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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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MICHLER'S KETONE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

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REPORT ON THE BIOASSAY OF MICHLER'S KETONE
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 Michler's ketone 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 Michler's ketone was conducted by Litton Bionetics, Inc., Kensington, 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. F. M. Garner (4) 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 analyses were performed by Litton Bionetics, Inc. (4), and Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

Histopathologic examinations were performed at Litton Bionetics, Inc. (4). Dr. R. L. Schueler (8) reviewed the liver lesions of the rats and Dr. Sagartz (8) reviewed those of the mice. The diagnoses included in this report represent the interpretation of these pathologists.

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. R. M. Helfand (7) and Dr. J. P. Dirkse, III (10) 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.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. A. R. Patel (1), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay for the possible carcinogenicity of technical-grade Michler's ketone was conducted using Fischer 344 rats and B6C3F1 mice. Michler's ketone was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of Michler's ketone were respectively, 500 and 250 ppm for male rats, 1000 and 500 ppm for female rats, and 2500 and 1250 ppm for mice of both sexes. The compound was administered to rats and mice for 78 weeks. The period of compound administration was followed by an observation period of 28 weeks for male and high dose female rats, 29 weeks for low dose female rats and 13 weeks for mice.

There were significant positive associations between the concentrations of Michler's ketone administered and mortality in rats and mice of both sexes. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. There was distinct dose-related mean body weight depression in female rats and in mice of both sexes, and the mean body weight among dosed male rats was slightly lower than that in controls, indicating that the concentrations of Michler's ketone administered to these animals in this bioassay may have approximated the maximum tolerated concentrations.

There were significant positive associations between the concentrations of Michler's ketone administered and the incidences of hepatocellular carcinomas in both sexes of rats and in female mice and hemangiosarcomas in male mice. In all of these cases the high dose to control Fisher exact comparison was also significant.

Under the conditions of this bioassay, dietary administration of Michler's ketone was carcinogenic to male and female Fischer 344 rats and female B6C3F1 mice, causing hepatocellular carcinomas, and to male B6C3F1 mice, causing hemangiosarcomas.

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I. INTRODUCTION

Michler's ketone (Figure 1) (NCI No. C02006), a dye intermediate and derivative of dimethylaniline, was selected for bioassay by the National Cancer Institute because of the elevated incidence of bladder cancer noted among dye manufacturing industry workers (Anthony and Thomas, 1970; Wynder et al., 1963). Aromatic amino compounds are one of several classes of chemicals thought to be responsible for the increased cancer risk in this industry (Clayson and Garner, 1976).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is bis[4-(dimethylamino)phenyl]methanone.* It is also called, 4,4'-bis(dimethylamino)benzophenone; p,p'-bis(dimethylamino)benzophenone; bis[p-(N,N'-dimethylamino)phenyl] ketone; and tetramethyldiaminobenzophenone.

Michler's ketone is used in the manufacture of at least 13 dyes and pigments: C.I. (Colour Index) Acid Blue 34; C.I. Acid Blue 86; C.I. Acid Blue 88; C.I. Acid Violet 15; C.I. Acid Violet 38; Acid Violet 2B; C.I. Basic Orange 23; C.I. Basic Violet 3; C.I. Basic Yellow 2; Ceres Blue I; Fanal Blue 3B supra; C.I. Solvent Violet 9; and C.I. Solvent Yellow 34 (Society of Dyers and Colourists, 1956).

Specific production data for Michler's ketone are not available; however, this compound is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) by one U.S. company (U.S. International Trade Commission, 1977). C.I. Basic Violet 3,

*The CAS registry number is 90-94-8.

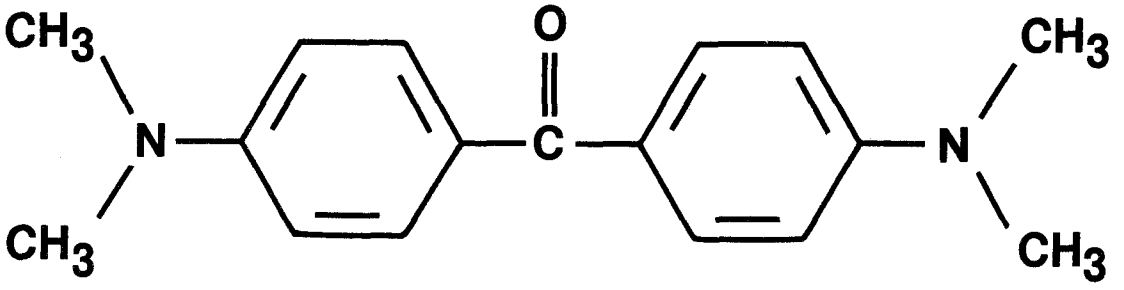

FIGURE 1
CHEMICAL STRUCTURE OF MICHLER'S KETONE

C.I. Basic Yellow 2, and C.I. Solvent Violet 9 are also produced in commercial quantities (U.S. International Trade Commission, 1977), although not necessarily from Michler's ketone (Society of Dyers and Colourists, 1956).

The potential for exposure to Michler's ketone is greatest for workers in facilities which manufacture the compound or any of the dyestuffs for which Michler's ketone is an intermediate.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade Michler's ketone was purchased from E.I. Dupont de Nemours & Company, Wilmington, Delaware. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland, and by Midwest Research Institute, Kansas City, Missouri. The following are the results of the Litton Bionetics, Inc., analyses. The experimentally determined range in melting point, 172° to 174°C, was compared to the literature value of 172°C (Grasselli and Ritchey, 1975). Thin-layer chromatography was performed utilizing two solvent systems (i.e., chloroform:ammonium hydroxide:methanol and benzene:methanol). Each plate was visualized with ultraviolet and visible light, iodine vapor, and ferric chloride-ferricyanide spray. The plate developed with the first solvent system revealed one single spot, while the plate developed with the second solvent system revealed one large and one faint spot. The results of infrared analysis were consistent with those expected based on the structure of the compound. The results of nuclear magnetic resonance analysis indicated a purity of 85 percent. Ultraviolet/visible analysis revealed λ_{max} at 245 and 370 nm with respective molar extinction coefficients of 1.62×10^4 and 3.33×10^4.

The results of the analyses performed by Midwest Research Institute subsequent to completion of the bioassay follow. Thin-layer chromatography was performed utilizing two solvent systems (i.e.,

isopropanol and benzene:methanol). Each plate was visualized with visible and ultraviolet light and ferric chloride-ferricyanide spray. The plate developed with the first solvent system revealed the major spot and three impurities, while the plate developed with the second solvent system revealed the major spot and four impurities. The results of elemental analysis were within 3 percent of those expected based on the molecular formula of the compound, $C_{17}H_{20}N_2O$. Titration of the amine group with perchloric acid indicated a purity of 99 percent. Vapor-phase chromatography revealed one major peak and 10 minor impurities. The results of nuclear magnetic resonance analysis were consistent with the structure of the compound and showed no detectible aldehyde proton resonance in either deuterated chloroform or deuterated pyridine.

A representative of DuPont Haskell Laboratory has stated that the Michler's ketone used in this bioassay is not typical of the usual DuPont technical-grade product. In a summary statement, he indicated the following: "The lot differs from our normal technical product in its behavior in the Ames test. Unlike normal DuPont technical Michler's ketone, which is negative in the Ames test for mutagenicity, the sample used in the NCI bioassay is positive. The cause of the difference is unknown."

