Tumor	111	95	111
Thyroid: Follicular-Cell Carcinoma[b]	4/48(0.08)	4/49(0.08)	5/48(0.10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.980	1.250
Lower Limit	---	0.192	0.285
Upper Limit	---	4.972	5.939
Weeks to First Observed Tumor	106	111	80
Thyroid: Follicular-Cell Adenoma or Carcinoma[b]	5/48(0.10)	8/49(0.16)	10/48(0.20)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.567	1.960
Lower Limit	---	0.489	0.677
Upper Limit	---	5.678	6.920
Weeks to First Observed Tumor	106	95	80

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Spleen: Hemangiosarcoma[b]	4/47(0.09)	3/18(0.17)	0/12(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[e]	---	1.958	0.000
Lower Limit	---	0.307	0.000
Upper Limit	---	10.117	3.880
Weeks to First Observed Tumor	90	95	---
Subcutaneous Tissue: Hemangiosarcoma[b]	4/49(0.08)	4/49(0.08)	1/50(0.02)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[e]	---	1.000	0.245
Lower Limit	---	0.198	0.005
Upper Limit	---	5.077	2.362
Weeks to First Observed Tumor	72	65	109

[a]Treated groups received time-weighted average doses of 4125 and 8000 ppm in feed.

[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 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 control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in a control group.

[e]The 95% confidence interval of the relative risk between a treated group and the control group.

[f]The probability level for departure from linear trend is given when $P < 0.05$ for a comparison.

TABLE 4

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	2/50(0.04)	4/50(0.08)	0/49(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	2.000	0.000
Lower Limit	---	0.301	0.000
Upper Limit	---	21.316	3.448
Weeks to First Observed Tumor	109	108	---
Kidney: Transitional-Cell Carcinoma[b]	0/50(0.00)	0/12(0.00)	1/10(0.10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	---	Infinite
Lower Limit	---	---	0.265
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	111
Kidney: Mixed Tumor, Malignant[b]	1/50(0.02)	1/12(0.08)	0/10(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	4.167	0.000
Lower Limit	---	0.055	0.000
Upper Limit	---	298.098	86.113
Weeks to First Observed Tumor	111	109	---

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Urinary Bladder: Papilloma, NOS[b]	0/49(0.00)	1/10(0.10)	0/12(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.017	---	---
Relative Risk (Control)[e]	---	Infinite	---
Lower Limit	---	0.262	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	111	---
Pituitary: Chromophobe Adenoma[b]	15/50(0.30)	5/12(0.42)	4/12(0.33)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.389	1.111
Lower Limit	---	0.463	0.307
Upper Limit	---	2.937	2.629
Weeks to First Observed Tumor	100	111	100
Thyroid: Follicular-Cell Adenoma[b]	0/50(0.00)	3/50(0.06)	0/49(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.014	---	---
Relative Risk (Control)[e]	---	Infinite	---
Lower Limit	---	0.428	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	111	---

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma[b]	1/50(0.02)	4/50(0.08)	0/49(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.025	---	---
Relative Risk (Control)[e]	---	4.000	0.000
Lower Limit	---	0.412	0.000
Upper Limit	---	192.807	19.032
Weeks to First Observed Tumor	111	111	---
Thyroid: Follicular-Cell Adenoma or Carcinoma[b]	1/50(0.02)	7/50(0.14)	0/49(0.00)
P Values[c,d]	N.S.	P = 0.028	N.S.
Departure from Linear Trend[f]	P = 0.001	---	---
Relative Risk (Control)[e]	---	7.000	0.000
Lower Limit	---	0.953	0.000
Upper Limit	---	308.322	19.032
Weeks to First Observed Tumor	111	111	---
Thyroid: C-Cell Adenoma or Carcinoma[b]	5/50(0.10)	4/50(0.08)	2/49(0.04)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.800	0.408
Lower Limit	---	0.169	0.040
Upper Limit	---	3.499	2.359
Weeks to First Observed Tumor	111	111	111

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Adenocarcinoma, NOS[b]	0/50(0.00)	3/50(0.06)	3/49(0.06)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	Infinite	Infinite
Lower Limit	---	0.428	0.436
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	111	111
Mammary Gland: Fibroadenoma[b]	15/50(0.30)	22/50(0.44)	12/49(0.24)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.039	---	---
Relative Risk (Control)[e]	---	1.467	0.816
Lower Limit	---	0.831	0.390
Upper Limit	---	2.644	1.668
Weeks to First Observed Tumor	87	71	100
Uterus: Endometrial Stromal Polyp[b]	4/49(0.08)	4/19(0.04)	2/16(0.13)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	2.579	1.531
Lower Limit	---	0.525	0.146
Upper Limit	---	12.079	9.297
Weeks to First Observed Tumor	111	106	111

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Adenocarcinoma,NOS[b]	0/49(0.00)	0/19(0.00)	1/16(0.06)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	---	Infinite
Lower Limit	---	---	0.163
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	111
Ovary: Luteoma[b]	0/49(0.00)	1/11(0.09)	0/11(0.00)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.025	---	---
Relative Risk (Control)[e]	---	Infinite	---
Lower Limit	---	0.238	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	111	---
Subcutaneous Tissue: Hemangiosarcoma[b]	3/50(0.06)	0/50(0.00)	1/40(0.02)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.000	0.340
Lower Limit	---	0.000	0.007
Upper Limit	---	1.663	4.062
Weeks to First Observed Tumor	89	---	111

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Spleen: Hemangiosarcoma[b]	0/50(0.00)	2/12(0.17)	0/11(0.00)
P Values[c,d]	N.S.	P = 0.035	N.S.
Departure from Linear Trend[f]	P = 0.003	---	---
Relative Risk (Control)[e]	---	Infinite	---
Lower Limit	---	1.246	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	107	---

[a]Treated groups received time-weighted average doses of 4125 and 7917 ppm in feed.

[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 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 control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in a control group.

[e]The 95% confidence interval on the relative risk between a treated group and the control group.

[f]The probability level for departure from linear trend is given when $P < 0.05$ for a comparison.

based upon the observed tumor incidence rates. In many 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 many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of a significantly increased rate of tumor incidence induced in rats by trifluralin that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

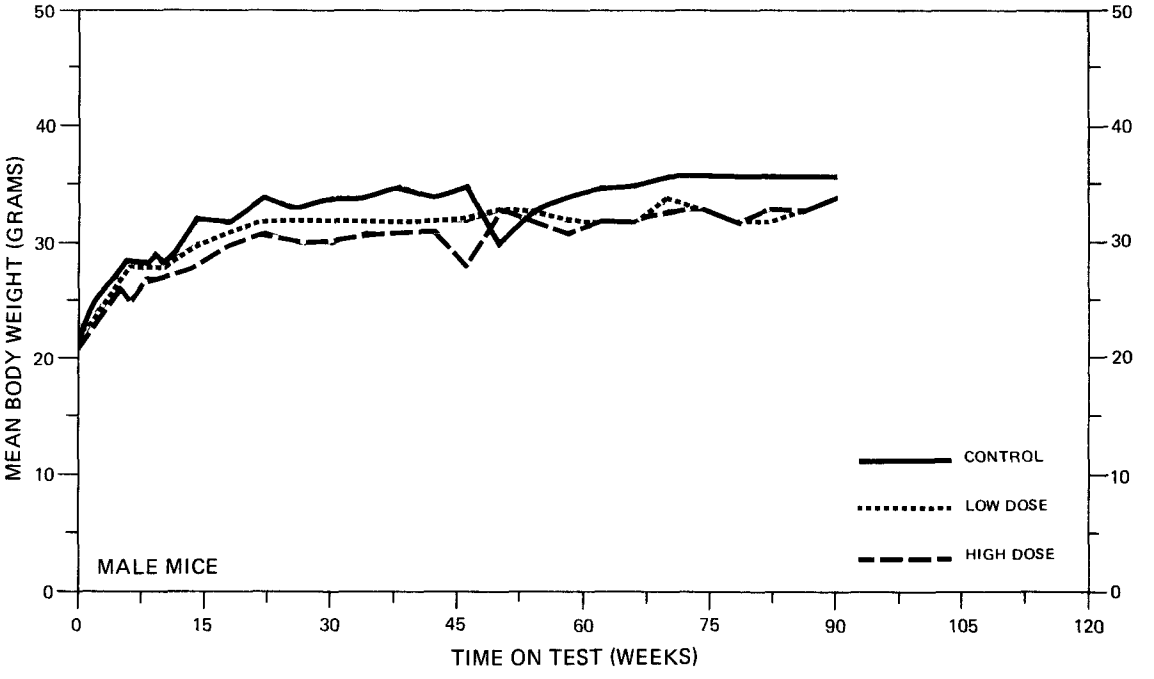
Distinct, dose-related weight gain retardation was evident in female mice throughout the bioassay. General weight gain depression did occur among the treated males, although it was not obviously dose-related (Figure 3).

