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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 PIVALOLACTONE FOR POSSIBLE CARCINOGENICITY

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

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

CONTRIBUTORS: This bioassay of pivalolactone was conducted by Litton Bionetics, Inc., Bethesda, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. S. M. Garner (4,5) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly. Chemical analysis was performed by Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

Histopathologic examinations were performed by Dr. A. DePaoli (4) at Litton Bionetics, Inc., the pathology narratives were written by Dr. A. DePaoli (4), and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (8).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (9); the statistical analysis was performed by Mr. W. W. Belew (7,10) and Mr. R. M. Helfand (7), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (11).

This report was prepared at METREK, a Division of The MITRE Corporation (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), task leader Ms. P. Walker (7), senior biologist Mr. M. Morse (7), biochemist Mr. S. C. Drill (7), and technical editor Ms. P. A. Miller (7). The final report was reviewed by members of the participating organizations.

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SUMMARY

The bioassay of pivalolactone for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. Pivalolactone in water was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The high and low dosages of pivalolactone utilized were, respectively, 300 and 150 mg/kg/day for rats and 150 and 75 mg/kg/day for mice. After a 103-week period of compound administration for rats and a 102-week period of compound administration for mice, rats were observed for 2 additional weeks and mice for 1 additional week. Twenty animals of each sex and species were placed on test as vehicle controls.

There was no significant positive association between dosage and mortality for either rats or mice, and in both species, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. Compound-related mean body weight depression was not observed in either sex of either species. In addition, no adverse clinical signs were observed among dosed mice. This evidence, plus the relatively fast decomposition of pivalolactone in water, suggests the possibility that the animals, and in particular the mice, may have been able to tolerate a higher dose.

Statistically significant incidences of squamous-cell papillomas and squamous-cell carcinomas of the forestomach were observed in rats but not in mice. No other rare or unusual tumors were observed in either species.

Under the conditions of this bioassay, pivalolactone was found to be carcinogenic to both male and female Fischer 344 rats, producing squamous-cell carcinomas and squamous-cell papillomas of the forestomach. This study provided no evidence for the carcinogenicity of pivalolactone in B6C3F1 mice of either sex.

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I. INTRODUCTION

Pivalolactone (Figure 1) (NCI No. C04126), an intermediate in polymer preparation, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to β-propiolactone, a well-documented direct acting carcinogen (International Agency for Research on Cancer, 1974).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 3,3-dimethyl-2-oxethanone.* It is also called 3,3-dimethyl-2-oxetanone; 3,3-dimethyl-β-propiolactone; dimethyl propiolactone; and pivalic acid lactone.

Pivalolactone has been used to prepare copolymers used in sutures, prosthetic devices, etc. (Schmitt and Epstein, 1976); to block and graft copolymers with acrylics (Shor and Van Dyk, 1975), isoprene and butadiene (Foss et al., 1976; Foss, 1975), and ethylenemethacrylic acid-vinyl acetate polymer (Sundet et al., 1976); and to prepare polyoxymethylenes (Radici et al., 1975a; Radici et al., 1975b). Pivalolactone has also been used as a plasticizer for cyanoacrylate adhesive (Brinkmann and Inoehl, 1975).

Specific production data for pivalolactone are not available; however, this compound does not appear to be produced or sold in commercial quantities (in excess of 1000 pounds or $1000 in value annually) in the United States (U.S. International Trade Commission, 1977).

*The CAS registry number is 1955-45-9.

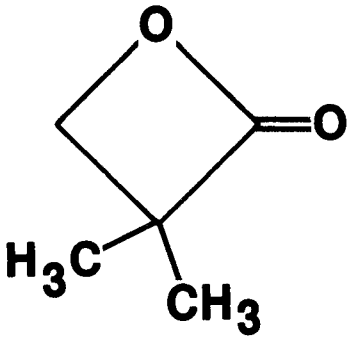


FIGURE 1
CHEMICAL STRUCTURE OF PIVALOLACTONE

The potential for exposure to pivalolactone is greatest for workers engaged in the preparation of this compound or pivalolactone copolymers.

II. MATERIALS AND METHODS

A. Chemicals

Pivalolactone was purchased from Shell Laboratories, Amsterdam, Holland. The manufacturer indicated that 15 ppm of the complex of BF_3 and tribenzylamine were added as a stabilizer to prevent polymerization. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. Thin-layer chromatographic plates utilizing two solvent systems (petroleum ether:dioxane and methanol) and visualized by hydroxylamine-ferric chloride each yielded one spot. Infrared and nuclear magnetic resonance analyses were consistent with that expected on the basis of the structure. Ultraviolet/visible spectrophotometric data indicated no absorbance between 210 and 800 nm, as would be expected. High-pressure liquid chromatography indicated the presence of two impurities.

A second batch of the compound was purchased from the same supplier. Thin-layer chromatography utilizing the same solvent systems as the first batch showed only one spot. High-pressure liquid chromatography showed the presence of two impurities. Infrared and nuclear resonance analyses were consistent with that expected on the basis of the structure. Ultraviolet/visible analysis again showed no peaks, as would be expected. These results suggested that the second batch was similar in purity to the first.

A lactone colorimetric analysis of the stability of pivalolactone in water was performed by Midwest Research Institute. The results indicated that a 0.5 mg/ml solution of pivalolactone in water was decomposed (97.1 percent) after 24 hours.

Throughout this report, the term pivalolactone is used to refer to this material.

B. Dosage Preparation

Fresh solutions of pivalolactone in distilled water (Borden Polar Water Company, Beltsville, Maryland) were prepared on each day that intubation was performed. Excess portions of the mixtures were disposed of rather than stored. The concentration of pivalolactone in distilled water ranged from 1.5 to 3 percent in rats, and from 0.68 to 1.36 percent in mice.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All rats were supplied by A. R. Schmidt, Madison, Wisconsin. All mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined for visible signs of disease or

parasites. Obviously ill or runted animals were culled. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were placed on test at this time. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 20° to 26°C and the relative humidity was maintained between 45 and 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

All rats were housed four per cage by sex and all mice five per cage by sex. Throughout the study, dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous stainless steel mesh lid over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding were provided twice weekly. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used in polycarbonate cages for the entire study.

Acidulated water (pH 2.5) was supplied to animals in water bottles filled by an automated metering device, which was checked daily for diluting accuracy. Water bottles were changed twice weekly and sipper tubes were washed at weekly intervals. All animals were supplied with Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois) in hanging stainless steel hoppers, which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* EDTA trisodium salt (150-38-9); rats receiving I.P. injections of methiodal sodium (126-31-8); and other rats intubated with lithocholic acid (434-13-9).

All dosed and control mice were housed in a room with other mice receiving diets containing 2,4-dimethoxyaniline hydrochloride (54150-69-5); 4'-(chloroacetyl)-acetanilide (140-49-8); nithiazide (139-94-6); p-phenylenediamine dihydrochloride (624-18-0); 4-nitro-o-phenylenediamine (99-56-9); 1-phenyl-3-methyl-5-pyrazolone (89-25-8); and other mice intubated with trimethylphosphate (512-56-1); 3-(chloromethyl)pyridine hydrochloride (3099-31-8); and 2-(chloromethyl) pyridine hydrochloride (6959-47-3).

E. Gastric Intubation

Intubation was performed for three days per week on a mg/kg body weight basis, utilizing the most recently observed group mean body

*CAS registry numbers are given in parentheses.

weight as a guide for determining the dose. All animals were weighed and dosages adjusted once monthly, based on group mean body weight. Animals of each sex within a dosed group received the same dosage. Thus, although the ratio of dose to weight remained constant, the total dosage administered fluctuated with an increase or decrease in group mean body weight.

F. Selection of Initial Dose Levels

In order to establish the maximum tolerated dosages of pivalolactone for administration to dosed animals in the chronic study, 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. Pivalolactone mixed with distilled water was introduced by gavage to five of the six rat groups at dosages of 316, 464, 681, 1000, and 1470 mg/kg/day and to five of the six mouse groups at dosages of 68, 100, 147, 215, and 316 mg/kg/day. The sixth group of each species served as a control, receiving only distilled water by gavage. Intubation was performed three times per week for 7 weeks, followed by a 1-week observation period to detect any delayed toxicity. Individual body weights were recorded weekly. At the end of the observation period, all survivors were sacrificed and necropsied.

At a dosage of 1470 mg/kg/day, four male and four female rats died; one additional male rat died at a dosage of 1000 mg/kg/day. At the end of the subchronic test, the mean body weight gain of male

rats receiving 316 mg/kg/day was 23 percent greater than the mean body weight gain of their controls, while female rats receiving the same dosage displayed a mean body weight gain which was 4 percent greater than that of their controls. The high dose selected for administration to rats in the chronic bioassay was 300 mg/kg/day.