Throughout this report, the term Michler's ketone is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). Michler's ketone was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a proper amount was blended with an aliquot of the feed using a mortar and pestle. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

Dosed feed preparations containing 1000 and 250 ppm of Michler's ketone were analyzed spectrophotometrically for Michler's ketone. The mean result immediately after preparation was 99 percent of theoretical (ranging from 94 to 109 percent). After 10 days at 4°C, the mean result was 90 percent of theoretical (ranging from 87 to 93 percent).

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats and mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice, approximately 4 weeks old when received, were examined and any obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks and those 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

Animals were housed by species in rooms maintained at 22° to 26°C and 45 to 55 percent relative humidity. 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.).

Rats were housed four per cage by sex and mice were housed 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 piece of stainless steel mesh 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 (Ab-sorb-dri® hardwood chip bedding, Wilner Wood Products Company, Norway, Maine) were provided twice weekly.

Acidulated water (pH 2.5) was supplied to animals in water bottles which were changed and washed twice weekly. Sipper tubes were

washed at weekly intervals. During the period of chemical administration, dosed and control animals received treated or untreated Wayne Lab-Blox® meal as appropriate. The feed was supplied 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* p-chloroaniline (106-47-8); trimethylthiourea (2489-77-2); and p-nitrosodiphenylamine (156-10-5).

All dosed and control mice were housed in a room with other mice receiving diets containing 2-nitro-p-phenylenediamine (5307-14-2); 4,4'-methylenebis(N,N-dimethyl)benzenamine (101-61-1); p-chloroaniline (106-47-8); 5-chloro-o-toluidine (95-79-4); N-phenyl-p-phenylenediamine hydrochloride (2198-59-6); 1-phenyl-2-thiourea (103-85-5); trimethylthiourea (2489-77-2); dibutyltin diacetate (1067-33-0); and 3-chloro-p-toluidine (95-74-9).

E. Selection of Initial Concentrations

To establish the concentrations of Michler's ketone for administration to dosed 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. Michler's ketone was incorporated into the

*CAS registry numbers are given in parentheses.

basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 680, 1465, 3155, 6800 and 14,665 ppm and to five of the six mouse groups in concentrations of 440, 1390, 3000, 6480 and 13,900 ppm. The remaining group of each species served as a control group, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 4 weeks, followed by a 2-week observation period during which all animals were fed the basal laboratory diet. Individual body weights were recorded twice weekly throughout the study. Upon termination of the study all survivors were euthanized and necropsied.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

Concentration (ppm)	Mean Body Weight Gain (%)*		Survival**	
	Males	Females	Males	Females
14,665	--	--	0/5	0/5
6,800	-118	--	2/5	0/5
3,155	-111	-49	5/5	3/5
1,465	- 69	-32	5/5	5/5
680	- 40	- 1	5/5	5/5
0	--	--	5/5	5/5

No other clinical abnormalities which could be attributed to administration of the compond were observed. The high concentrations

*+ is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.
**Number of animals observed/number of animals originally in group.

selected for administration to dosed rats in the chronic bioassay were 500 and 1000 ppm for males and females, respectively.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the mouse groups at the end of the subchronic test.

MOUSE SUBCHRONIC STUDY RESULTS

Concentration (ppm)	Mean Body Weight Gain (%)*		Survival**	
	Males	Females	Males	Females
13,900	-15	-3	4/5	4/5
6,480	-15	0	5/5	5/5
3,000	- 8	-8	5/5	5/5
1,390	- 2	+4	5/5	5/5
440	+ 4	+5	5/5	5/5
0	--	--	5/5	5/5

No other clinical abnormalities which could be attributed to administration of the compound were observed. The high concentration selected for administration to dosed mice in the chronic bioassay was 2500 ppm.

F. Experimental Design

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

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*+ is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

**Number of animals observed/number of animals originally in group.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
MICHLER'S KETONE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	MICHLER'S KETONE CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	106
LOW DOSE	50	250 0	78	28
HIGH DOSE	50	500 0	78	28
FEMALE				
CONTROL	20	0	0	106
LOW DOSE	50	500 0	78	29
HIGH DOSE	50	1000 0	78	28

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
MICHLER'S KETONE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	MICHLER'S KETONE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	91
LOW DOSE	50	1250	78	
		0		13
HIGH DOSE	50	2500	78	
		0		13
FEMALE				
CONTROL	20	0	0	91
LOW DOSE	50	1250	78	
		0		13
HIGH DOSE	50	2500	78	
		0		13

[a]Concentrations given in parts per million.

Rats were approximately 6 weeks old at the time the test was initiated and all were placed on test on the same day. The dietary concentrations of Michler's ketone administered to male rats were 500 and 250 ppm. Throughout this report those male rats receiving the former concentration are referred to as the high dose group and those receiving the latter concentration are referred to as the low dose group. The dietary concentrations of Michler's ketone administered to female rats were 1000 and 500 ppm. Throughout this report those female rats receiving the former concentration are referred to as the high dose group and those receiving the latter concentration are referred to as the low dose group. Dosed rats were supplied with feed containing Michler's ketone for 78 weeks followed by a 28-week observation period for males and high dose females and a 29-week observation period for low dose females.

Mice were approximately 6 weeks old at the time the test was initiated and all were placed on test on the same day. The dietary concentrations of Michler's ketone administered were 2500 and 1250 ppm. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Dosed mice were supplied with feed containing Michler's ketone for 78 weeks followed by a 13-week observation period.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights of rats were recorded once a week for the first 6 weeks, every 2 weeks for the next 6 weeks, at monthly intervals for the next 8 weeks, and once every 2 months thereafter. Body weights of mice were recorded once a week for the first 6 weeks, every 2 weeks for the next 6 weeks, at monthly intervals for the next 8 weeks, once every 2 months for the next 27 weeks, and at monthly intervals for the remainder of the bioassay. All animals were inspected twice daily. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group.

All moribund animals, animals that developed large, palpable masses that jeopardized their health, or animals that survived until the end of the bioassay were euthanized using carbon dioxide. Necropsies were immediately performed on these animals and on all animals found dead during the bioassay. Gross and microscopic examinations were performed on 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.

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) 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