Appearance and behavior for the treated and untreated mice were generally comparable throughout the test. Clinical observations associated with group-housing and aging were observed in comparable numbers of treated and control animals during the study. These included: sores on parts of the body; localized or generalized alopecia; penile, anal, or vulvar irritation with occasional prolapse or discharge; hunched appearance; rough or stained fur; abdominal distension or bloating; and palpable or subcutaneous nodules and/or tissue masses. Several treated and control male mice showed subcutaneous abscesses (probably traumatic injury from fighting), some of which evidently drained and healed to form fibrotic nodules.

B. Survival

The estimated probabilities of survival for male and female mice in the control and trifluralin-treated groups are shown in Figure 4.

For male mice the Tarone test did not indicate a significant association between increased dosage and elevated mortality. Nonetheless, the actual survival in the high dose group was lower than in the other groups: 52 percent of the low dose and 55 percent of the



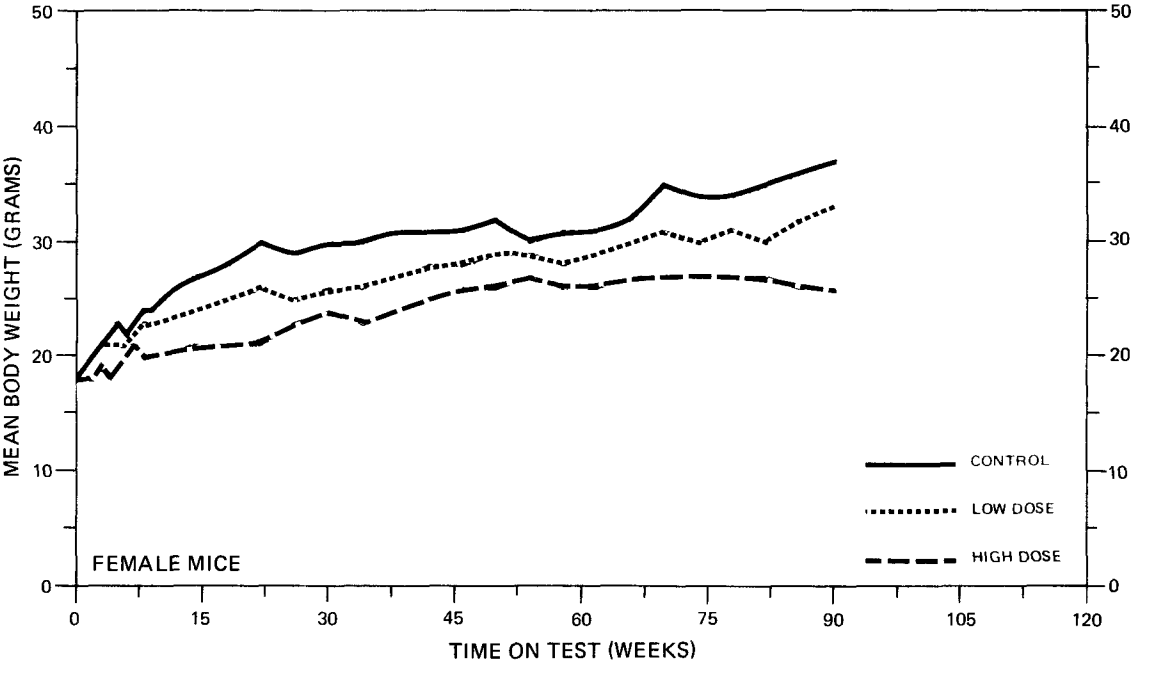
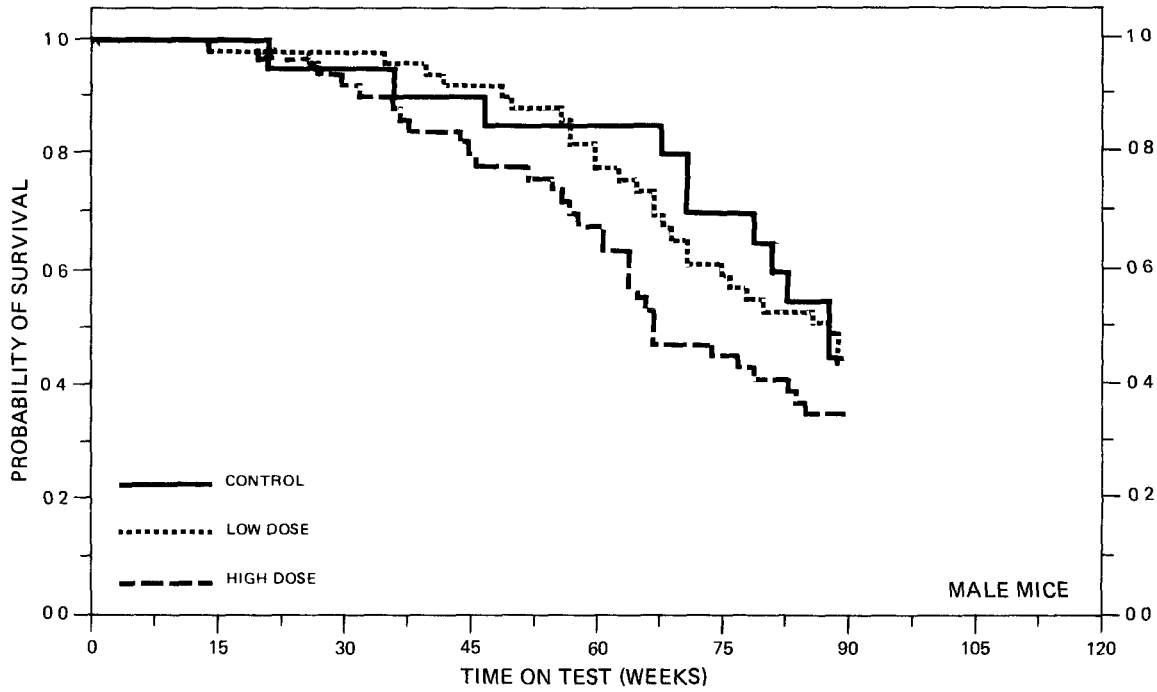