At a dosage of 316 mg/kg/day, two male and three female mice died. At the end of the subchronic test, the mean body weight gain of male mice receiving 215 mg/kg/day was 7 percent greater than the mean body weight gain of their controls, while females receiving the same dosage displayed a mean body weight gain which was 18 percent less than that of their controls. The mean body weight gain of male mice receiving 147 mg/kg/day was 5 percent greater than that of their controls, while females receiving the same dosage displayed a mean body weight gain which was 11 percent less than that of their controls. The high dose selected for administration to mice in the chronic bioassay was 150 mg/kg/day.

G. Experimental Design

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

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dosages administered were 300 and 150 mg/kg/day. Throughout this report, those rats receiving the former dosage are referred to as the high dose

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
PIVALOLACTONE GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	PIVALOLACTONE DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0	-	105[b]
LOW DOSE	50	150	103	
		0		2
HIGH DOSE	50	300	103	
		0		2
FEMALE				
CONTROL	20	0	-	105[b]
LOW DOSE	50	150	103	
		0		2
HIGH DOSE	50	300	103	
		0		2

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

[b]Gavaged with distilled water 3 times per week for 103 weeks.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
PIVALOLACTONE GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	PIVALOLACTONE DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0	-	103[b]
LOW DOSE	50	75	102	
		0		1
HIGH DOSE	50	150	102	
		0		1
FEMALE				
CONTROL	20	0	-	103[b]
LOW DOSE	50	75	102	
		0		1
HIGH DOSE	50	150	102	
		0		1

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

[b] Gavaged with distilled water 3 times per week for 102 weeks.

groups and those receiving the latter dosage are referred to as the low dose groups. All dosed rats were administered pivalolactone at the dosages indicated for 103 weeks, followed by a 2-week observation period.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dosages administered were 150 and 75 mg/kg/day. Throughout this report, those mice receiving the former dosage are referred to as the high dose groups and those receiving the latter dosage are referred to as the low dose groups. All dosed mice were administered pivalolactone at the dosages indicated for 102 weeks, followed by a 1-week observation period.

Vehicle control animals were intubated with 10 ml/kg distilled water three times per week. All survivors were sacrificed and necropsied at the end of the observation period.

H. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected twice daily for mortality. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group. Rats were weighed at monthly intervals throughout the bioassay. Body weights of mice were recorded once a week for the first 4 weeks, and once monthly for the remainder of the bioassay.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were sacrificed. A necropsy was performed on each animal regardless of whether it died, was sacrificed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide asphyxiation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

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), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

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 recorded in each group at the time that the test was initiated.

I. Data Recording and Statistical Analyses

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

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

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

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

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was

used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week

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

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group

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

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

There was no evidence of dose-related mean body weight depression in either male or female rats (Figure 2).

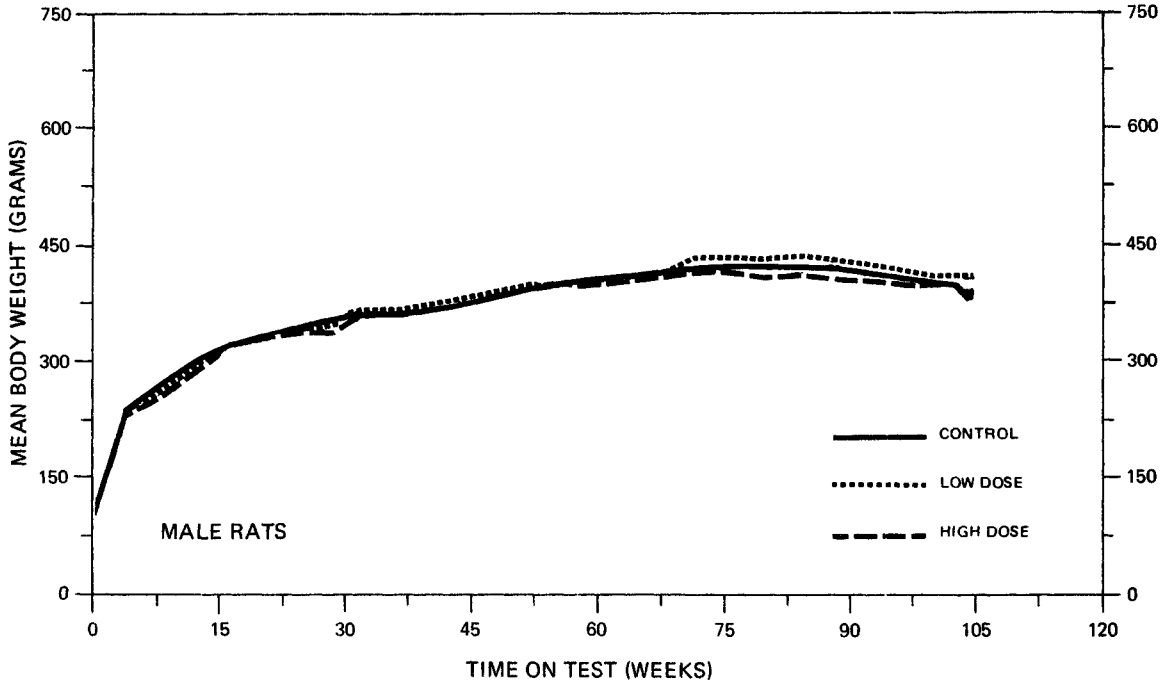
No abnormal clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and pivalolactone-dosed groups are shown in Figure 3. For male rats the Tarone test for association between dosage and mortality and the Cox tests comparing both high dose to control and low dose to control did not show any significant associations between dosage and mortality. For females the Tarone test indicated a significant ($P = 0.004$) dose-related trend for accelerated mortality. However, a significant ($P = 0.026$) departure from linear trend was also present due to the relatively high survival of the low dose group. The Cox tests comparing high dose to control and low dose to control were not significant.

Adequate numbers of male rats were at risk from late-developing tumors, as 62 percent (31/50) of the high dose, 76 percent (38/50) of the low dose, and 75 percent (15/20) of the control group survived on test until the termination of the study.

For female rats, 56 percent (28/50) of the high dose, 86 percent (43/50) of the low dose, and 80 percent (16/20) of the control group



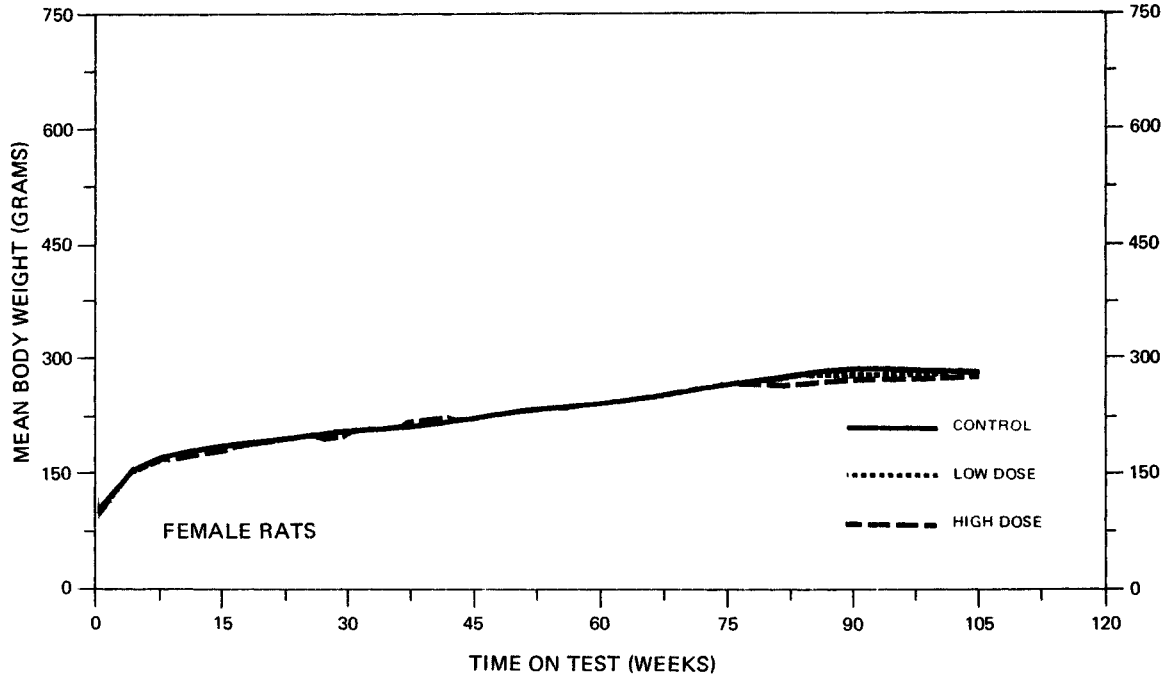
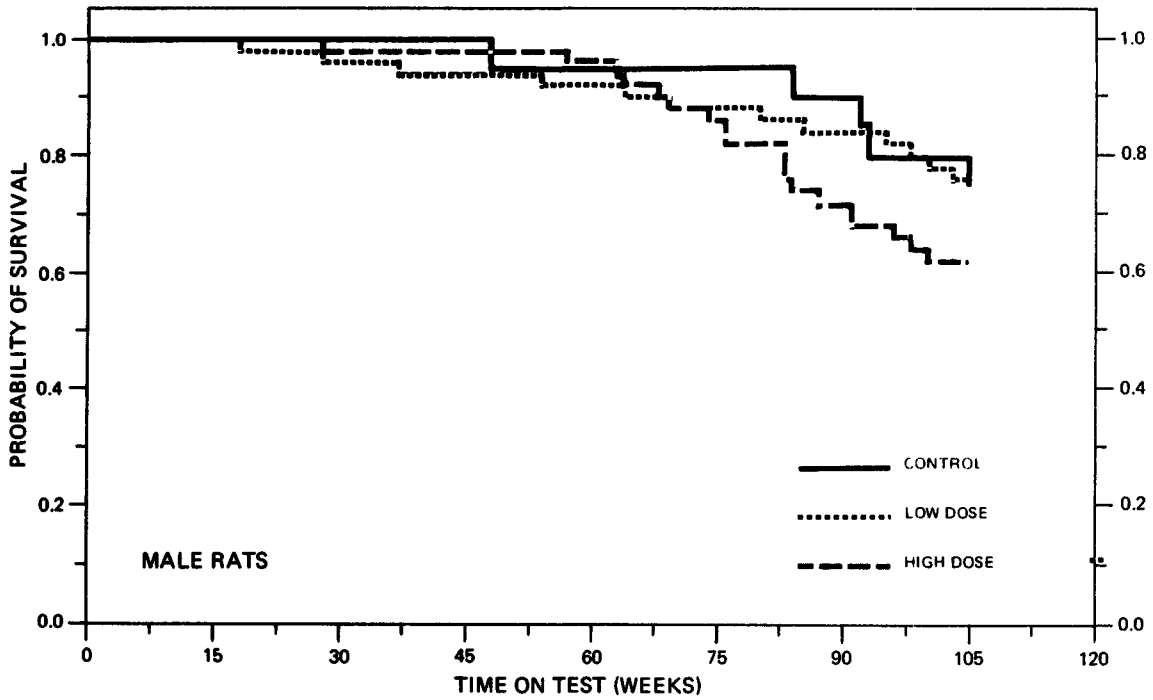