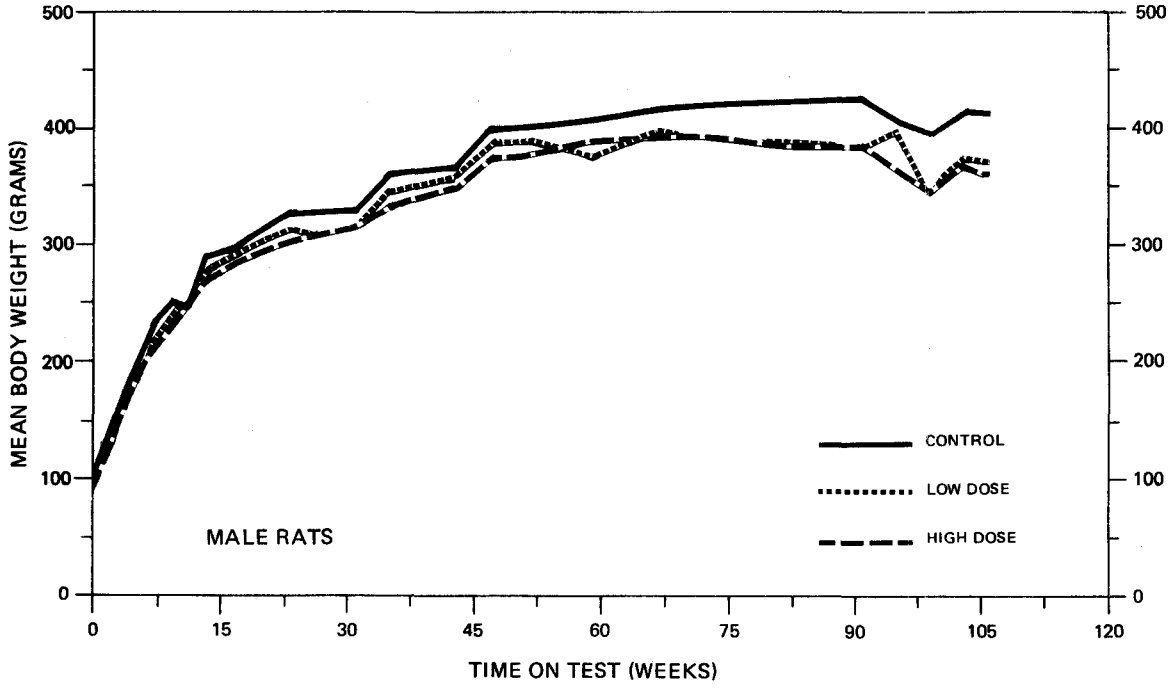
In female rats distinct and consistent dose-related mean body weight depression was apparent throughout the bioassay. Although the concentrations of Michler's ketone fed to male rats were one-half the concentrations fed to females, the mean body weights among the high and low dose males were slightly lower than the mean body weight for the male control rats (Figure 2).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and Michler's ketone-dosed groups are shown in Figure 3. The Tarone test for association between dosage and mortality was significant for males ($P = 0.003$) and females ($P < 0.001$). For female rats, the departure from linear trend was also significant ($P < 0.001$), reflecting the elevated mortality in the high dose group. This was supported by the Cox test which indicated a significant comparison ($P < 0.001$) between the high dose female group and the control although not between the low dose female group and the control.

There were adequate numbers of male rats at risk from late-developing tumors as 68 percent (34/50) of the high dose, 82 percent (41/50) of the low dose, and 100 percent (20/20) of the controls survived on test until the termination of the study. Of those males



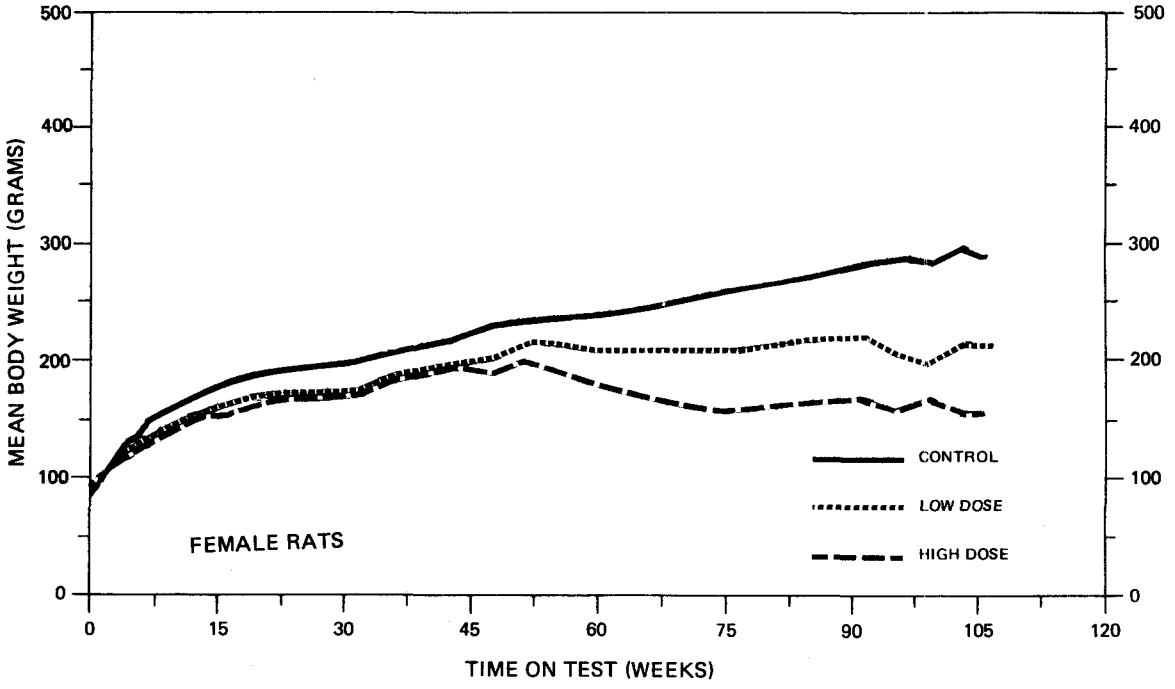
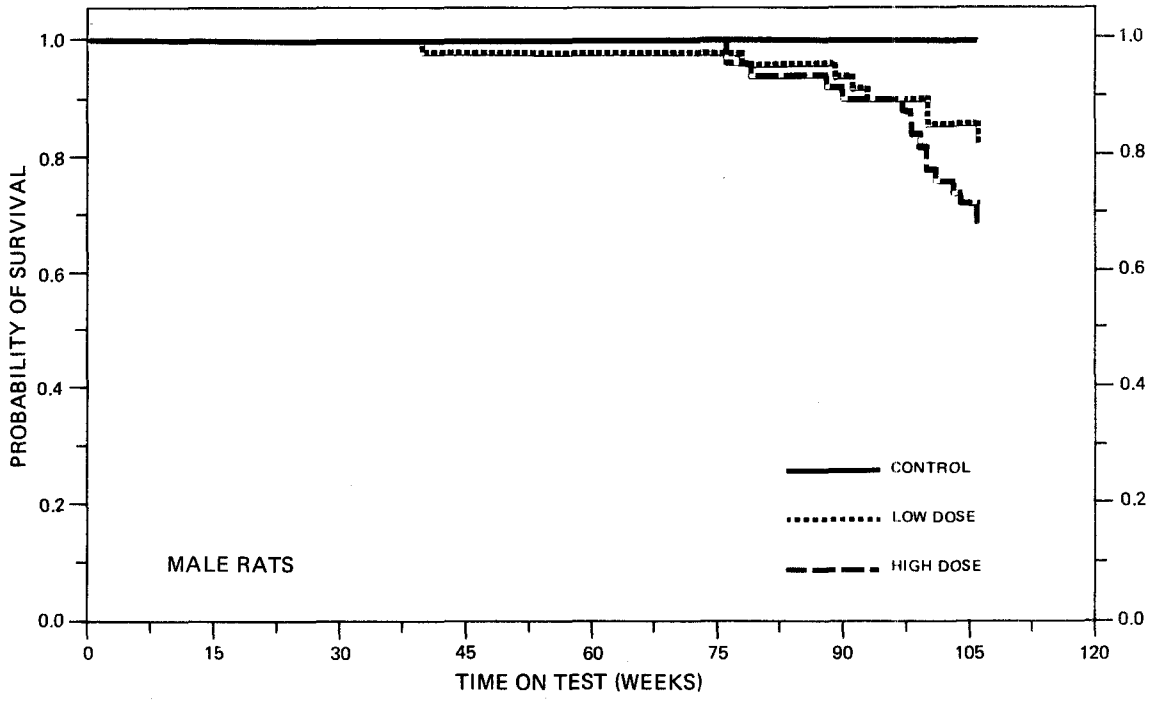