FIGURE 3
GROWTH CURVES FOR TRIFLURALIN CHRONIC STUDY MICE



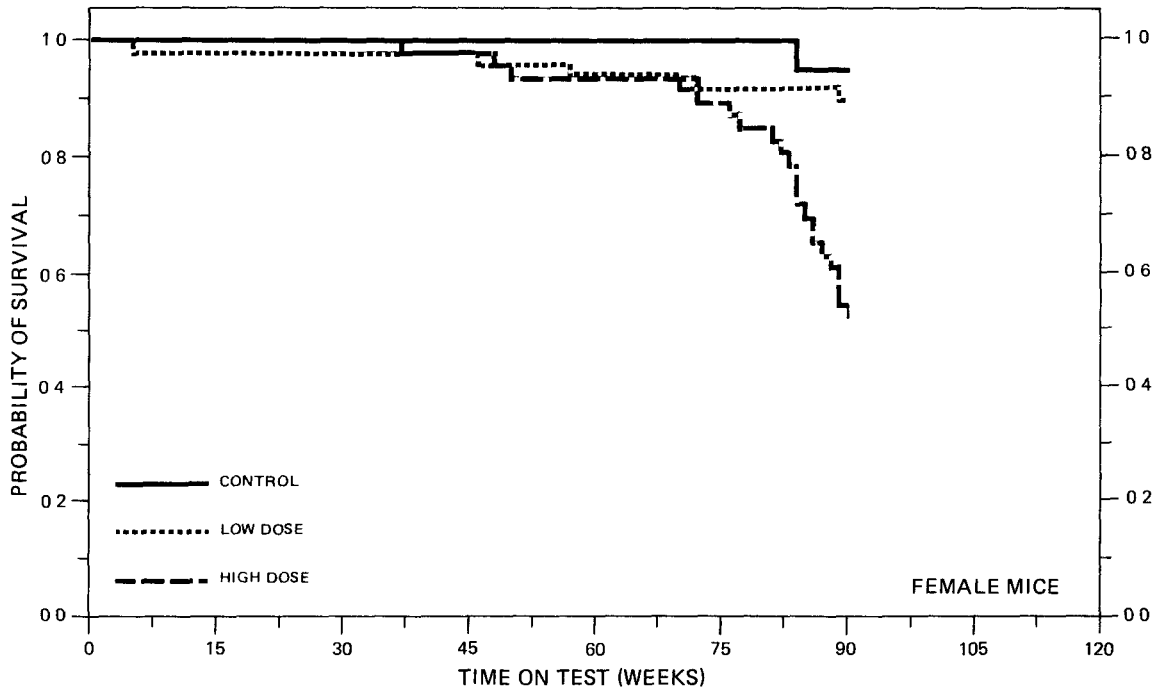


FIGURE 4
SURVIVAL COMPARISONS OF TRIFLURALIN CHRONIC STUDY MICE

control survived at least 86 weeks compared to only 34 percent of the high dose group.

For female mice the Tarone test indicated a significant ($P < 0.001$) dose-mortality association. The actual survival, however, was adequate for meaningful statistical analysis, as 62 percent of the high dose, 92 percent of the low dose, and 95 percent of the control group survived at least 85 weeks.

C. Pathology

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

Hepatocellular carcinomas occurred in 4/19 (21 percent) male controls, 12/47 (26 percent) low dose males, 9/49 (18 percent) high dose males, 0/20 female controls, 12/47 (26 percent) low dose females, and 21/44 (48 percent) high dose females. Hepatocellular adenomas occurred in 2/47 low dose males and 3/47 low dose females. The hepatocellular carcinomas varied greatly in appearance. Some lesions contained well-differentiated hepatic cells that had a relatively uniform arrangement of the cords, and others had very anaplastic liver cells with large hyperchromatic nuclei, often with inclusion bodies and with vacuolated, pale cytoplasm. Arrangement of the neoplastic liver cells varied from short, stubby cords to nests of hepatic cells and occasionally acinar formation. Mitotic figures were often present. Some of the tumors were characterized by discrete

areas of highly anaplastic cells. The hepatic neoplasms observed in the control mice were not different in appearance from those noted in the treated mice.

Squamous-cell carcinomas of the stomach occurred in 4/45 low dose females and 1/44 high dose females. This neoplasm did not occur in male or female control mice or in the low or high dose male mice.

Microscopically the squamous-cell carcinomas of the stomach were characterized by acanthosis of the surface epithelium along with increased keratin. At the base of the epithelial layer, there were papillary cords and nests of anaplastic squamous epithelial cells supported by dense bands of fibrous connective tissue. The neoplasms invaded and replaced the lamina propria, muscularis mucosa, and muscular layers. In one mouse there was metastasis of the squamous-cell carcinoma to the adjacent organs.

A squamous-cell papilloma occurred in 1/47 low dose males. The papilloma consisted of a finger-like stalk of fibrous connective tissue, which was covered by squamous epithelial cells and projected into the lumen of the stomach.

Other neoplasms that occurred in the mice were similar in kind and number to those seen in aged untreated B6C3F1 mice.

Acanthosis and hyperkeratosis occurred in the forestomach of 6/45 low dose females, 1/47 high dose males and 1/44 high dose females.

Other inflammatory, degenerative, and proliferative lesions occurred without appreciable difference in frequency in the control

and treated animals and were the usual lesions found in aged mice of this strain.

Findings of this histopathologic examination provide evidence for carcinogenicity of trifluralin in female mice. Administration of trifluralin was associated with an increased incidence of hepatocellular neoplasms. It was also associated with squamous-cell carcinomas of the forestomach in these animals. Acanthosis and hyperkeratosis were present in stomach sections from seven treated female mice.

D. Statistical Analyses of Results

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

Two control groups were used for statistical analyses: the control group originally assigned to the trifluralin bioassay in the experimental design (designated in this section as the "matched" control group) and a pooled control group that combined the controls from the studies of trifluralin, pentachloronitrobenzone, and p,p'-TDE. The control mice used for the pool were of the same strain, were from the same supplier, were housed in the same room, were tested concurrently for more than 1 year, and were diagnosed by the same pathologists.

In female mice the Cochran-Armitage test indicated a significant ($P < 0.001$) association between increased dosage and an elevated

TABLE 5

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

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibrosarcoma[b]	8/58(0.14)	5/20(0.25)	7/47(0.15)	9/48(0.19)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[e]	---	---	1.080	1.359
Lower Limit	---	---	0.358	0.505
Upper Limit	---	---	3.145	3.725
Relative Risk (Matched Control)[e]	---	---	0.596	0.750
Lower Limit	---	---	0.192	0.268
Upper Limit	---	---	2.150	2.567
Weeks to First Observed Tumor	63	71	67	45
Lung: Alveolar/Bronchiolar Adenoma[b]	1/57(0.02)	0/19(0.00)	0/10(0.00)	1/10(0.10)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[e]	---	---	0.000	5.700
Lower Limit	---	---	0.000	0.075
Upper Limit	---	---	98.160	398.973
Relative Risk (Matched Control)[e]	---	---	---	Infinite
Lower Limit	---	---	---	0.104
Upper Limit	---	---	---	Infinite
Weeks to First Observed Tumor	90	---	---	90

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	8/57(0.14)	4/19(0.21)	12/47(0.26)	9/49(0.18)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[e]	---	---	1.819	1.309
Lower Limit	---	---	0.748	0.484
Upper Limit	---	---	4.677	3.589
Relative Risk (Matched Control)[e]	---	---	1.213	0.872
Lower Limit	---	---	0.437	0.288
Upper Limit	---	---	4.669	3.535
Weeks to First Observed Tumor	79	83	88	67
Liver: Hepatocellular Adenoma or Carcinoma[b]	8/57(0.14)	4/19(0.21)	14/47(0.30)	9/49(0.18)
P Values[c,d]	N.S.	N.S.	P = 0.043*	N.S.
Relative Risk (Pooled Control)[e]	---	---	2.122	1.309
Lower Limit	---	---	0.913	0.484
Upper Limit	---	---	5.296	3.589
Relative Risk (Matched Control)[e]	---	---	1.415	0.872
Lower Limit	---	---	0.532	0.288
Upper Limit	---	---	5.322	3.535
Weeks to First Observed Tumor	79	83	88	67

TABLE 5 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 2000 and 3744 ppm in feed.