FIGURE 2
GROWTH CURVES FOR PIVALOLACTONE CHRONIC STUDY RATS



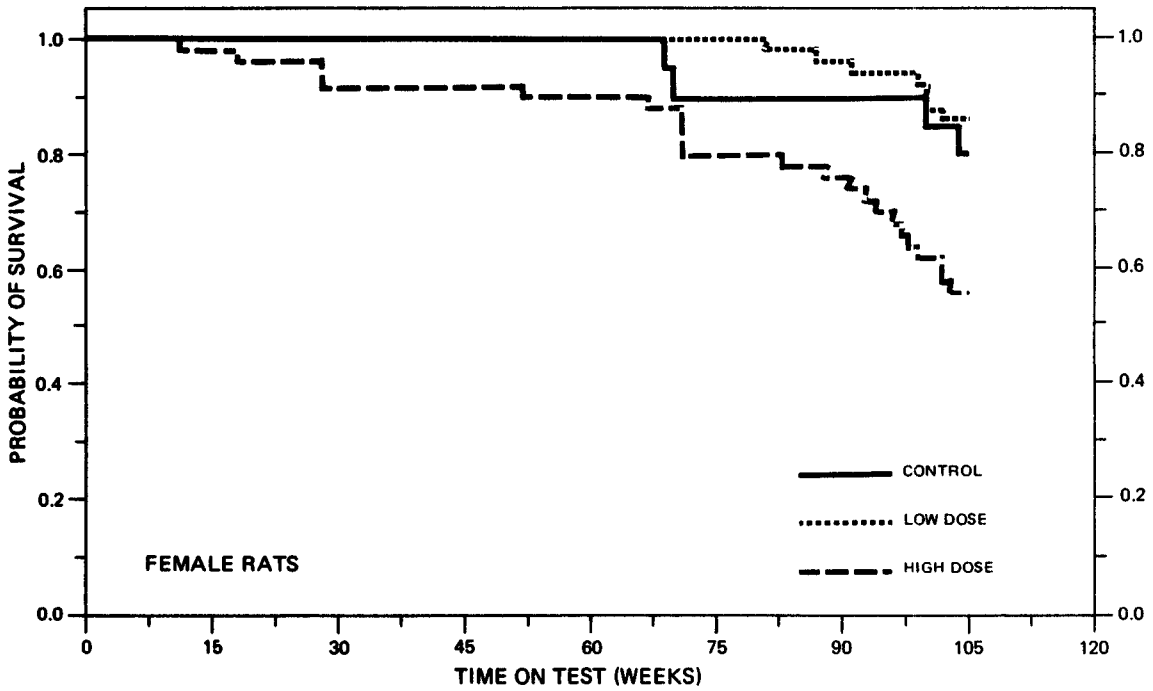


FIGURE 3
SURVIVAL COMPARISONS OF PIVALOLACTONE CHRONIC STUDY RATS

survived on test until termination of the study, thus providing adequate numbers at risk from late-developing tumors.

C. Pathology

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

There was an increased incidence of gastric neoplasms in the dosed groups when compared with the controls. Other neoplasms occurred with a greater frequency in dosed rats than controls. These lesions, however, often occur spontaneously in Fischer 344 rats.

In addition to the neoplastic lesions, a large number of degenerative, proliferative and inflammatory changes were encountered in dosed and control animals (Appendix C). Most of these nonneoplastic lesions are commonly seen in aged laboratory rats. However, a greater number of proliferative changes occurred in the stomach of dosed rats than in their controls.

The incidences of the neoplastic and proliferative lesions in the stomach were as follows:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Stomachs Examined Histopathologically	(19)	(49)	(48)	(20)	(50)	(50)
Squamous-Cell Carcinoma	0	1	7	0	0	2
Squamous-Cell Papilloma	0	5	14	0	2	9
Epithelial Hyperplasia	5	39	14	1	32	26
Inflammation	3	11	3	3	9	4

Only the larger proliferative gastric lesions were noted during the gross examination. These were usually reported as single papilliferous growths, although occassional multiple growths were noted in the same animal. In most animals gross changes were not evident.

Microscopically, the proliferative lesions usually were focal, located in the limiting ridge of the forestomach. Most squamous-cell carcinomas were papillary. The lining epithelium was well differentiated, markedly thickened and variably keratinized. Chords of epithelial cells extended from the surface into the submucosa and the muscularis mucosa. Invading cells were usually hyperchromatic and somewhat atypical with variable mitotic activity. The squamous-cell papillomas were usually smaller, did not demonstrate local invasion, cellular atypia or any degree of mitotic activity. Squamous-cell hyperplasia varied greatly from minimal thickening of the squamous

epithelium to marked focal thickening of the lining epithelium with early papillary proliferation. The hyperplastic changes, while usually focal and involving the limiting ridge, occasionally were multifocal and affected other portions of the forestomach. The hyperplastic changes were least severe (minimal to mild) in the affected control animals, becoming more pronounced in the dosed groups. A mild focal gastritis was associated with these proliferative changes. The inflammation was characterized by infiltration of the mucosa in the region of proliferation with a few granulocytes and lymphocytes. The focal gastritis was most evident in the early proliferative lesions.

While mild hyperplastic changes were noted in the control groups, the increased incidence and severity of these hyperplastic lesions in the dosed groups, coupled with the occurrence of papillomas and carcinomas in the dosed animals, suggests that pivalolactone was responsible for both neoplasms and increased hyperplasia.

The results of this pathologic examination indicate that pivalolactone was carcinogenic, inducing squamous-cell carcinomas, papillomas and focal hyperplasia in the forestomach of Fischer 344 rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of tumor in either sex where at least two such tumors were

TABLE 3

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	1/20(0.05)	4/50(0.08)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.600	0.800
Lower Limit	---	0.175	0.045
Upper Limit	---	77.169	46.273
Weeks to First Observed Tumor	105	105	105
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	5/50(0.10)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.667	0.533
Lower Limit	---	0.147	0.102
Upper Limit	---	4.014	3.410
Weeks to First Observed Tumor	84	95	69
Liver: Neoplastic Nodule[b]	0/20(0.00)	3/50(0.06)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.250	0.123
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	105

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Stomach: Squamous-Cell Carcinoma[b]	0/19(0.00)	1/49(0.02)	7/48(0.15)
P Values[c]	P = 0.011	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.021	0.804
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	98	98
Stomach: Squamous-Cell Papilloma or Squamous-Cell Carcinoma[b]	0/19(0.00)	6/49(0.12)	21/48(0.44)
P Values[c]	P < 0.001	N.S.	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.649	2.823
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	85	76
Pituitary: Chromophobe Adenoma[b]	2/18(0.11)	2/47(0.04)	3/44(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.383	0.614
Lower Limit	---	0.030	0.079
Upper Limit	---	5.033	6.973
Weeks to First Observed Tumor	84	103	105