FIGURE 2
GROWTH CURVES FOR MICHLER'S KETONE CHRONIC STUDY RATS



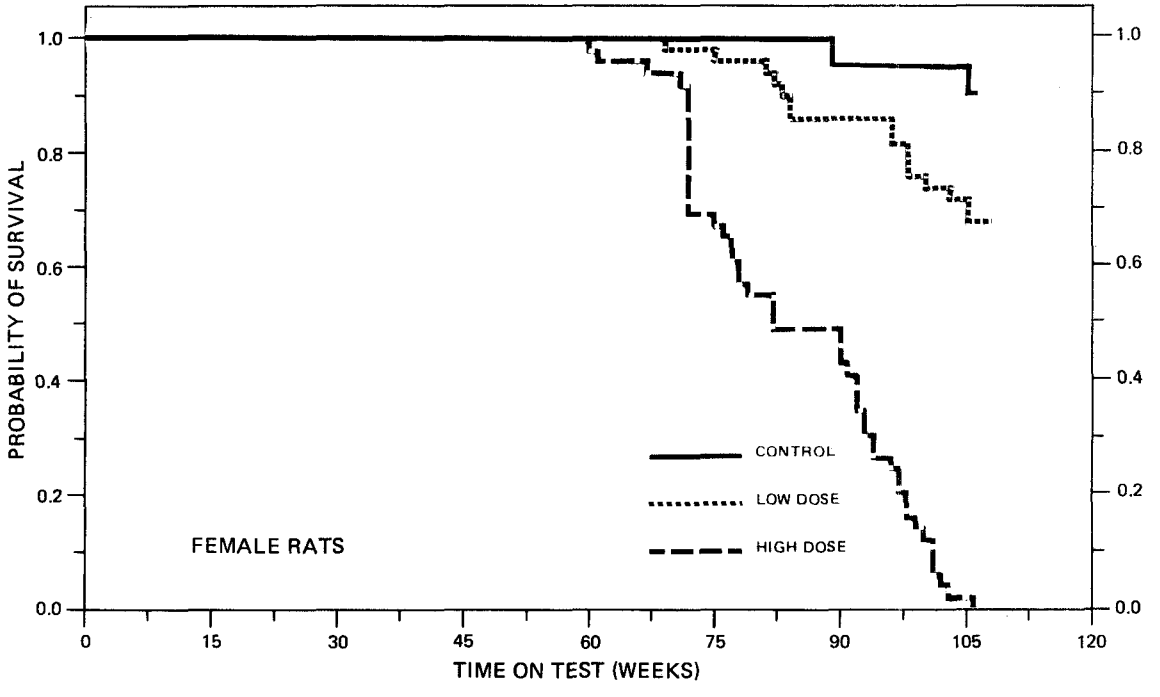


FIGURE 3
SURVIVAL COMPARISONS OF MICHLER'S KETONE CHRONIC STUDY RATS

that died prior to the end of the bioassay, all but one low dose male survived on test for at least 76 weeks.

There were also adequate numbers of female rats at risk from late-developing tumors. Although no high dose females survived until the termination of the bioassay, 98 percent (49/50) survived on test for at least 60 weeks. All low dose females survived on test for at least 69 weeks and 66 percent (33/50) survived until the termination of the study. Ninety percent (18/20) of the controls survived on test until the termination of the study. One low dose female was missing in week 107 and one high dose female was missing in week 35.

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).

While a variety of neoplasms were present in the control as well as dosed rats, there was a definite increase in the incidence of neoplasia in the dosed rats. Most of the high dose female rats became moribund and were killed or died before the end of the study with liver neoplasms which in many cases metastasized to the lungs. Virtually all of the high dose male and female rats and low dose female rats had neoplastic lesions of the liver as did nearly one-third of the low dose males. The morphologic spectrum included both neoplastic nodules and hepatocellular carcinomas.

The neoplastic nodules varied from a small compressing lesion within a single liver lobe to multiple nodules involving one or more lobes. The morphology of the neoplastic hepatocytes was inconstant between nodules as well as within the same nodule. Eosinophilic, clear, and basophilic cells, and trabecular patterns of well-differentiated hepatocytes were all seen.

The carcinomas were usually larger and involved one or more lobes. Many metastasized to the lung. The appearance of the neoplastic cells varied from fairly well-differentiated hepatocytes proliferating in cords of two or more cells thick to sheets and masses of pleomorphic anaplastic cells invading or completely replacing normal liver cells within the lobe. In some areas the neoplastic hepatocytes formed structures resembling acini or proliferating biliary epithelium. Large cystic spaces, hemorrhage and necrosis were frequently present.

The usual variety of nonneoplastic lesions seen in older rats were present in both the dosed and control rats. All were considered to be spontaneous lesions and not related to the administration of the chemical.

Based on the results of this pathology examination, Michler's ketone was carcinogenic to male and female Fischer 344 rats, inducing hepatocellular carcinomas, under the conditions of this bioassay.

D. Statistical Analyses of Results

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

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH MICHLER'S KETONE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin: Squamous-Cell Carcinoma[b]	0/20(0.00)	1/50(0.02)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.022	0.386
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	106	76
Liver: Hepatocellular Carcinoma[b]	0/20(0.00)	9/50(0.18)	40/50(0.80)
P Values[c]	P < 0.001	P = 0.039	P < 0.001
Departure from Linear Trend[e]	P = 0.016	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.096	5.727
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	76
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/20(0.00)	17/50(0.34)	43/50(0.86)
P Values[c]	P < 0.001	P = 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	2.256	6.247
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	76

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Carcinoma or Chromophobe Adenoma[b]	3/17(0.18)	4/39(0.10)	2/42(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.581	0.270
Lower Limit	---	0.114	0.025
Upper Limit	---	3.655	2.190
Weeks to First Observed Tumor	106	106	106
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	1/16(0.06)	3/33(0.09)	4/43(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.455	1.488
Lower Limit	---	0.132	0.167
Upper Limit	---	73.940	71.511
Weeks to First Observed Tumor	106	106	79
Pancreatic Islets: Islet-Cell Adenoma[b]	0/20(0.00)	3/47(0.06)	3/47(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.266	0.266
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	106	106

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor[b]	16/20(0.80)	43/49(0.88)	45/48(0.94)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.097	1.172
Lower Limit	---	0.880	0.948
Upper Limit	---	1.457	1.429
Weeks to First Observed Tumor	106	89	76

[a]Treated groups received doses of 250 or 500 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. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

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

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH MICHLER'S KETONE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/20(0.00)	41/47(0.87)	44/49(0.90)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	6.363	6.617
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	75	61
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/20(0.00)	46/47(0.98)	48/49(0.98)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	7.836	7.854
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	69	60
Pituitary: Chromophobe Adenoma[b]	4/16(0.25)	3/44(0.07)	0/31(0.00)
P Values[c]	P = 0.005(N)	N.S.	P = 0.010(N)
Relative Risk (Control)[d]	---	0.273	0.000
Lower Limit	---	0.047	0.000
Upper Limit	---	1.470	0.539
Weeks to First Observed Tumor	89	84	---

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma[b]	2/20(0.10)	3/46(0.07)	0/41(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.652	0.000
Lower Limit	---	0.083	0.000
Upper Limit	---	7.437	1.632
Weeks to First Observed Tumor	105	100	---
Mammary Gland: Fibroadenoma or Adenoma NOS[b]	5/20(0.25)	2/49(0.04)	0/49(0.00)
P Values[c]	P = 0.001(N)	P = 0.019(N)	P = 0.001(N)
Relative Risk (Control)[d]	---	0.163	0.000
Lower Limit	---	0.017	0.000
Upper Limit	---	0.918	0.319
Weeks to First Observed Tumor	105	69	---

[a]Treated groups received doses of 500 or 1000 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. 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 $P < 0.005$.

every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or Michler's ketone-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male rats the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of hepatocellular carcinomas. This was supported by a significant ($P < 0.001$) Fisher exact test comparing high dose to control. The low dose to control Fisher exact comparison had a P value of 0.039 which was not significant under the Bonferroni criterion. The test for departure from linear trend was also significant ($P = 0.016$) at this site.