[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 to the pooled control group (*) or to the matched control group (**) is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in the treated group(s) than in the control group.

[e]The 95% confidence interval on the relative risk between each treated group and the specified control group.

TABLE 6

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

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	0/59(0.00)	0/19(0.00)	6/43(0.14)	3/30(0.10)
P Values[c,d]	P = 0.026	N.S.	P = 0.005*	P = 0.036*
Relative Risk (Pooled Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	2.194	1.184
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	0.740	0.397
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	90	90
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/59(0.00)	0/19(0.00)	7/43(0.16)	3/30(0.10)
P Values[c,d]	P = 0.026	N.S.	P = 0.002*	P = 0.036*
Departure from Linear Trend[f]	P = 0.024	---	---	---
Relative Risk (Pooled Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	2.661	1.184
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	0.899	0.397
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	90	90

TABLE 6 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Lymphoma[b]	3/60(0.05)	1/20(0.05)	4/50(0.08)	5/44(0.11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[e]	---	---	1.600	2.273
Lower Limit	---	---	0.283	0.467
Upper Limit	---	---	10.441	13.894
Relative Risk (Matched Control)[e]	---	---	1.600	2.273
Lower Limit	---	---	0.175	0.284
Upper Limit	---	---	77.169	104.874
Weeks to First Observed Tumor	90	90	90	70
Liver: Hepatocellular Adenoma[b]	0/60(0.00)	0/20(0.00)	3/47(0.06)	0/44(0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[f]	P = 0.010	P = 0.047	---	---
Relative Risk (Pooled Control)[e]	---	---	Infinite	---
Lower Limit	---	---	0.767	---
Upper Limit	---	---	Infinite	---
Relative Risk (Matched Control)[e]	---	---	Infinite	---
Lower Limit	---	---	0.267	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	90	---

TABLE 6 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/60(0.00)	0/20(0.00)	12/47(0.26)	21/44(0.48)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001* P = 0.009**	P < 0.001* P < 0.001**
Relative Risk (Pooled Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	4.674	9.274
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	1.630	3.228
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	90	70
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/60(0.00)	0/20(0.00)	15/47(0.32)	21/44(0.48)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001* P = 0.002**	P < 0.001* P < 0.001**
Relative Risk (Pooled Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	5.980	9.274
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	2.092	3.228
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	90	70

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Stomach: Squamous-Cell Carcinoma[b]	0/60(0.00)	0/20(0.00)	4/45(0.09)	1/44(0.02)
P Values[c,d]	N.S.	N.S.	P = 0.031*	N.S.
Departure from Linear Trend[f]	P = 0.016	---	---	---
Relative Risk (Pooled Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	1.232	0.073
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[e]	---	---	Infinite	Infinite
Lower Limit	---	---	0.428	0.025
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	89	76

[a]Treated groups received time-weighted average doses of 2740 and 5192 ppm in feed.

[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 to the pooled control group (*) or to the matched control group (**) is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in the treated group(s) than in the control group.

[e]The 95% confidence interval on the relative risk between each treated group and the specified control group.

[f]The probability level for departure from linear trend is given when $P < 0.05$ for a comparison.

incidence of hepatocellular carcinoma whether comparing to the matched control or the pooled control. The Fisher exact tests were significant ($P < 0.01$) in comparisons of either of the dosed groups to either of the controls; for all comparisons the lower limit of the confidence interval on the relative risk was greater than the value one.

Among females alveolar/bronchiolar adenomas were observed only in the treated mice. The Cochran-Armitage test indicated a significant ($P = 0.026$) association between dosage and incidence when comparing to the pooled control. The Fisher exact test indicated that incidence in the low dose group was significantly ($P = 0.005$) higher than incidence among pooled controls; for this comparison the lower limit of the confidence interval on the relative risk was greater than the value one. For the high dose comparison the probability level was $P = 0.036$, a marginal result which was not significant under the Bonferroni criteria.

Based upon these results the statistical conclusion is that the administration of trifluralin was associated with an increased incidence of hepatocellular carcinomas and of alveolar/bronchiolar adenomas in female B6C3F1 mice under the conditions of this experiment.

In females the Fisher exact test comparing the incidence of squamous-cell carcinomas of the stomach in the low dose to that in the pooled control group had a significance level of $P = 0.031$, a

marginal result which was not significant under the Bonferroni criteria.

Similarly, in male mice the Fisher exact test comparing the incidence of hepatocellular carcinomas or adenomas in the low dose to that in the pooled control had a probability level of P = 0.043, a marginal result which was not significant under the principles of the Bonferroni inequality.

No statistical tests for other body sites in mice of either sex indicated a significant positive association between trifluralin administration and tumor incidence.

Because of the relatively poor survival observed in the high dose male mouse group, additional analyses were conducted. These calculations excluded all mice that died before 52 weeks except in the case where the tumor of interest was observed earlier than 52 weeks; for this case all mice were excluded that died before the earliest tumor of interest was observed. The results of these time-adjusted analyses are given in Table 7. None of the results were statistically significant.

To provide additional insight, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5, 6, and 7, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an

TABLE 7

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH TRIFLURALIN[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibrosarcoma[b,f]	5/17(0.29)	7/43(0.16)	9/40(0.23)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.553	0.765
Lower Limit	---	0.184	0.283
Upper Limit	---	1.969	2.561
Weeks to First Observed Tumor	71	67	45
Lung: Alveolar/Bronchiolar Adenoma[b]	0/16(0.00)	0/10(0.00)	1/10(0.10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	---	Infinite
Lower Limit	---	---	0.089
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	90
Liver: Hepatocellular Carcinoma[b]	4/16(0.25)	12/42(0.29)	9/38(0.24)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.143	0.947
Lower Limit	---	0.428	0.325
Upper Limit	---	4.323	3.742
Weeks to First Observed Tumor	83	88	67

TABLE 7 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Adenoma or Carcinoma[b]	4/16(0.25)	14/42(0.33)	9/38(0.24)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.333	0.947
Lower Limit	---	0.519	0.325
Upper Limit	---	4.922	3.742
Weeks to First Observed Tumor	83	88	67

[a]Treated groups received time-weighted average doses of 2000 and 3744 ppm in feed.

[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 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 control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in a control group.

[e]The 95% confidence interval on the relative risk between a treated group and the control group.

[f]If the first tumor of interest was observed earlier than 52 weeks, only those animals which died in the weeks before the tumor was observed were excluded for the analysis.

upper limit greater than one, indicating the theoretical possibility of a significantly increased rate of tumor incidence induced in mice by trifluralin that could not be established under the conditions of this test.

V. DISCUSSION

Under the conditions of this bioassay, dietary administration of trifluralin was associated with elevated incidences of hepatocellular carcinoma and of alveolar/bronchiolar adenoma in female mice. Survival was adequate among all groups except male mice; therefore, in addition to the standard statistical analyses, a time-adjusted analysis was performed for male mouse groups.

Neoplasms that developed in both the male and female treated rats were types that have occurred spontaneously in this strain and were considered to be unrelated to trifluralin treatment. The statistical tests did not indicate significant positive results for any neoplasm in the male or female rats.