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	3/20(0.15)	4/48(0.08)	2/49(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.556	0.272
Lower Limit	---	0.106	0.025
Upper Limit	---	3.546	2.233
Weeks to First Observed Tumor	105	105	105
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	1/20(0.05)	6/48(0.13)	2/46(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.500	0.870
Lower Limit	---	0.339	0.049
Upper Limit	---	112.370	50.196
Weeks to First Observed Tumor	105	105	105
Testis: Interstitial-Cell Tumor[b]	12/20(0.60)	42/50(0.84)	40/49(0.82)
P Values[c]	N.S.	P = 0.035	N.S.
Relative Risk (Control)[d]	---	1.400	1.361
Lower Limit	---	0.980	0.946
Upper Limit	---	2.115	2.096
Weeks to First Observed Tumor	92	69	64

TABLE 3 (CONCLUDED)

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[a]Treated groups received doses of 150 or 300 mg/kg by gavage.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the 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. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

TABLE 4

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/20(0.20)	1/50(0.02)	1/50(0.02)
P Values[c]	P = 0.010(N)	P = 0.021(N)	P = 0.021(N)
Departure from Linear Trend[e]	P = 0.032	---	---
Relative Risk (Control)[d]	---	0.100	0.100
Lower Limit	---	0.002	0.002
Upper Limit	---	0.944	0.944
Weeks to First Observed Tumor	70	105	83
Stomach: Squamous-Cell Carcinoma[b]	0/20(0.00)	0/50(0.00)	2/50(0.04)
P Values[c]	N.S.	---	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.123
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	97
Stomach: Squamous-Cell Papilloma or Squamous-Cell Carcinoma[b]	0/20(0.00)	2/50(0.04)	11/50(0.22)
P Values[c]	P = 0.002	N.S.	P = 0.017
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.123	1.384
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	94

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	8/19(0.42)	22/47(0.47)	10/46(0.22)
P Values[c]	P = 0.026(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	1.112	0.516
Lower Limit	---	0.608	0.231
Upper Limit	---	2.423	1.302
Weeks to First Observed Tumor	70	100	18
Adrenal: Pheochromocytoma[b]	1/19(0.05)	3/49(0.06)	0/48(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.163	0.000
Lower Limit	---	0.103	0.000
Upper Limit	---	59.809	7.392
Weeks to First Observed Tumor	105	105	---
Thyroid: C-Cell Adenoma[b]	0/18(0.00)	2/47(0.04)	3/47(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.118	0.241
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	96

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	0/20(0.00)	1/50(0.02)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.022	0.250
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	105
Uterus: Endometrial Stromal Polyp[b]	8/20(0.40)	6/50(0.12)	10/49(0.20)
P Values[c]	N.S.	P = 0.012(N)	N.S.
Departure from Linear Trend[e]	P = 0.019	---	---
Relative Risk (Control)[d]	---	0.300	0.510
Lower Limit	---	0.104	0.224
Upper Limit	---	0.871	1.298
Weeks to First Observed Tumor	69	105	28

[a]Treated groups received doses of 150 or 300 mg/kg by gavage.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the 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. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

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

observed in at least one of the control or pivalolactone-dosed groups and where such tumors were observed in at least 5 percent of the group.

The Cochran-Armitage test indicated a significant positive association between dose and the combined incidence of squamous-cell papillomas or squamous-cell carcinomas of the forestomach for both males ($P < 0.001$) and females ($P = 0.002$). The Fisher exact tests comparing high dose to control were also significant in both males ($P < 0.001$) and females ($P = 0.017$). Based on these results the administration of pivalolactone was associated with the increased incidence of squamous-cell neoplasms of the forestomach in both male and female rats.

For male rats the Fisher exact test comparing the incidence of interstitial-cell tumors of the testis in the low dose group to that in the control group had a probability level of $P = 0.035$, a marginal result which was not significant under the Bonferroni criterion.

For female rats the possibility of a negative association between chemical administration and tumor incidence was observed for the combined incidence of leukemia or malignant lymphoma.

A significant negative trend was also indicated by the Cochran-Armitage test for pituitary chromophobe adenomas in female rats. The Fisher exact tests, however, were not significant. A significant negative Fisher exact test for the incidence of endometrial stromal

polyps of the uterus was also found for low dose female rats, but no other tests were significant.

In summary, the statistical findings were that the administration of pivalolactone was associated with the increased incidence of squamous-cell neoplasms of the forestomach in both male and female rats.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

No consistent dose-related mean body weight depression was apparent in either male or female mice (Figure 4).

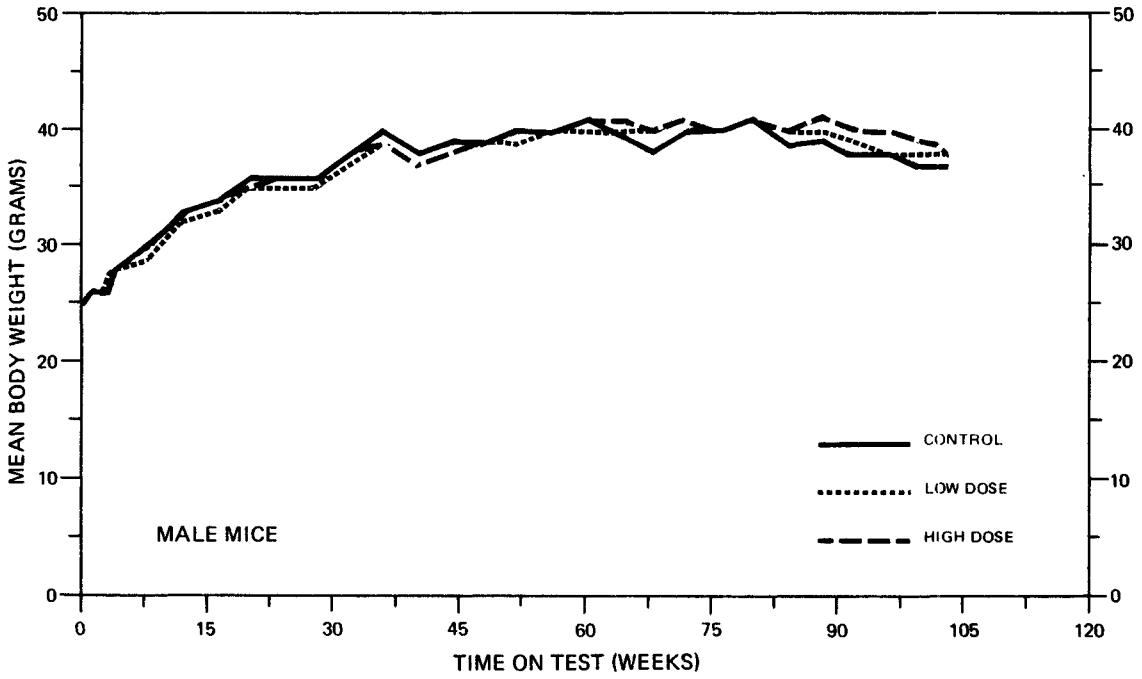
No abnormal clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and pivalolactone-dosed groups are shown in Figure 5. For both male and female mice the Tarone test did not indicate a positive association between dosage and mortality. For male mice, a surprisingly significant (P = 0.0152) negative trend was indicated by the Tarone test, with the survival of the high dose and low dose males higher than that of the control group.

Although one low dose male mouse was missing in week 14, adequate numbers of male mice were at risk from late-developing tumors, as 90 percent (45/50) of the high dose, 84 percent (42/50) of the low dose, and 65 percent (13/20) of the control group survived on test until the termination of the study.

Although two low dose female mice were missing in week 16, 72 percent (36/50) of the high dose, 72 percent (36/50) of the low dose, and 80 percent (16/20) of the control group survived on test until termination of the study, providing adequate numbers at risk from late-developing tumors.