The Cochran-Armitage test also indicated a significant ($P < 0.001$) positive association between dose and the incidence of a combination of hepatocellular carcinomas or neoplastic nodules in male rats. The Fisher exact test comparing high dose to control was significant ($P < 0.001$) as was the low dose to control comparison ($P = 0.001$).

In female rats the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of hepatocellular carcinomas. The Fisher exact tests were significant when comparing high dose to control ($P < 0.001$) and low dose to control ($P < 0.001$). The test for departure from linear trend was also significant ($P < 0.001$).

For female rats the Cochran-Armitage test also indicated a significant ($P < 0.001$) positive association between dose and the incidence of a combination of hepatocellular carcinomas or neoplastic nodules. This was supported by significant Fisher exact tests comparing high dose to control ($P < 0.001$) and low dose to control ($P < 0.001$). Here again the test for departure from linear trend was significant ($P < 0.001$).

In female rats the Cochran-Armitage test indicated a significant negative association between dose and the incidence of chromophobe adenomas of the pituitary. The Fisher exact test comparing high dose to control supported this negative association but the Fisher exact test comparing low dose to control did not meet the Bonferroni criterion. Also in females a significant negative association was indicated by the Cochran-Armitage test between dose and the incidence of a combination of fibroadenomas or adenomas NOS of the mammary gland. This negative association was supported by both the Fisher exact tests.

Based on these statistical results the administration of Michler's ketone was carcinogenic to both male and female Fischer 344 rats under the conditions of this bioassay. The administration of the compound was associated with a significantly increased incidence of hepatocellular carcinomas and also with a significantly increased incidence of a combination of hepatocellular carcinomas or neoplastic nodules of the liver in both male and female rats.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Distinct and consistent dose-related mean body weight depression was apparent in both male and female mice after week 30 (Figure 4).

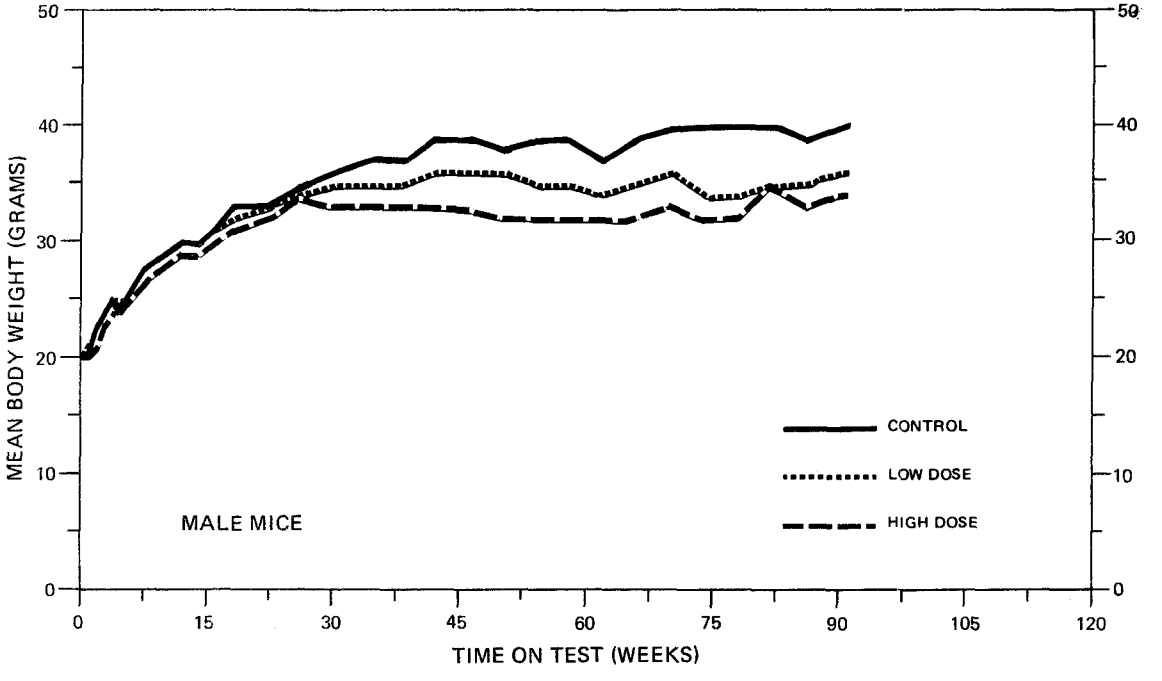
No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and Michler's ketone-dosed groups are shown in Figure 5. The Tarone test for association between dosage and mortality was significant for males ($P < 0.001$) and females ($P = 0.005$). For male mice the departure from linear trend was also significant ($P = 0.004$), reflecting the elevated mortality in the high dose group. This was supported by the Cox test which indicated a significant comparison ($P < 0.001$) between the high dose male group and the control although not between the low dose male group and the control.

Despite the elevated mortality in the high dose group, adequate numbers of male mice were at risk from late-developing tumors. While only 14 percent (7/50) of the high dose group was available until the termination of the study, all high dose males survived on test for at least 66 weeks. Eighty percent (40/50) of the low dose and 90 percent (18/20) of the controls survived on test until the end of the bioassay.

There were adequate numbers of female mice at risk from late-developing tumors, as 66 percent (33/50) of the high dose, 84