Among the mice, hepatocellular carcinomas were detected in 4/19 (21 percent), 12/47 (26 percent), and 9/49 (18 percent) of the control, low dose, and high dose males, respectively, and in 0/20, 12/47 (26 percent), and 21/44 (48 percent) of the control, low dose, and high dose females, respectively. Statistical evaluation of these incidences, using either the matched or pooled controls, revealed a significant association between increased dosage and elevated incidence of hepatocellular carcinomas in females. These results were supported by positive tests for significance of tumor incidence at each dose level. A statistically significant positive association between dose and tumor incidence was also demonstrated, using the pooled controls, for alveolar/bronchiolar adenomas in female mice.

The increased incidence of alveolar/bronchiolar adenomas was demonstrated significantly for the low dose group, but only marginally for the high dose group. Evaluation of the incidences of hepatocellular carcinoma in males (4/19, 12/47, and 9/49 in the control, low dose, and high dose groups, respectively) provided no statistically significant positive associations.

Squamous-cell carcinomas of the stomach were observed in 4/45 low dose female and 1/44 high dose female mice but none were observed in control or male mice. Although the incidences of squamous-cell carcinomas were not found to be statistically significant, these tumors are only infrequently detected in B6C3F1 mice. The historical incidence, collated from all untreated female mice of this strain used in the NCI Bioassay Program, of squamous-cell carcinomas of the stomach (i.e., 0/1985) indicates the rarity of these tumors. In addition, it is felt that the frequency with which related proliferative and dysplastic stomach lesions (i.e., hyperkeratosis and acanthosis) were observed in the treated animals provided supplementary evidence for concluding that occurrence of these tumors was treatment-related.

The possibility that the carcinogenic action of trifluralin in female B6C3F1 mice may have been due to the action of a contaminant, specifically dipropylnitrosamine, cannot be discounted. This nitrosamine, established as a liver, esophagus, and tongue carcinogen in BD-rats by Druckery et al. (1967), was present as a component of the

tested trifluralin in concentrations of 84 to 88 ppm (Olin, 1976). These concentrations were detected more than 5.5 years after the compound had been purchased and over 3.5 years after the animal dosing phase of the bioassay. As dipropylnitrosamine is a low molecular weight nitrosamine and, therefore, quite volatile, it is not unreasonable to suspect that a considerably higher concentration of the contaminant may have actually been present in the purchased compound when utilized in the bioassay.

Evaluation of the results of this bioassay indicates that technical-grade trifluralin is a carcinogen in female B6C3F1 mice, being associated in these animals with an elevated incidence of hepatocellular neoplasms, alveolar/bronchiolar adenomas and squamous-cell carcinomas of the stomach. Under the conditions of this bioassay sufficient evidence was not provided for the carcinogenicity or tumorigenicity of trifluralin in male B6C3F1 mice or in Osborne-Mendel rats of either sex.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH TRIFLURALIN

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

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	46	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(49)	(50)
HEMANGIOPERICYTOMA, MALIGNANT	1 (2%)		
*SUBCUT TISSUE	(49)	(49)	(50)
FIBROMA	1 (2%)	1 (2%)	1 (2%)
FIBROSARCOMA	1 (2%)	1 (2%)	1 (2%)
LIPOSARCOMA		1 (2%)	
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA	4 (8%)	4 (8%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(49)
FOLLICULAR-CELL CARCINOMA, METAS			1 (2%)
MIXED TUMOR, MALIGNANT	1 (2%)		
HEMANGIOSARCOMA, METASTATIC	2 (4%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(49)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		3 (6%)	
#SPLEEN	(47)	(18)	(12)
HEMANGIOSARCOMA	4 (9%)	3 (17%)	
#KIDNEY	(47)	(11)	(12)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (9%)	
CIRCULATORY SYSTEM			
#HEART	(47)	(10)	(11)
HEMANGIOSARCOMA	1 (2%)		

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
HEMANGIOSARCOMA, METASTATIC	1 (2%)		
*PAMPINIFORM PLEXUS	(49)	(49)	(50)
LIPOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(49)
HEMANGIOSARCOMA			1 (2%)
HEMANGIOSARCOMA, METASTATIC	1 (2%)		
#STOMACH	(46)	(28)	(28)
HEMANGIOSARCOMA			1 (4%)
URINARY SYSTEM			
#KIDNEY	(47)	(11)	(12)
TUBULAR-CELL ADENOMA			1 (8%)
LIPOMA	2 (4%)		
HEMANGIOSARCOMA	1 (2%)		
#URINARY BLADDER	(46)	(13)	(11)
PAPILLOMA, NOS	3 (7%)	1 (8%)	1 (9%)
ENDOCRINE SYSTEM			
#PITUITARY	(41)	(11)	(11)
SQUAMOUS CELL CARCINOMA, INVASIV			1 (9%)
CHROMOPHOBE ADENOMA	4 (10%)	3 (27%)	
#ADRENAL	(46)	(11)	(10)
PHEOCHROMOCYTOMA		1 (9%)	
#THYROID	(48)	(49)	(48)
FOLLICULAR-CELL ADENOMA	3 (6%)	4 (8%)	5 (10%)
FOLLICULAR-CELL CARCINOMA	4 (8%)	4 (8%)	5 (10%)
C-CELL ADENOMA			1 (2%)
C-CELL CARCINOMA		1 (2%)	1 (2%)
#PARATHYROID	(46)	(49)	(48)
ADENOMA, NOS	1 (2%)		
#PANCREATIC ISLETS	(46)	(11)	(12)
ISLET-CELL ADENOMA	1 (2%)	1 (9%)	

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(49)	(50)
ADENOCARCINOMA, NOS	2 (4%)	1 (2%)	
FIBROADENOMA	1 (2%)		
#PROSTATE	(34)	(10)	(10)
HEMANGIOSARCOMA, METASTATIC	1 (3%)		
*SEMINAL VESICLE	(49)	(49)	(50)
HEMANGIOSARCOMA, METASTATIC	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(47)	(49)	(48)
GLIOMA, NOS	1 (2%)		
ASTROCYTOMA		1 (2%)	1 (2%)
*TRIGEMINAL NERVE	(49)	(49)	(50)
SQUAMOUS CELL CARCINOMA, INVASIV			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(49)	(49)	(50)
FIBROSARCOMA	1 (2%)		
*MUSCLE OF BACK	(49)	(49)	(50)
FIBROSARCOMA			1 (2%)
*MUSCLE OF HEAD	(49)	(49)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
*MUSCLE OF THORAX	(49)	(49)	(50)
HEMANGIOSARCOMA	1 (2%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(49)	(50)
LIPOMA	1 (2%)		1 (2%)

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

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
*MESENTERY	(49)	(49)	(50)
LIPOMA			2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(49)	(50)
HEMANGIOSARCOMA	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	24	20	17
MORIBUND SACRIFICE	2	4	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	24	26	31
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	28	22	22
TOTAL PRIMARY TUMORS	41	32	26
TOTAL ANIMALS WITH BENIGN TUMORS	13	10	13
TOTAL BENIGN TUMORS	17	12	13
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	16	12
TOTAL MALIGNANT TUMORS	24	20	13
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		2
TOTAL SECONDARY TUMORS	6		3
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH TRIFLURALIN

	CONTROL (VEH) 01-F001	LOW DOSE 01-F002	HIGH DOSE 01-F003
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	50	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA			1 (2%)
BASAL-CELL CARCINOMA	1 (2%)		
FIBROMA	2 (4%)	4 (8%)	
LIPOMA	1 (2%)	1 (2%)	
HEMANGIOSARCOMA	3 (6%)		1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(49)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(12)	(11)
HEMANGIOSARCOMA		2 (17%)	
#CERVICAL LYMPH NODE	(48)	(10)	(10)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
NEOPLASTIC NODULE	1 (2%)		