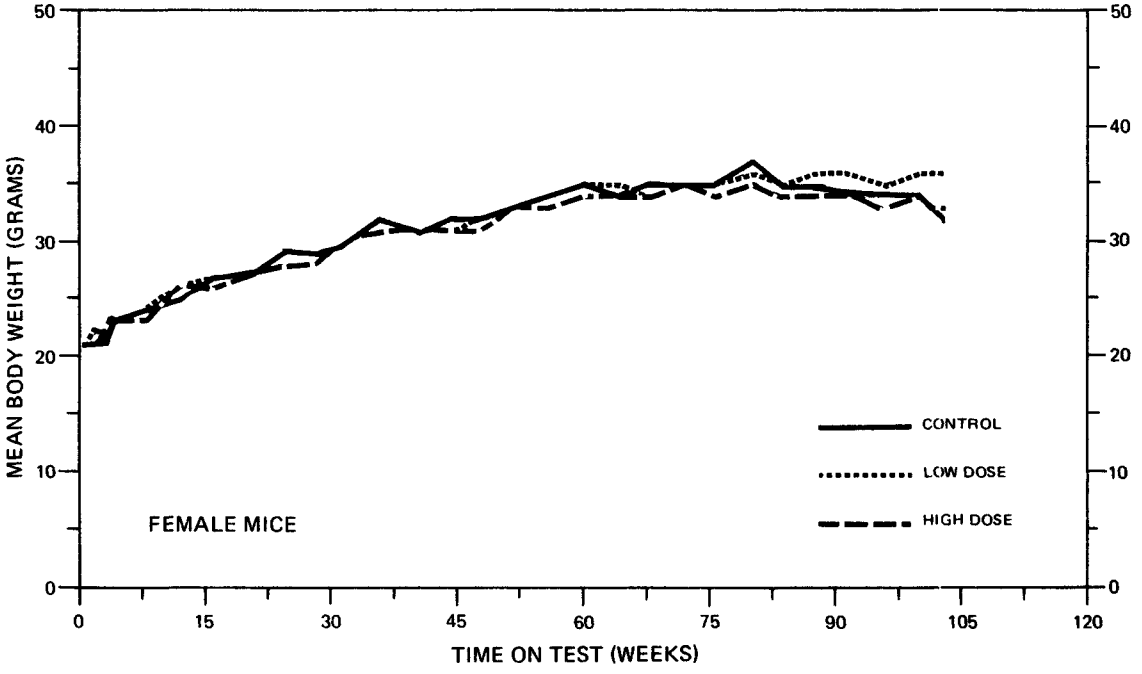


FIGURE 4
GROWTH CURVES FOR PIVALOLACTONE CHRONIC STUDY MICE

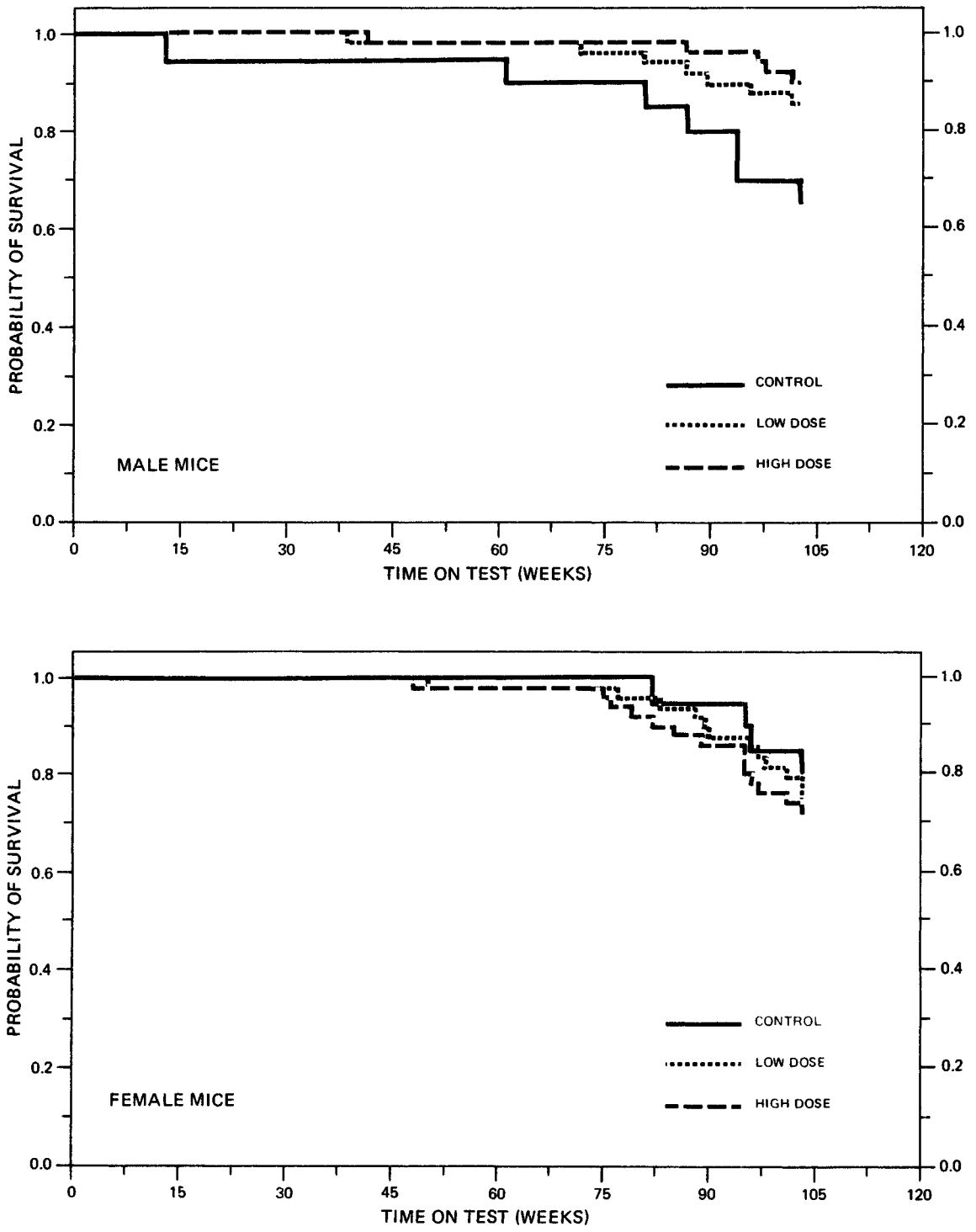


FIGURE 5
SURVIVAL COMPARISONS OF PIVALOLACTONE CHRONIC STUDY MICE

C. Pathology

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

A variety of tumors occurred spontaneously, both in the control and dosed groups. Most frequently affected were the liver, lung and hematopoietic system. These lesions, however, are not uncommon in aged mice of this strain, as can be seen from their occurrence in controls. The significant proliferative gastric lesions present in rats were not noted in mice.

In addition to the neoplastic lesions, a number of proliferative, inflammatory and degenerative changes were also encountered in animals of the dosed and control groups (Appendix D). These nonneoplastic lesions are seen commonly in aged B6C3F1 mice, and were not related to administration of the chemical.

The results of this pathologic examination indicate that pivalolactone had no observable carcinogenic effect in 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 is included for every type of tumor in either sex where at least two such tumors were observed in at least one of the control or pivalolactone-dosed groups and where such tumors were observed in at least 5 percent of the group.

TABLE 5

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	2/20(0.10)	6/49(0.12)	10/49(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.224	2.041
Lower Limit	---	0.248	0.498
Upper Limit	---	11.802	18.154
Weeks to First Observed Tumor	103	87	103
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	10/20(0.50)	10/49(0.20)	12/50(0.24)
P Values[c]	N.S.	P = 0.017(N)	P = 0.035(N)
Relative Risk (Control)[d]	---	0.408	0.480
Lower Limit	---	0.195	0.244
Upper Limit	---	0.936	1.059
Weeks to First Observed Tumor	61	72	87
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma[b]	4/20(0.20)	10/49(0.20)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.020	0.400
Lower Limit	---	0.346	0.085
Upper Limit	---	4.068	1.984
Weeks to First Observed Tumor	103	72	103

TABLE 5 (CONCLUDED)

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[a]Treated groups received doses of 75 or 150 mg/kg by gavage.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the 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. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

TABLE 6

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	0/20(0.00)	4/48(0.08)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.402	0.123
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	96	103
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/20(0.20)	13/48(0.27)	19/50(0.38)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.354	1.900
Lower Limit	---	0.495	0.752
Upper Limit	---	5.170	6.909
Weeks to First Observed Tumor	82	83	75

[a]Treated groups received doses of 75 or 150 mg/kg by gavage.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the 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. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

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

In male mice the Cochran-Armitage test indicated a significant negative association between dose and the incidence of hepatocellular carcinomas. The Fisher exact tests, however, were not significant. Also, the Fisher exact test indicated a negative association between the incidence of leukemia or malignant lymphoma in low dose male mice and that in the control group. No other tests, however, were significant under the Bonferroni criterion.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by pivalolactone that could not be established under the conditions of this test.

V. DISCUSSION

The survival among dosed rats was not significantly less than the survival among their controls, and there were no positive associations between dosage and mortality for mice of either sex. Adequate numbers of rats and mice of both sexes survived long enough to be at risk from late-developing tumors. Depression of mean body weight was not observed in dosed groups of either species. In addition, no adverse clinical signs were observed among dosed mice. This evidence, plus the relatively fast decomposition of pivalolactone in water, suggests the possibility that the animals, and in particular the mice, may have been able to tolerate a higher dose.

The administration of pivalolactone to male and female rats was associated with increased incidences of hyperplasia, squamous-cell papillomas, and squamous-cell carcinomas of the forestomach. The Cochran-Armitage test indicated significant positive associations between pivalolactone dosage and the combined incidence of squamous-cell papillomas and squamous-cell carcinomas of the forestomach in male and female rats. The Fisher exact tests indicated that the number of high dose rats with squamous-cell papilloma or squamous-cell carcinoma of the forestomach was significant for both males and females. These tumors were combined for statistical analysis since they are assumed to have a common pathogenesis. They do not commonly occur in control Fischer 344 rats and are, therefore, considered indicative of a carcinogenic effect of pivalolactone.