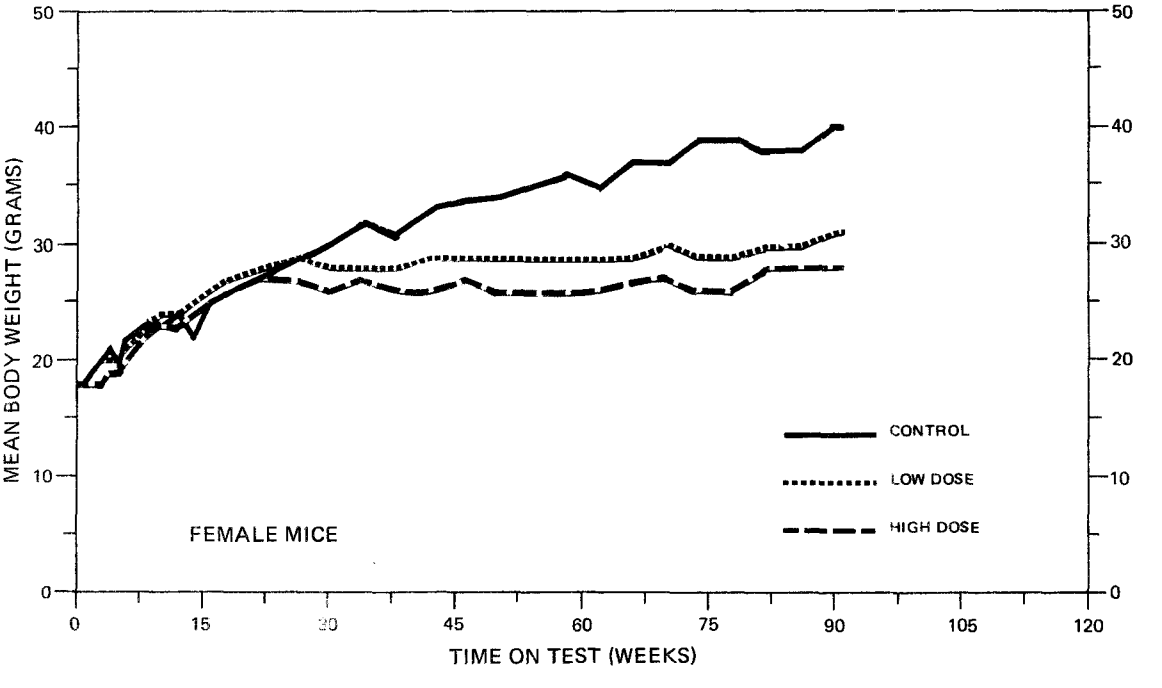
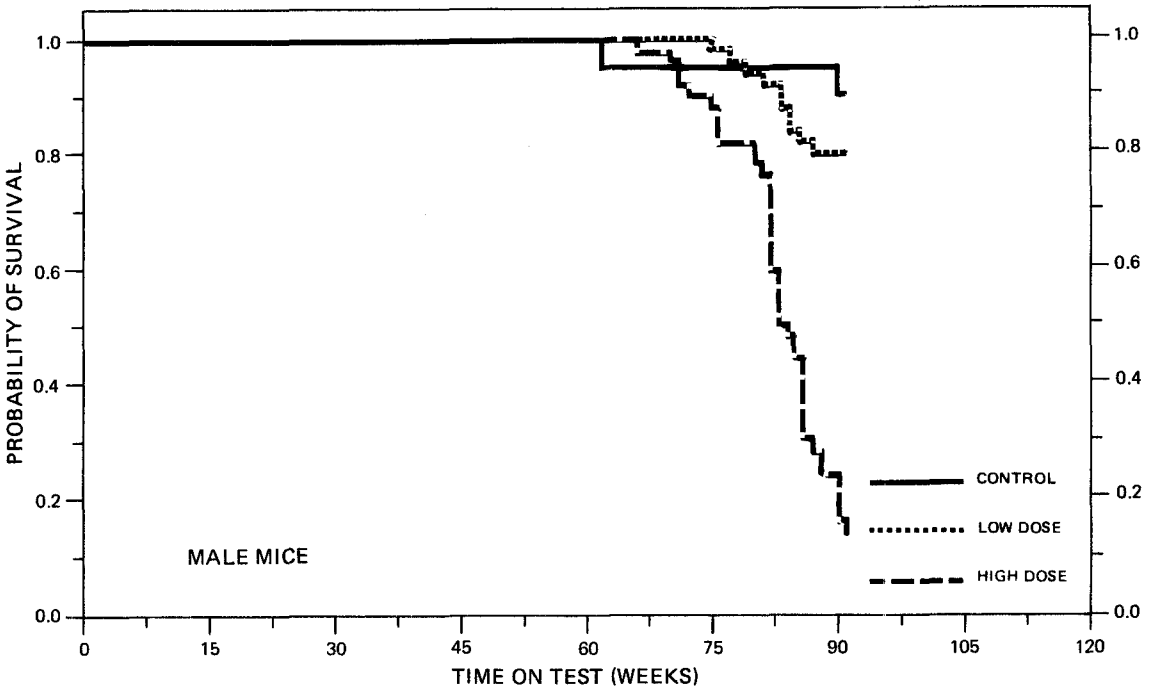


FIGURE 4
GROWTH CURVES FOR MICHLER'S KETONE CHRONIC STUDY MICE



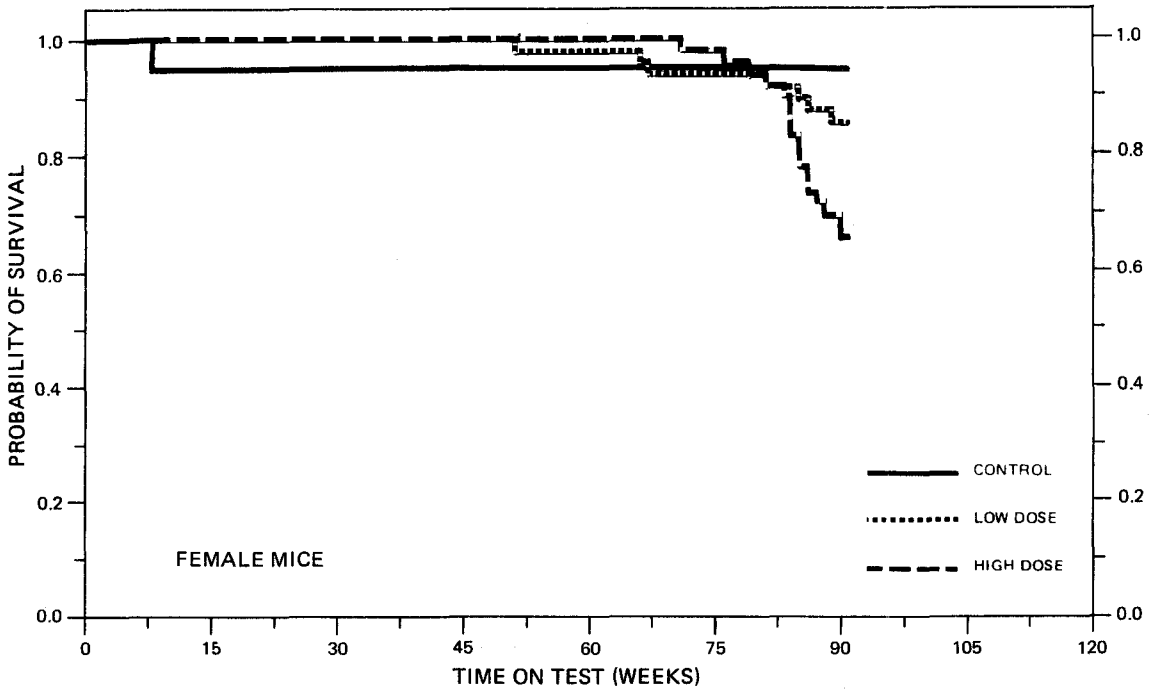


FIGURE 5
SURVIVAL COMPARISONS OF MICHLER'S KETONE CHRONIC STUDY MICE

percent (42/50) of the low dose and 90 percent (18/20) of the controls survived on test until the termination of the study. One low dose female was missing in week 50 and one control female was missing in week 59.

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).

While a variety of neoplasms was present in all groups of mice, there was a definite increase in the incidence of neoplasia in dosed mice of both sexes.

Approximately 50 percent of the high dose male mice developed malignant mesenchymal tumors, predominantly hemangiosarcomas involving the subcutis. Grossly, these appeared as raised, firm 1 to 2 cm nodules on the midline between the shoulders or about the head and neck. When excised they were found to be spongy and hemorrhagic. Microscopically, there were lakes of red blood cells bordered by proliferating spindle-shaped cells with scanty cytoplasm and large, strongly basophilic pleomorphic nuclei. Occasionally these tumors appeared as sheets of spindle cells with numerous mitotic figures and formed few vascular spaces. These lesions infiltrated the subcutaneous adipose tissue and invaded the underlying muscle. Metastatic lesions appeared as vascular spaces surrounded by cells similar to those previously described.