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 01-F001	LOW DOSE 01-F002	HIGH DOSE 01-F003
#STOMACH	(50)	(19)	(25)
HEMANGIOSARCOMA		1 (5%)	
#DUODENUM	(50)	(10)	(10)
HEMANGIOSARCOMA	1 (2%)		
*ANUS	(50)	(50)	(49)
FIBROSARCOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(12)	(10)
TRANSITIONAL-CELL CARCINOMA			1 (10%)
LIPOMA	1 (2%)		
MIXED TUMOR, MALIGNANT	1 (2%)	1 (8%)	
HAMARTOMA+	1 (2%)		
#URINARY BLADDER	(49)	(10)	(12)
PAPILLOMA, NOS		1 (10%)	
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(12)	(12)
CHROMOPHOBE ADENOMA	15 (30%)	5 (42%)	4 (33%)
#THYROID	(50)	(50)	(49)
FOLLICULAR-CELL ADENOMA		3 (6%)	
FOLLICULAR-CELL CARCINOMA	1 (2%)	4 (8%)	
C-CELL ADENOMA	5 (10%)	2 (4%)	1 (2%)
C-CELL CARCINOMA		2 (4%)	1 (2%)
#PARATHYROID	(48)	(49)	(49)
ADENOMA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
ADENOCARCINOMA, NOS		3 (6%)	3 (6%)
FIBROADENOMA	15 (30%)	22 (44%)	12 (24%)
#UTERUS	(49)	(19)	(16)
ADENOCARCINOMA, NOS			1 (6%)

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 01-F001	LOW DOSE 01-F002	HIGH DOSE 01-F003
ENDOMETRIAL STROMAL POLYP	4 (8%)	4 (21%)	2 (13%)
#OVARY	(49)	(11)	(11)
CARCINOMA,NOS	1 (2%)		
LUTEOMA		1 (9%)	
GRANULOSA-CELL TUMOR	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL VISCERA	(50)	(50)	(49)
HEMANGIOSARCOMA	1 (2%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	14	12	15
MORIBUND SACRIFICE			3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	38	31
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (VEH) 01-F001	LOW DOSE 01-F002	HIGH DOSE 01-F003
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	36	39	22
TOTAL PRIMARY TUMORS	56	59	28
TOTAL ANIMALS WITH BENIGN TUMORS	31	32	18
TOTAL BENIGN TUMORS	44	44	20
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	14	7
TOTAL MALIGNANT TUMORS	10	15	8
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2		
TOTAL UNCERTAIN TUMORS	2		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH TRIFLURALIN

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

	CONTROL (VEH) 02-M001	LOW DOSE 02-M002	HIGH DOSE 02-M003
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	20	47	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	47	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(47)	(48)
FIBROSARCOMA	5 (25%)	7 (15%)	9 (19%)
HEMANGIOSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(19)	(10)	(10)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (10%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(47)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
*MUSCLE HIP/THIGH	(20)	(47)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(47)	(49)
HEPATOCELLULAR ADENOMA		2 (4%)	
HEPATOCELLULAR CARCINOMA	4 (21%)	12 (26%)	9 (18%)
#STOMACH	(18)	(47)	(47)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
URINARY SYSTEM			
NONE			

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

TABLE B1 (CONTINUED)

	CONTROL (VEH) 02-M001	LOW DOSE 02-M002	HIGH DOSE 02-M003
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF BACK	(20)	(47)	(48)
FIBROSARCOMA			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	7	26	31
MORIBUND SACRIFICE	4	1	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	22	16
ANIMAL MISSING		1	1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

	CONTROL (VEH) 02-M001	LOW DOSE 02-M002	HIGH DOSE 02-M003
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	20	17
TOTAL PRIMARY TUMORS	10	23	22
TOTAL ANIMALS WITH BENIGN TUMORS		3	1
TOTAL BENIGN TUMORS		3	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	19	17
TOTAL MALIGNANT TUMORS	10	20	21
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH TRIFLURALIN

	CONTROL (VEH) 02-F001	LOW DOSE 02-F004	HIGH DOSE 02-F005
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			4
ANIMALS NECROPSIED	20	50	44
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	42
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(44)
FIBROSARCOMA		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(43)	(30)
ALVEOLAR/BRONCHIOLAR ADENOMA		6 (14%)	3 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(44)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)	2 (4%)	3 (7%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#KIDNEY	(20)	(46)	(44)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(44)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
HEPATOCELLULAR ADENOMA		3 (6%)	
HEPATOCELLULAR CARCINOMA		12 (26%)	21 (48%)

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

TABLE B2 (CONTINUED)

	CONTROL (VEH) 02-F001	LOW DOSE 02-F004	HIGH DOSE 02-F005
#STOMACH	(20)	(45)	(44)
SQUAMOUS CELL CARCINOMA		4 (9%)	1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(18)	(15)	(9)
PHEOCHROMOCYTOMA	1 (6%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(44)
ADENOCARCINOMA, NOS		1 (2%)	
#UTERUS	(20)	(49)	(41)
ENDOMETRIAL STROMAL POLYP		1 (2%)	
#OVARY	(20)	(47)	(39)
CYSTADENOCARCINOMA, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2 (CONCLUDED)

	CONTROL (VEH) 02-F001	LOW DOSE 02-F004	HIGH DOSE 02-F005
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	4	22
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	45	24
ANIMAL MISSING			4
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	24	26
TOTAL PRIMARY TUMORS	2	34	31
TOTAL ANIMALS WITH BENIGN TUMORS	1	9	3
TOTAL BENIGN TUMORS	1	10	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	20	25
TOTAL MALIGNANT TUMORS	1	24	28
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH TRIFLURALIN

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

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	46	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(49)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
*SUBCUT TISSUE	(49)	(49)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
ABSCESS, NOS	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(4)	(13)	(19)
INFLAMMATION, NOS	4 (100%)	6 (46%)	6 (32%)
#LUNG	(49)	(49)	(49)
PNEUMONIA, CHRONIC MURINE	20 (41%)	21 (43%)	37 (76%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(47)	(18)	(12)
FIBROSIS	1 (2%)		
ATROPHY, NOS		1 (6%)	
HYPERPLASIA, NOS		1 (6%)	
HEMATOPOIESIS	1 (2%)	2 (11%)	
#MESENTERIC L. NODE	(45)	(11)	(10)
CYST, NOS	1 (2%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(47)	(10)	(11)
INFLAMMATION, NOS	14 (30%)	1 (10%)	
FIBROSIS	1 (2%)		

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
*AORTA	(49)	(49)	(50)
ARTERIOSCLEROSIS, NOS	4 (8%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND		(1)	
ABSCESS, NOS		1 (100%)	
#LIVER	(49)	(49)	(49)
CYST, NOS	2 (4%)	1 (2%)	2 (4%)
INFLAMMATION, NOS	3 (6%)		1 (2%)
METAMORPHOSIS FATTY	3 (6%)	1 (2%)	1 (2%)
FOCAL CELLULAR CHANGE		1 (2%)	
HYPERPLASIA, NOS	5 (10%)		
ANGIECTASIS			1 (2%)
*BILE DUCT	(49)	(49)	(50)
DILATATION, NOS			1 (2%)
HYPERPLASIA, NOS	3 (6%)	1 (2%)	1 (2%)
#PANCREAS	(46)	(11)	(12)
PERIARTERITIS	5 (11%)	1 (9%)	6 (50%)
#ESOPHAGUS	(1)		
INFLAMMATION, NOS	1 (100%)		
#STOMACH	(46)	(28)	(28)
INFLAMMATION, NOS	1 (2%)	3 (11%)	2 (7%)
ULCER, FOCAL		1 (4%)	1 (4%)
#COLON	(46)	(10)	(10)
INFLAMMATION, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(47)	(11)	(12)
CYST, NOS		1 (9%)	
PYELONEPHRITIS, NOS	2 (4%)		1 (8%)
PYONEPHROSIS	1 (2%)		
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	37 (79%)	4 (36%)	10 (83%)
#URINARY BLADDER	(46)	(13)	(11)
INFLAMMATION, NOS	1 (2%)		1 (9%)