No tumors occurred in mice at significantly higher incidences in dosed groups than in control groups. No rare or unusual tumors were observed during the histopathologic examination of mice.

No indications were found in the literature for the carcinogenicity in rodents of BF_3 or tribenzylamine, stabilizers added to the chemical by the manufacturer.

These results indicate that under the conditions of this bioassay, pivalolactone was carcinogenic in male and female Fischer 344 rats, causing a significant increase in the combined incidence of squamous-cell carcinomas and squamous-cell papillomas at the site of chemical administration, the forestomach. This study provided no evidence for the carcinogenicity of pivalolactone in B6C3F1 mice of either sex.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH PIVALOLACTONE

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

	CONTROL (VEH) 11-1505	LOW DOSE 11-1503	HIGH DOSE 11-1501
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
SEBACEOUS ADENOMA			1 (2%)
SARCOMA, NOS		1 (2%)	1 (2%)
FIBROMA	1 (5%)	4 (8%)	2 (4%)
LIPOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(20)	(50)	(49)
NEOPLASM, NOS, METASTATIC		1 (2%)	
CARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	
SEBACEOUS ADENOCARCINOMA, METAST			1 (2%)
ADENOSQUAMOUS CARCINOMA		1 (2%)	
SARCOMA, NOS, METASTATIC		1 (2%)	
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*BRAIN	(20)	(49)	(49)
MALIGNANT RETICULOSIS			1 (2%)
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)		1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)	1 (2%)	
LEUKEMIA,NOS	1 (5%)	3 (6%)	3 (6%)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1505	LOW DOSE 11-1503	HIGH DOSE 11-1501
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(46)	(44)
SARCOMA, NOS	1 (5%)		
#LIVER	(20)	(50)	(50)
NEOPLASTIC NODULE		3 (6%)	2 (4%)
#PANCREAS	(20)	(48)	(46)
ACINAR-CELL ADENOMA			1 (2%)
#STOMACH	(19)	(49)	(48)
SQUAMOUS CELL PAPILLOMA		5 (10%)	14 (29%)
SQUAMOUS CELL CARCINOMA		1 (2%)	7 (15%)
#LARGE INTESTINE	(20)	(49)	(50)
ADENOCA IN ADENOMATOUS POLYP		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(47)	(44)
CHROMOPHOBE ADENOMA	2 (11%)	2 (4%)	3 (7%)
ACIDOPHIL ADENOMA	1 (6%)		
#ADRENAL	(20)	(48)	(49)
CARCINOMA, NOS		1 (2%)	
PHEOCHROMOCYTOMA	2 (10%)	4 (8%)	2 (4%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (5%)		
#THYROID	(20)	(48)	(46)
C-CELL ADENOMA	1 (5%)	5 (10%)	1 (2%)
C-CELL CARCINOMA		1 (2%)	1 (2%)
#PARATHYROID	(13)	(31)	(25)
ADENOMA, NOS	1 (8%)		
#PANCREATIC ISLETS	(20)	(48)	(46)
ISLET-CELL ADENOMA		1 (2%)	1 (2%)

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1505	LOW DOSE 11-1503	HIGH DOSE 11-1501
REPRODUCTIVE SYSTEM			
*TESTIS	(20)	(50)	(49)
INTERSTITIAL-CELL TUMOR	12 (60%)	42 (84%)	40 (82%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
EPENDYMOMA	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF LEG	(20)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
*ABDOMINAL VISCERA	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
*MESENTERY	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
MESOTHELIOMA, MALIGNANT			1 (2%)

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

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 11-1505	LOW DOSE 11-1503	HIGH DOSE 11-1501
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	6	15
MORIBUND SACRIFICE	3	6	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	38	31
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	46	46
TOTAL PRIMARY TUMORS	26	63	84
TOTAL ANIMALS WITH BENIGN TUMORS	17	43	44
TOTAL BENIGN TUMORS	20	65	65
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	13	15
TOTAL MALIGNANT TUMORS	6	14	16
TOTAL ANIMALS WITH SECONDARY TUMORS#		4	1
TOTAL SECONDARY TUMORS		4	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		4	2
TOTAL UNCERTAIN TUMORS		4	3
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 PIVALOI ACTONE

	CONTROL (VEH) 11-1506	LOW DOSE 11-1504	HIGH DOSE 11-1502
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOCA/SQUAMOUS METAPLASIA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA		1 (2%)	1 (2%)
FIBROADENOMA			2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	3 (15%)	1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
LEUKEMIA,NOS	1 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#STOMACH	(20)	(50)	(50)
SQUAMOUS CELL PAPILLOMA		2 (4%)	9 (18%)

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 11-1506	LOW DOSE 11-1504	HIGH DOSE 11-1502
SQUAMOUS CELL CARCINOMA			2 (4%)
ADENOCARCINOMA, NOS		1 (2%)	
SARCOMA, NOS			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(19)	(44)	(47)
LEIOMYOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(46)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
CHROMOPHOBE ADENOMA	8 (42%)	22 (47%)	10 (22%)
CHROMOPHOBE CARCINOMA			1 (2%)
#ADRENAL	(19)	(49)	(48)
PHEOCHROMOCYTOMA	1 (5%)	3 (6%)	
#THYROID	(18)	(47)	(47)
ADENOMA, NOS			1 (2%)
FOLLICULAR-CELL ADENOMA			2 (4%)
C-CELL ADENOMA		2 (4%)	3 (6%)
#PANCREATIC ISLETS	(20)	(46)	(48)
ISLET-CELL ADENOMA			2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS	1 (5%)	1 (2%)	1 (2%)
CYSTADENOMA, NOS		1 (2%)	
INTRADUCTAL PAPILLOMA		1 (2%)	
FIBROADENOMA		1 (2%)	3 (6%)
#UTERUS	(20)	(50)	(49)
ADENOCARCINOMA, NOS	1 (5%)		
LEIOMYOSARCOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP	8 (40%)	6 (12%)	10 (20%)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(50)	(49)
CARCINOMA, NOS		1 (2%)	

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 11-1506	LOW DOSE 11-1504	HIGH DOSE 11-1502
ADENOCARCINOMA, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
SARCOMA, NOS, METASTATIC			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		4	19
MORIBUND SACRIFICE	4	3	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	43	28
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (VEH) 11-1506	LOW DOSE 11-1504	HIGH DOSE 11-1502
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	35	34
TOTAL PRIMARY TUMORS	23	48	51
TOTAL ANIMALS WITH BENIGN TUMORS	13	30	32
TOTAL BENIGN TUMORS	18	40	45
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	8	5
TOTAL MALIGNANT TUMORS	5	8	6
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	1
TOTAL SECONDARY TUMORS		2	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 B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH PIVALOLACTONE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICF TREATED WITH PIVALOLACTONE

	CONTROL (VEH) 22-2505	LOW DOSE 22-2503	HIGH DOSE 22-2501
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	6 (12%)	10 (20%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)	2 (4%)	2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (10%)	3 (6%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)	1 (2%)	2 (4%)
LEUKEMIA,NOS	1 (5%)	1 (2%)	1 (2%)
UNDIFFERENTIATED LEUKEMIA	1 (5%)		
#SPLEEN	(18)	(47)	(50)
HEMANGIOSARCOMA			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (6%)		
#LYMPH NODE	(19)	(48)	(46)
MALIG.LYMPHOMA, UNDIFFER-TYPE		2 (4%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#MESENTERIC L. NODE	(19)	(48)	(46)
MALIG.LYMPHOMA, UNDIFFER-TYPE			2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
#PEYERS PATCH	(18)	(46)	(49)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)

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

TABLE B1 (CONTINUED)

	CONTROL (VEH) 22-2505	LOW DOSE 22-2503	HIGH DOSE 22-2501
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
HEPATOCELLULAR ADENOMA	1 (5%)	2 (4%)	3 (6%)
HEPATOCELLULAR CARCINOMA	3 (15%)	8 (16%)	1 (2%)
#STOMACH	(20)	(48)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(16)	(37)	(48)
CORTICAL ADENOMA		1 (3%)	
#THYROID	(13)	(23)	(36)
FOLLICULAR-CELL ADENOMA			1 (3%)
REPRODUCTIVE SYSTEM			
#TESTIS	(17)	(45)	(49)
INTERSTITIAL-CELL TUMOR		1 (2%)	
SEMINOMA/DYSGERMINOMA		1 (2%)	
HEMANGIOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE B1 (CONTINUED)

	CONTROL (VEH) 22-2505	LOW DOSE 22-2503	HIGH DOSE 22-2501
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	7	5	3
MORIBUND SACRIFICE		2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	42	45
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

	CONTROL (VEH) 22-2505	LOW DOSE 22-2503	HIGH DOSE 22-2501
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	24	25
TOTAL PRIMARY TUMORS	16	30	29
TOTAL ANIMALS WITH BENIGN TUMORS	2	10	14
TOTAL BENIGN TUMORS	3	10	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	17	13
TOTAL MALIGNANT TUMORS	13	20	14
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* 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 PIVALOLACTONE