The majority of the female mice had liver lesions (hepatocellular adenomas and hepatocellular carcinomas). Adenomatous tumors usually involved a single lobe and consisted of expanding cords of neoplastic cells distinctly demarcated from contiguous normal liver tissue. The hepatocytes varied widely in their histologic appearance. Some were well-differentiated, while others were slightly basophilic, eosinophilic or of the vacuolated cell type. In the carcinomas, the hepatocytes grew in plates of two or more cells thick or in irregular masses with accompanying areas of hemorrhage and necrosis. Glandular differentiation was present in some cases. The individual neoplastic hepatocytes were often fairly well-differentiated, but bizarre pleomorphic megalocytes occurred and mitoses were frequent in some of the carcinomas.

The nonneoplastic lesions usually seen in older mice were present in the dosed and control groups and were not considered to be related to the action of the test chemical.

Based on the results of this pathology examination, Michler's ketone was carcinogenic in B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH MICHLER'S KETONE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin and Subcutaneous Tissue: Fibrosarcoma[b]	0/19(0.00)	2/50(0.04)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.117	0.238
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	83	84
Skin and Subcutaneous Tissue: Fibrosarcoma or Sarcoma NOS[b]	0/19(0.00)	2/50(0.04)	6/50(0.12)
P Values[c]	P = 0.041	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.117	0.636
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	83	80
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	2/19(0.11)	2/49(0.04)	4/49(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.388	0.776
Lower Limit	---	0.031	0.125
Upper Limit	---	5.108	8.165
Weeks to First Observed Tumor	90	91	88

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/19(0.05)	5/50(0.10)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.900	0.760
Lower Limit	---	0.238	0.043
Upper Limit	---	87.985	43.961
Weeks to First Observed Tumor	91	91	66
Circulatory System: Hemangiosarcoma[b]	0/19(0.00)	5/50(0.10)	20/50(0.40)
P Values[c]	P < 0.001	N.S.	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.501	2.568
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	79	71
Circulatory System: Hemangiosarcoma or Hemangioma[b]	0/19(0.00)	5/50(0.10)	23/50(0.46)
P Values[c]	P < 0.001	N.S.	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.501	2.988
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	79	71

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/19(0.00)	6/49(0.12)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.649	0.248
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	83	71
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	3/19(0.16)	8/49(0.16)	9/48(0.19)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.034	1.187
Lower Limit	---	0.288	0.346
Upper Limit	---	5.620	6.318
Weeks to First Observed Tumor	91	83	71

[a]Treated groups received doses of 1250 or 2500 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. 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 MICHLER'S KETONE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/19(0.05)	7/49(0.14)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.714	1.140
Lower Limit	---	0.393	0.101
Upper Limit	---	119.544	58.635
Weeks to First Observed Tumor	91	67	85
Circulatory System: Hemangiosarcoma[b]	2/19(0.11)	0/49(0.00)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.041	---	---
Relative Risk (Control)[d]	---	0.000	0.380
Lower Limit	---	0.000	0.030
Upper Limit	---	1.303	5.009
Weeks to First Observed Tumor	91	---	79
Circulatory System: Hemangiosarcoma or Hemangioma[b]	2/19(0.11)	0/49(0.00)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.036	---	---
Relative Risk (Control)[d]	---	0.000	0.570
Lower Limit	---	0.000	0.073
Upper Limit	---	1.303	6.511
Weeks to First Observed Tumor	91	---	79

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/19(0.00)	16/49(0.33)	38/50(0.76)
P Values[c]	P < 0.001	P = 0.002	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	2.053	5.150
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	81	79
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	0/19(0.00)	41/49(0.84)	49/50(0.98)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	5.756	7.489
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	66	71

[a]Treated groups received doses of 1250 or 2500 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. 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.

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

In male mice the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of hemangiosarcomas of the circulatory system. This result was supported by a significant ($P < 0.001$) Fisher exact test comparing high dose to control. Also in the circulatory system, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of a combination of hemangiosarcomas and hemangiomas. Again this was supported by a significant ($P < 0.001$) Fisher exact test comparing high dose to control.

For male mice the Cochran-Armitage test also indicated a significant ($P = 0.041$) positive association between dose and the incidence of a combination of fibrosarcomas and sarcomas NOS of the skin and subcutaneous tissue. However, neither of the Fisher exact tests were significant.

In female mice the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of hepatocellular carcinomas. This result was supported by a significant ($P < 0.001$) Fisher exact test comparing high dose to control and also by a significant ($P = 0.002$) low dose to control comparison.

For female mice the Cochran-Armitage test also indicated a significant ($P < 0.001$) positive association between dose and the incidence of a combination of hepatocellular carcinomas and hepatocellular adenomas. This was supported by both a significant ($P < 0.001$) Fisher exact test comparing high dose to control and a significant ($P < 0.001$) low dose to control comparison. The test for departure from linear trend was also significant ($P < 0.001$).

In female mice the tests for departure from linear trend were significant both for hemangiosarcomas ($P = 0.041$) of the circulatory system and also for the combination of hemangiosarcomas and hemangiomas ($P = 0.036$).

Based on these statistical results, the administration of Michler's ketone was carcinogenic to both male and female mice under the conditions of this bioassay. The administration of the compound was associated with a significantly increased incidence of hemangiosarcomas of the circulatory system, hemangiosarcomas or hemangiomas of the circulatory system, and fibrosarcomas or sarcomas NOS of the skin and subcutaneous tissue in male mice. For female mice the administration of the compound was associated with a significantly increased incidence of hepatocellular carcinomas and with the incidence of a combination of hepatocellular carcinomas and hepatocellular adenomas.

V. DISCUSSION

There were significant positive associations between the concentrations of Michler's ketone administered and mortality in rats and mice of both sexes. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. There was distinct dose-related mean body weight depression in female rats and in both sexes of mice. The mean body weight of dosed male rats was slightly lower than that of controls, indicating that the concentrations of Michler's ketone administered to these animals in this bioassay may have approximated the maximum tolerated concentrations.

All of the high dose female rats died prior to the end of the bioassay, and the majority of them (44/49) had hepatocellular carcinomas. In addition, virtually all of the high dose males and low dose females and one-third of the low dose males had neoplastic liver lesions. There was also considerable metastasis to the lung among the high dose rats. There were significant positive associations between the concentrations administered and the incidences of hepatocellular carcinomas in male rats (i.e., 0/20, 9/50 and 40/50 in the control, low dose, and high dose, respectively), in female rats (i.e., 0/20, 41/47 and 44/49 in the control, low dose, and high dose, respectively), and in female mice (i.e., 0/19, 16/49 and 38/50 in the control, low dose, and high dose, respectively). In all cases the high dose to control Fisher exact comparison supported the finding, and for the female rats

and female mice the low dose to control Fisher exact comparisons were also significant.

Among male mice there was a significant positive association between concentration administered and the incidence of hemangiosarcomas (i.e., 0/19, 5/50 and 20/50 in the control, low dose, and high dose, respectively). The high dose to control Fisher exact comparison for these neoplasms at this site was also significant.