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
*URETHRA	(49)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(41)	(11)	(11)
DILATATION, NOS		1 (9%)	
CYST, NOS	1 (2%)	1 (9%)	
#ADRENAL	(46)	(11)	(10)
ANGIECTASIS	8 (17%)	5 (45%)	3 (30%)
#ADRENAL CORTEX	(46)	(11)	(10)
DEGENERATION, NOS			1 (10%)
#THYROID	(48)	(49)	(48)
CYST, NOS	1 (2%)		
HYPERPLASIA, C-CELL	1 (2%)	3 (6%)	4 (8%)
HYPERPLASIA, FOLLICULAR-CELL	4 (8%)	8 (16%)	5 (10%)
#PARATHYROID	(46)	(49)	(48)
HYPERPLASIA, NOS	2 (4%)	3 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(49)	(50)
GALACTOCELE	1 (2%)		
CYST, NOS	1 (2%)		
#PROSTATE	(34)	(10)	(10)
INFLAMMATION, NOS	9 (26%)	1 (10%)	2 (20%)
*SEMINAL VESICLE	(49)	(49)	(50)
INFLAMMATION, NOS	1 (2%)		
#TESTIS	(44)	(11)	(10)
ATROPHY, NOS	9 (20%)	2 (18%)	2 (20%)
*EPIDIDYMIS	(49)	(49)	(50)
GRANULOMA, SPERMATIC			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(47)	(49)	(48)
INFLAMMATION, NOS		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
#CEREBRUM	(47)	(49)	(48)
ABSCESS, NOS		1 (2%)	
#BRAIN	(47)	(49)	(48)
HYDROCEPHALUS, NOS			1 (2%)
ATROPHY, NOS		1 (2%)	
*ACCESSORY NERVE	(49)	(49)	(50)
INFLAMMATION, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(49)	(49)	(50)
INFLAMMATION, NOS			1 (2%)
PANNUS			1 (2%)
CATARACT			2 (4%)
PHTHISIS BULBI	1 (2%)		
*HARDERIAN GLAND	(49)	(49)	(50)
INFLAMMATION, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(49)	(49)	(50)
DEGENERATION, NOS	1 (2%)		
BODY CAVITIES			
*ABDOMINAL WALL	(49)	(49)	(50)
HYPERPLASIA, NOS			1 (2%)
*PLEURA	(49)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	
*PERICARDIUM	(49)	(49)	(50)
INFLAMMATION, NOS	5 (10%)		1 (2%)
*MESENTERY	(49)	(49)	(50)
PERIARTERITIS	2 (4%)		1 (2%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE C1 (CONCLUDED)

	CONTROL (VEH) 01-M001	LOW DOSE 01-M002	HIGH DOSE 01-M003
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	1
NECROPSY PERF/NO HISTO PERFORMED		1	
AUTO/NECROPSY/NO HISTO		2	1
AUTOLYSIS/NO NECROPSY	1	1	

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS
IN FEMALE RATS TREATED WITH TRIFLURALIN

	CONTROL (VEH) 01-F001	LOW DOSE 01-F002	HIGH DOSE 01-F003
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	50	47
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
EPIDERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#TRACHEA	(5)	(7)	(13)
INFLAMMATION, NOS	5 (100%)	2 (29%)	5 (38%)
#LUNG	(50)	(50)	(49)
INFLAMMATION, NOS			9 (18%)
PNEUMONIA, CHRONIC MURINE	15 (30%)	30 (60%)	30 (61%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(50)	(10)	(10)
METAMORPHOSIS FATTY	1 (2%)		
#SPLEEN	(50)	(12)	(11)
HEMATOPOIESIS	5 (10%)	1 (8%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(12)	(12)
INFLAMMATION, NOS		1 (8%)	
DEGENERATION, NOS	2 (4%)		1 (8%)
#ENDOCARDIUM	(50)	(12)	(12)
HYPERPLASIA, NOS	1 (2%)		
*AORTA	(50)	(50)	(49)
ARTERIOSCLEROSIS, NOS	1 (2%)		

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 01-F001	LOW DOSE 01-F002	HIGH DOSE 01-F003
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
CYST, NOS			3 (6%)
INFLAMMATION, NOS	4 (8%)		
CIRRHOSIS, NOS		1 (2%)	
METAMORPHOSIS FATTY	1 (2%)	3 (6%)	
FOCAL CELLULAR CHANGE	1 (2%)		
ANGIECTASIS		5 (10%)	3 (6%)
*BILE DUCT	(50)	(50)	(49)
DILATATION, NOS	1 (2%)	3 (6%)	1 (2%)
HYPERPLASIA, NOS	2 (4%)	3 (6%)	
#PANCREAS	(50)	(11)	(11)
PERIARTERITIS			1 (9%)
#STOMACH	(50)	(19)	(25)
INFLAMMATION, NOS			1 (4%)
ULCER, NOS		1 (5%)	1 (4%)
ULCER, FOCAL	5 (10%)	1 (5%)	3 (12%)
CALCIUM DEPOSIT	1 (2%)		1 (4%)
ACANTHOSIS		1 (5%)	
#LARGE INTESTINE	(49)	(10)	(9)
PARASITISM	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(12)	(10)
PYELONEPHRITIS, NOS	1 (2%)		
PYONEPHROSIS		1 (8%)	
INFLAMMATION, CHRONIC	23 (46%)	4 (33%)	7 (70%)
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(12)	(12)
CYST, NOS			1 (8%)
ANGIECTASIS	1 (2%)		
#ADRENAL	(50)	(10)	(10)
ANGIECTASIS	17 (34%)	2 (20%)	3 (30%)
#ADRENAL CORTEX	(50)	(10)	(10)
DILATATION, NOS		1 (10%)	

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 01-F001	LOW DOSE 01-F002	HIGH DOSE 01-F003
#THYROID	(50)	(50)	(49)
HYPERPLASIA, C-CELL	2 (4%)	2 (4%)	
HYPERPLASIA, FOLLICULAR-CELL	2 (4%)	1 (2%)	3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
GALACTOCELE		1 (2%)	
HYPERPLASIA, NOS	1 (2%)		
#UTERUS	(49)	(19)	(16)
HYDROMETRA	9 (18%)	1 (5%)	
CYST, NOS			1 (6%)
INFLAMMATION, NOS	2 (4%)	1 (5%)	3 (19%)
#UTERUS/ENDOMETRIUM	(49)	(19)	(16)
INFLAMMATION, NOS		1 (5%)	
HYPERPLASIA, CYSTIC	3 (6%)	6 (32%)	2 (13%)
#OVARY	(49)	(11)	(11)
CYST, NOS	2 (4%)	2 (18%)	2 (18%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(49)
SYNECHIA, NOS		1 (2%)	
CATARACT		1 (2%)	
*EYE/CORNEA	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
*EYE/RETINA	(50)	(50)	(49)
ATROPHY, NOS		1 (2%)	
*EYE/LACRIMAL GLAND	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*FACIAL MUSCLE	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	