	CONTROL (VEH) 22-2506	LOW DOSE 22-2504	HIGH DOSE 22-2502
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	
ANIMALS NECROPSIED	20	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	48	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(48)	(50)
ADNEXAL CARCINOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (6%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
ADNEXAL CARCINOMA, METASTATIC			1 (2%)
RHABDOMYOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(48)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)	4 (8%)	3 (6%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)	4 (8%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (2%)	5 (10%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (5%)		2 (4%)
LEUKEMIA,NOS		1 (2%)	1 (2%)
#SPLEEN	(19)	(43)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#LYMPH NODE	(19)	(47)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#MESENTERIC L. NODE	(19)	(47)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	

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

TABLE B2 (CONTINUED)

	CONTROL (VEH) 22-2506	LOW DOSE 22-2504	HIGH DOSE 22-2502
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#LIVER	(19)	(48)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(48)	(50)
HEPATOCELLULAR CARCINOMA		2 (4%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(18)	(40)	(46)
ADENOCARCINOMA, NOS	1 (6%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(47)	(49)
LEIOMYOMA	1 (5%)		
LEIOMYOSARCOMA			1 (2%)
#OVARY	(19)	(46)	(46)
CYSTADENOMA, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(48)	(50)
ADENOMA, NOS		1 (2%)	

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

TABLE B2 (CONTINUED)

	CONTROL (VEH) 22-2506	LOW DOSE 22-2504	HIGH DOSE 22-2502
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(48)	(50)
RHABDOMYOSARCOMA		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	7	9
MORIBUND SACRIFICE	1	5	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	36	36
ANIMAL MISSING		2	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (VEH) 22-2506	LOW DOSE 22-2504	HIGH DOSE 22-2502
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	20	22
TOTAL PRIMARY TUMORS	6	22	23
TOTAL ANIMALS WITH BENIGN TUMORS	1	4	2
TOTAL BENIGN TUMORS	1	5	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	16	21
TOTAL MALIGNANT TUMORS	5	17	21
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

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

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

	CONTROL (VEH) 11-1505	LOW DOSE 11-1503	HIGH DOSE 11-1501
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(20)	(50)	(49)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
#LUNG	(20)	(50)	(49)
MINERALIZATION		1 (2%)	
CONGESTION, NOS		1 (2%)	2 (4%)
EDEMA, NOS		1 (2%)	1 (2%)
HEMORRHAGE			1 (2%)
PNEUMONIA, CHRONIC MURINE	14 (70%)	10 (20%)	3 (6%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(48)	(48)
MYELOFIBROSIS	1 (5%)		
#SPLEEN	(20)	(50)	(49)
FIBROSIS, FOCAL			1 (2%)
#CERVICAL LYMPH NODE	(20)	(49)	(48)
LYMPHANGIECTASIS		1 (2%)	
#MESENTERIC L. NODE	(20)	(49)	(48)
LYMPHANGIECTASIS		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(50)	(48)
INFLAMMATION, FOCAL			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1505	LOW DOSE 11-1503	HIGH DOSE 11-1501
FIBROSIS	5 (25%)	13 (26%)	13 (27%)
NECROSIS, NOS	1 (5%)		1 (2%)
*VEIN	(20)	(50)	(50)
THROMBOSIS, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
CONGESTION, NOS		1 (2%)	
HEPATITIS, TOXIC	1 (5%)		
METAMORPHOSIS FATTY	1 (5%)	3 (6%)	
BASOPHILIC CYTO CHANGE		3 (6%)	1 (2%)
FOCAL CELLULAR CHANGE		3 (6%)	1 (2%)
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
NECROSIS, NOS		2 (4%)	
#PANCREAS	(20)	(48)	(46)
INFLAMMATION, CHRONIC			1 (2%)
FIBROSIS, FOCAL			1 (2%)
#PANCREATIC ACINUS	(20)	(48)	(46)
ATROPHY, NOS		1 (2%)	
#ESOPHAGUS	(20)	(49)	(47)
INFLAMMATION, ACUTE NECROTIZING			2 (4%)
PARAKERATOSIS		1 (2%)	
#STOMACH	(19)	(49)	(48)
INFLAMMATION, NOS	3 (16%)		1 (2%)
INFLAMMATION, FOCAL		10 (20%)	1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
HYPERPLASIA, EPITHELIAL	5 (26%)	39 (80%)	14 (29%)
HYPERPLASIA, PAPILLARY			1 (2%)
#SMALL INTESTINE	(20)	(50)	(49)
CONGESTION, NOS			1 (2%)
HEMORRHAGE			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
#LARGE INTESTINE	(20)	(49)	(50)
NEMATODIASIS		2 (4%)	

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1505	LOW DOSE 11-1503	HIGH DOSE 11-1501
PARASITISM	6 (30%)	12 (24%)	8 (16%)
*COLON	(20)	(49)	(50)
PARASITISM		1 (2%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(49)
MINERALIZATION			1 (2%)
INFLAMMATION, CHRONIC	10 (50%)	30 (63%)	16 (33%)
#URINARY BLADDER	(17)	(43)	(43)
MUCOCELE	1 (6%)		
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(47)	(44)
CYST, NOS		1 (2%)	1 (2%)
ANGIECTASIS			1 (2%)
#ADRENAL	(20)	(48)	(49)
MINERALIZATION			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL CORTEX	(20)	(48)	(49)
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
ANGIECTASIS			1 (2%)
#ADRENAL MEDULLA	(20)	(48)	(49)
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(20)	(48)	(46)
HYPERPLASIA, C-CELL	1 (5%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYSTIC DUCTS			1 (2%)
#PROSTATE	(16)	(42)	(43)
INFLAMMATION, SUPPURATIVE			1 (2%)

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

TABLE C1 (CONCLUDED)

	CONTROL (VEH) 11-1505	LOW DOSE 11-1503	HIGH DOSE 11-1501
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
*TESTIS	(20)	(50)	(49)
MINERALIZATION	1 (5%)		1 (2%)
ATROPHY, NOS	3 (15%)	3 (6%)	3 (6%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(20)	(50)	(50)
INFLAMMATION, NOS			2 (4%)
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT			3 (6%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMOID REACTION			1 (2%)
ADIPOSE TISSUE			
INFLAMMATION, NECROTIZING			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	

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

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

	CONTROL (VEH) 11-1506	LOW DOSE 11-1504	HIGH DOSE 11-1502
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
MINERALIZATION		1 (2%)	
CONGESTION, NOS			3 (6%)
EDEMA, NOS			1 (2%)
HEMORRHAGE		1 (2%)	
BRONCHOPNEUMONIA, ACUTE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	5 (25%)	16 (32%)	8 (16%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(46)	(45)
OSTEOSCLEROSIS		1 (2%)	
#SPLEEN	(20)	(47)	(49)
NECROSIS, FOCAL	1 (5%)		
HEMOSIDEROSIS		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(48)
PERIARTERITIS		1 (2%)	
#MYOCARDIUM	(20)	(50)	(48)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, MULTIFOCAL		1 (2%)	
FIBROSIS	2 (10%)	4 (8%)	

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1506	LOW DOSE 11-1504	HIGH DOSE 11-1502
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(50)
INFLAMMATION, NECROTIZING	1 (5%)		
INFLAMMATION, CHRONIC NECROTIZIN		1 (2%)	
HEPATITIS, TOXIC			1 (2%)
NECROSIS, NOS	1 (5%)		
METAMORPHOSIS FATTY	1 (5%)	3 (6%)	1 (2%)
BASOPHILIC CYTO CHANGE		13 (28%)	7 (14%)
FOCAL CELLULAR CHANGE		1 (2%)	3 (6%)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
HYPERPLASIA, DIFFUSE		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(47)	(50)
METAMORPHOSIS FATTY			1 (2%)
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
#PANCREATIC ACINUS	(20)	(46)	(48)
ATROPHY, NOS		1 (2%)	
ATROPHY, FOCAL			1 (2%)
#STOMACH	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, FOCAL	2 (10%)	7 (14%)	3 (6%)
INFLAMMATION, CHRONIC	1 (5%)	1 (2%)	
HYPERPLASIA, EPITHELIAL	1 (5%)	32 (64%)	26 (52%)
HYPERPLASIA, PAPILLARY			2 (4%)
HYPERKERATOSIS			1 (2%)
#SMALL INTESTINE	(20)	(50)	(50)
HYPERPLASIA, FOCAL			1 (2%)
#LARGE INTESTINE	(20)	(49)	(49)
PARASITISM	8 (40%)	10 (20%)	17 (35%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
MINERALIZATION			2 (4%)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC	5 (25%)	9 (18%)	5 (10%)