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

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 01-F001	LOW DOSE 01-F002	HIGH DOSE 01-F003
BODY CAVITIES			
*PERICARDIUM	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL PCBPHOLOGY SUMMARY			
NO LESION REPORTED	1		2
ANIMAL MISSING/NO NECROPSY			1
AUTC/NECROPSY/NO HISTO			2

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

APPENDIX D

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

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

	CONTROL (VEH) 02-M001	LOW DOSE 02-M002	HIGH DOSE 02-M003
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	20	47	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	47	47
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(47)	(48)
INFLAMMATION, NOS			1 (2%)
ULCER, NOS	2 (10%)		
INFLAMMATION, CHRONIC	3 (15%)		
ULCER, CHRONIC	2 (10%)		
HYPERKERATOSIS			1 (2%)
ACANTHOSIS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(10)	(10)
INFLAMMATION, NOS			2 (20%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(10)	(10)
AMYLOIDOSIS	4 (22%)	5 (50%)	
HEMATOPOIESIS	2 (11%)	2 (20%)	
#MESENTERIC L. NODE	(13)	(7)	(9)
HEMORRHAGE			2 (22%)
ANGIECTASIS	1 (8%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(47)	(49)
CYST, NOS			1 (2%)

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

TABLE D1 (CONTINUED)

	CONTROL (VEH) 02-M001	LOW DOSE 02-M002	HIGH DOSE 02-M003
INFLAMMATION, NOS		4 (9%)	5 (10%)
NECROSIS, FOCAL			3 (6%)
AMYLOIDOSIS	4 (21%)	5 (11%)	
METAMORPHOSIS FATTY		1 (2%)	
HYPERPLASIA, NODULAR			1 (2%)
#LIVER/CENTRILOBULAR	(19)	(47)	(49)
NECROSIS, NOS	1 (5%)		
#STOMACH	(18)	(47)	(47)
ULCER, FOCAL		1 (2%)	
HYPERKERATOSIS			1 (2%)
ACANTHOSIS			1 (2%)
*RECTUM	(20)	(47)	(48)
PROLAPSE	2 (10%)		
INFLAMMATION, CHRONIC	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(47)	(49)
PYELONEPHRITIS, NOS			1 (2%)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (5%)		
INFLAMMATION, CHRONIC	3 (15%)	3 (6%)	
AMYLOIDOSIS		2 (4%)	
#KIDNEY/TUBULE	(20)	(47)	(49)
INFLAMMATION, CHRONIC			1 (2%)
#URINARY BLADDER	(15)	(10)	(10)
INFLAMMATION, CHRONIC	2 (13%)		
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(9)	(10)
AMYLOIDOSIS	1 (6%)		
REPRODUCTIVE SYSTEM			
*PENIS	(20)	(47)	(48)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC	1 (5%)		

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

TABLE D1 (CONCLUDED)

	CONTROL (VEH) 02-M001	LOW DOSE 02-M002	HIGH DOSE 02-M003
#PROSTATE	(12)		
INFLAMMATION, CHRONIC	1 (8%)		
*SEMINAL VESICLE	(20)	(47)	(48)
INFLAMMATION, NOS	1 (5%)		
#TESTIS	(19)	(10)	(10)
ATROPHY, NOS	1 (5%)		
*EPIDIDYMIS	(20)	(47)	(48)
GRANULOMA, SPERMATIC	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	25	15
ANIMAL MISSING/NO NECROPSY		1	1
AUTC/NECROPSY/HISTO PERF			2
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY		2	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 TRIFLURALIN

	CONTROL (VEH) 02-F001	LOW DOSE 02-F004	HIGH DOSE 02-F005
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			4
ANIMALS NECROPSIED	20	50	44
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	42
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(19)	(43)	(30)
INFLAMMATION, NOS		5 (12%)	8 (27%)
PNEUMONIA, CHRONIC MURINE		2 (5%)	1 (3%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(16)	(10)
HEMATOPOIESIS		1 (6%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(11)		
INFLAMMATION, NOS	1 (9%)		
#LIVER	(20)	(47)	(44)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS		2 (4%)	
NECROSIS, FOCAL		1 (2%)	
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASTIC NODULE	1 (5%)		
#PANCREAS	(19)	(15)	(10)
CYST, NOS		1 (7%)	

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

TABLE D2 (CONTINUED)

	CONTROL (VEH) 02-F001	LOW DOSE 02-F004	HIGH DOSE 02-F005
#STOMACH	(20)	(45)	(44)
HYPERKERATOSIS		6 (13%)	1 (2%)
ACANTHOSIS		6 (13%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(46)	(44)
PYELONEPHRITIS, NOS			1 (2%)
INFLAMMATION, CHRONIC	1 (5%)	2 (4%)	24 (55%)
#KIDNEY/TUBULE	(20)	(46)	(44)
CAST, NOS			1 (2%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(49)	(41)
HYDROMETRA	4 (20%)	21 (43%)	11 (27%)
INFLAMMATION, NOS		2 (4%)	1 (2%)
INFLAMMATION, SUPPURATIVE	2 (10%)		
ABSCESS, NOS	1 (5%)		
#UTERUS/ENDOMETRIUM	(20)	(49)	(41)
INFLAMMATION, SUPPURATIVE	2 (10%)		
HYPERPLASIA, CYSTIC	4 (20%)	3 (6%)	
#OVARY/OVIDUCT	(20)	(49)	(41)
INFLAMMATION, NOS			1 (2%)
#OVARY	(20)	(47)	(39)
CYST, NOS	1 (5%)	3 (6%)	2 (5%)
FOLLICULAR CYST, NOS	6 (30%)		
INFLAMMATION, SUPPURATIVE	3 (15%)		
ABSCESS, NOS	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D2 (CONCLUDED)

	CONTROL (VEH) 02-F001	LOW DOSE 02-F004	HIGH DOSE 02-F005
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	9	3
ANIMAL MISSING/NO NECROPSY			4
AUTO/NECROPSY/HISTO PERF	1	1	2
AUTC/NECROPSY/NO HISTO			2
AUTOLYSIS/NO NECROPSY			2

Review of the Bioassay of Trifluralin* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

November 28, 1977

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and 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 organic 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 NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which Trifluralin was reviewed.

The primary reviewer said that during the manufacture of Trifluralin, dipropylnitrosamine (DPNA) was formed and was contained in the technical-grade material as a by-product. DPNA was shown by Druckery to be carcinogenic in both rats and mice.

The primary reviewer noted that hepatocellular carcinomas in female mice was the only increased tumor incidence found among the Trifluralin-treated animals. Because the tested material contained DPNA, he said that it was not possible to interpret the results in terms of pur Trifluralin. He remarked, however, that the tested material was then the product of commerce.

An NCI staff member noted that, in addition to liver tumors, a statistically significant increase in the incidence of alveolar/bronchiolar adenomas was found. Also, there was an increased number of squamous-cell carcinomas of the stomach in female mice, which appeared to be treatment-related.

A consultant to Eli Lilly Company reported that the nitrosamine content had been reduced by 95 percent in the commercial Trifluralin currently marketed, as compared to the one tested in the NCI study. Since DPNA is formed

during the manufacture of Trifluralin, one Subgroup member commented that there may be high occupational exposure to the contaminated product.

A motion was made that the report on the bioassay of Trifluralin be accepted. It was added that attention should be given to the fact that the technical-grade Trifluralin tested contained dipropylnitrosamine, an experimental carcinogen known to induce tumors of the types found in the treated female mice. The motion was seconded and approved unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Lawrence Garfinkel, American Cancer Society
Henry C. Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
John H. Weisburger, American Health Foundation

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* 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.

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