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1506	LOW DOSE 11-1504	HIGH DOSE 11-1502
NEPHROPATHY, TOXIC		1 (2%)	
#KIDNEY/PELVIS	(20)	(49)	(50)
MINERALIZATION			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(46)
CYST, NOS	1 (5%)	2 (4%)	1 (2%)
HEMORRHAGIC CYST		1 (2%)	
ANGIECTASIS		2 (4%)	
#ADRENAL	(19)	(49)	(48)
FIBROSIS		1 (2%)	
METAMORPHOSIS FATTY			2 (4%)
ANGIECTASIS		1 (2%)	
#ADRENAL CORTEX	(19)	(49)	(48)
METAMORPHOSIS FATTY		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL MEDULLA	(19)	(49)	(48)
HYPERPLASIA, NOS			1 (2%)
#THYROID	(18)	(47)	(47)
HYPERPLASIA, C-CELL	1 (6%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYSTIC DUCTS		1 (2%)	1 (2%)
#UTERUS	(20)	(50)	(49)
CYST, NOS			1 (2%)
PYOMETRA	3 (15%)	1 (2%)	1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(50)	(49)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS	3 (15%)	2 (4%)	1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, NOS	1 (5%)	3 (6%)	1 (2%)
HYPERPLASIA, CYSTIC			2 (4%)

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

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 11-1506	LOW DOSE 11-1504	HIGH DOSE 11-1502
#OVARY/OVIDUCT	(20)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (5%)	1 (2%)	
#OVARY	(20)	(47)	(50)
CYST, NOS			3 (6%)
PAROVARIAN CYST	1 (5%)	4 (9%)	2 (4%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
HYDROCEPHALUS, INTERNAL			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT	1 (5%)	1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMOID REACTION			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	

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

APPENDIX D

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

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

	CONTROL (VEH) 22-2505	LOW DOSE 22-2503	HIGH DOSE 22-2501
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
FOREIGN BODY, NOS		1 (2%)	
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(49)
PNEUMONIA, ASPIRATION	1 (5%)		1 (2%)
PNEUMONIA, CHRONIC MURINE		7 (14%)	11 (22%)
PERIVASCULITIS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(47)	(50)
FIBROSIS, FOCAL	1 (6%)		
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
HEMATOPOIESIS		1 (2%)	
#LYMPH NODE	(19)	(48)	(46)
HYPERPLASIA, LYMPHOID		2 (4%)	
#MESENTERIC L. NODE	(19)	(48)	(46)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (5%)		1 (2%)
CIRCULATORY SYSTEM			
#MITRAL VALVE	(19)	(48)	(49)
DEGENERATION, HYALINE		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL (VEH) 22-2505	LOW DOSE 22-2503	HIGH DOSE 22-2501
*TESTICULAR ARTERY	(20)	(49)	(50)
DEGENERATION, HYALINE		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
EMBOLISM, NOS		1 (2%)	
NECROSIS, NOS	1 (5%)		
NECROSIS, FOCAL	2 (10%)		
HEPATOCYTOMEGALY		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(49)	(50)
METAMORPHOSIS FATTY	1 (5%)		
#LIVER/HEPATOCYTES	(20)	(49)	(50)
HYPERPLASIA, NOS			1 (2%)
#ESOPHAGUS	(19)	(48)	(48)
INFLAMMATION, NOS		1 (2%)	
#STOMACH	(20)	(48)	(50)
INFLAMMATION, NOS			1 (2%)
#SMALL INTESTINE	(18)	(46)	(49)
NEMATODIASIS			1 (2%)
#PEYERS PATCH	(18)	(46)	(49)
ABSCESS, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (6%)	3 (7%)	3 (6%)
#LARGE INTESTINE	(19)	(49)	(49)
NEMATODIASIS		7 (14%)	14 (29%)
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(50)
GLOMERULONEPHRITIS, NOS		1 (2%)	
SCLEROSIS			1 (2%)
PERIVASCULITIS		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(13)	(23)	(36)
CYSTIC FOLLICLES			3 (8%)

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

TABLE D1 (CONTINUED)

	CONTROL (VEH) 22-2505	LOW DOSE 22-2503	HIGH DOSE 22-2501
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(49)	(50)
CYST, NOS			1 (2%)
#TESTIS	(17)	(45)	(49)
ATROPHY, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(49)
PSAMMOMA BODIES		2 (4%)	10 (20%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(49)	(50)
PARASITISM		1 (2%)	
BODY CAVITIES			
*MESENTERY	(20)	(49)	(50)
PERIARTERITIS			1 (2%)
NECROSIS, FAT		2 (4%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
PERIVASCULITIS			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	12	9
ANIMAL MISSING/NO NECROPSY		1	

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

TABLE D1 (CONCLUDED)

	CONTROL (VEH) 22-2505	LOW DOSE 22-2503	HIGH DOSE 22-2501
AUTO/NECROPSY/HISTO PERF	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 PIVALOLACTONE

	CONTROL (VEH) 22-2506	LOW DOSE 22-2504	HIGH DOSE 22-2502
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	
ANIMALS NECROPSIED	20	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	48	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(48)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(50)
PNEUMONIA, ASPIRATION		1 (2%)	
PNEUMONIA, CHRONIC MURINE	10 (50%)	5 (10%)	12 (24%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(43)	(48)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID	1 (5%)		
#LYMPH NODE	(19)	(47)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MANDIBULAR L. NODE	(19)	(47)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(19)	(47)	(49)
HYPERPLASIA, NOS	1 (5%)		
HYPERPLASIA, RETICULUM CELL			1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(46)	(49)
FIBROSIS		1 (2%)	

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

TABLE D2 (CONTINUED)

	CONTROL (VEH) 22-2506	LOW DOSE 22-2504	HIGH DOSE 22-2502
DIGESTIVE SYSTEM			
#LIVER	(19)	(48)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		2 (4%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY		2 (4%)	
HEMATOPOIESIS			2 (4%)
#PANCREAS	(19)	(43)	(49)
DILATATION/DUCTS	1 (5%)		
#STOMACH	(19)	(48)	(50)
HYPERPLASIA, EPITHELIAL		1 (2%)	
#PEYERS PATCH	(19)	(47)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
#COLON	(19)	(47)	(50)
NEMATODIASIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(49)
HYDRONEPHROSIS			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR		5 (10%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
PERIARTERITIS			1 (2%)
INFARCT, NOS		1 (2%)	
#URINARY BLADDER	(18)	(43)	(45)
INFLAMMATION, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(34)	(36)
ANGIECTASIS			1 (3%)
#THYROID	(7)	(31)	(29)
CYSTIC FOLLICLES		1 (3%)	2 (7%)
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(48)	(50)
HYPERPLASIA, FOCAL		1 (2%)	

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

TABLE D2 (CONTINUED)

	CONTROL (VEH) 22-2506	LOW DOSE 22-2504	HIGH DOSE 22-2502
*UTERUS/ENDOMETRIUM	(19)	(47)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, NOS	1 (5%)	1 (2%)	3 (6%)
HYPERPLASIA, CYSTIC	11 (58%)	16 (34%)	22 (45%)
*OVARY	(19)	(46)	(46)
CYST, NOS	4 (21%)	3 (7%)	9 (20%)
NERVOUS SYSTEM			
*BRAIN	(19)	(48)	(50)
PSAMMOMA BODIES	2 (11%)		3 (6%)
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(48)	(50)
HYPERPLASIA, EPITHELIAL	1 (5%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(48)	(50)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(48)	(50)
HYPERPLASIA, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID	1 (5%)	2 (4%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		6	4
ANIMAL MISSING/NO NECROPSY		2	

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

TABLE D2 (CONCLUDED)

	CONTROL (VEH) 22-2506	LOW DOSE 22-2504	HIGH DOSE 22-2502
AUTO/NECROPSY/HISTO PERF	1		

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

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

June 29, 1978

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

The reviewer noted that the substance is a structural analog of the human carcinogen Beta-Propiolactone. He agreed with the conclusion in the report that Pivalolactone was carcinogenic in treated rats under the conditions of test. He expressed surprise that it also was not carcinogenic in the treated mice. The reviewer conjectured that the Pivalolactone may have hydrolyzed to an innocuous substance by the time it was administered to the mice, thus accounting for its lack of carcinogenic activity. He pointed out that Beta-Propiolactone hydrolyzes to propionic acid in the presence of water. The reviewer recommended that an analysis be done to determine the half-life of Pivalolactone in water. The results of such an analysis might be helpful in assessing the dose of the test substance administered to the mice. The reviewer noted the small control group size and the route of exposure as experimental shortcomings. Despite these deficiencies, he said that Pivalolactone should be considered to pose a carcinogenic risk to man. He moved that the report on the bioassay of Pivalolactone be accepted as written.

A Subgroup member suggested that the lack of a carcinogenic response in the mice may have been due to a vitamin deficiency. Another Subgroup member expressed concern regarding the lack of knowledge about the precise identity of the test substance administered. The reviewer explained that his concern was not in its identity but rather whether the Pivalolactone decomposed to an innocuous substance. A staff member said that the program could undertake some analysis of the stability of Pivalolactone in water. It was agreed to accept the report on the bioassay Pivalolactone as written. It was noted, however, that additional information on the half-life of the compound in water was desired.

Clearinghouse Members present:

Arnold L. Brown (Chairman), Mayo Clinic
Paul Nettesheim, National Institute of Environmental Health Sciences
Verne Ray, Pfizer Medical Research Laboratory
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

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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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