Under the conditions of this bioassay, dietary administration of Michler's ketone was carcinogenic to male and female Fischer 344 rats and female B6C3F1 mice, causing hepatocellular carcinomas, and to male B6C3F1 mice, causing hemangiosarcomas.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH MICHLER'S KETONE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH MICHLER'S KETONE

	CONTROL (UNTR) 11-1025	LOW DOSE 11-1023	HIGH DOSE 11-1021
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%)	4 (8%)
FIBROMA		1 (2%)	
FIBROADENOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA		1 (2%)	
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(19)	(50)	(48)
CARCINOMA, NOS		1 (2%)	
#LUNG	(19)	(50)	(48)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	9 (19%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	1 (2%)	
C-CELL CARCINOMA, METASTATIC			1 (2%)
SARCOMA, NOS, METASTATIC			1 (2%)
FIBROSARCOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (5%)		
#LIVER	(20)	(50)	(50)
LYMPHOCYTIC 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 (UNTR) 11-1025	LOW DOSE 11-1023	HIGH DOSE 11-1021
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
NEOPLASTIC NODULE		8 (16%)	3 (6%)
HEPATOCELLULAR CARCINOMA		9 (18%)	40 (80%)
#BILE DUCT	(20)	(50)	(50)
BILE DUCT ADENOMA			1 (2%)
#STOMACH WALL	(20)	(47)	(49)
LEIOMYOMA			1 (2%)
#DUODENUM	(20)	(46)	(49)
LEIOMYOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
GANGLIONEUROMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(39)	(42)
CHROMOPHOBE ADENOMA	3 (18%)	3 (8%)	2 (5%)
CHROMOPHOBE CARCINOMA		1 (3%)	
#ADRENAL	(18)	(47)	(47)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA	1 (6%)		1 (2%)
#THYROID	(16)	(33)	(43)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA	1 (6%)	3 (9%)	3 (7%)
C-CELL CARCINOMA			1 (2%)
#PARATHYROID	(2)	(13)	(14)
ADENOMA, NOS		1 (8%)	
#PANCREATIC ISLETS	(20)	(47)	(47)
ISLET-CELL ADENOMA		3 (6%)	3 (6%)
REPRODUCTIVE SYSTEM			
#TESTIS	(20)	(49)	(48)
INTERSTITIAL-CELL TUMOR	16 (80%)	43 (88%)	45 (94%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1025	LOW DOSE 11-1023	HIGH DOSE 11-1021
NERVOUS SYSTEM			
#CEREBRUM	(20)	(50)	(49)
OSTEOSARCOMA		1 (2%)	
#BRAIN	(20)	(50)	(49)
GLIOMA, NOS		1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
SARCOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		8	12
MORIBUND SACRIFICE		1	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	20	41	34
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 11-1025	LOW DOSE 11-1023	HIGH DOSE 11-1021
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	50	49
TOTAL PRIMARY TUMORS	23	82	111
TOTAL ANIMALS WITH BENIGN TUMORS	17	46	46
TOTAL BENIGN TUMORS	21	56	59
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	17	43
TOTAL MALIGNANT TUMORS	2	18	49
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	10
TOTAL SECONDARY TUMORS		1	12
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		8	3
TOTAL UNCERTAIN TUMORS		8	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 MICHLER'S KETONE

	CONTROL (UNTR) 11-1026	LOW DOSE 11-1024	HIGH DOSE 11-1022
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	20	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(49)
SQUAMOUS CELL CARCINOMA			1 (2%)
FIBROMA	1 (5%)		
*SUBCUT TISSUE	(20)	(49)	(49)
FIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(48)
HEPATOCELLULAR CARCINOMA, METAST		8 (17%)	25 (52%)
ACINAR-CELL CARCINOMA, METASTATI			1 (2%)
HEMATOPOIETIC SYSTEM			
#SMALL INTESTINE	(20)	(46)	(46)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(42)	(44)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(49)
NEOPLASTIC NODULE		5 (11%)	5 (10%)
HEPATOCELLULAR CARCINOMA		41 (87%)	44 (90%)
#PANCREAS	(20)	(44)	(40)
ACINAR-CELL CARCINOMA			1 (3%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1026	LOW DOSE 11-1024	HIGH DOSE 11-1022
#SMALL INTESTINE	(20)	(46)	(46)
ADENOCARCINOMA, NOS		1 (2%)	
URINARY SYSTEM			
#URINARY BLADDER	(18)	(34)	(28)
PAPILLARY ADENOMA			1 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(44)	(31)
CHROMOPHOBE ADENOMA	4 (25%)	3 (7%)	
#ADRENAL	(20)	(46)	(41)
PHECCHROMOCYTOMA	2 (10%)	3 (7%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(49)
ADENOMA, NOS	1 (5%)	1 (2%)	
FIBROADENOMA	4 (20%)	1 (2%)	
#UTERUS	(20)	(44)	(38)
LEICMYCMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP		2 (5%)	
#OVARY	(20)	(44)	(35)
LUTEOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1026	LOW DOSE 11-1024	HIGH DOSE 11-1022
BODY CAVITIES			
*PERITONEUM	(20)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		14	31
MORIBUND SACRIFICE	2	2	18
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	33	
ANIMAL MISSING		1	1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	46	48
TOTAL PRIMARY TUMORS	12	60	53
TOTAL ANIMALS WITH BENIGN TUMORS	7	11	1
TOTAL BENIGN TUMORS	12	13	1
TOTAL ANIMALS WITH MALIGNANT TUMORS		42	44
TOTAL MALIGNANT TUMORS		42	47
TOTAL ANIMALS WITH SECONDARY TUMORS#		9	26
TOTAL SECONDARY TUMORS		9	27
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		5	5
TOTAL UNCERTAIN TUMORS		5	5
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 MICHLER'S KETONE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH MICHLER'S KETONE

	CONTROL (UNTR) 22-2025	LOW DOSE 22-2023	HIGH DOSE 22-2021
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	19	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(19)	(50)	(50)
FIBROSARCOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
*SUBCUT TISSUE	(19)	(50)	(50)
SARCOMA, NOS			3 (6%)
FIBROSARCOMA		1 (2%)	3 (6%)
RHAEDOMYOSARCOMA			1 (2%)
HEMANGIOMA			3 (6%)
HEMANGIOSARCOMA		2 (4%)	15 (30%)
RESPIRATORY SYSTEM			
*LARYNX	(19)	(50)	(50)
SARCOMA, NOS			1 (2%)
#LUNG	(19)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST		3 (6%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	2 (4%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		1 (2%)
FIBROSARCOMA, METASTATIC			1 (2%)
HEMANGIOSARCOMA			1 (2%)
HEMANGIOSARCOMA, METASTATIC			3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (2%)	
LEUKEMIA,NOS		1 (2%)	
LYMPHOCYTIC LEUKEMIA			1 (2%)

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

TABLE B1 (CONTINUED)

	CONTROL(UNTR) 22-2025	LOW DOSE 22-2023	HIGH DOSE 22-2021
GRANULOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(18)	(49)	(44)
SARCOMA, NOS	1 (6%)		
HEMANGIOSARCOMA		3 (6%)	3 (7%)
#MESENTERIC L. NODE	(17)	(35)	(9)
MALIGNANT LYMPHOMA, NOS		2 (6%)	
CIRCULATORY SYSTEM			
#HEART	(18)	(46)	(43)
RHABDOMYOSARCOMA			1 (2%)
#MYOCARDIUM	(18)	(46)	(43)
HEMANGIOSARCOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(48)
HEPATOCELLULAR ADENOMA	3 (16%)	2 (4%)	6 (13%)
HEPATOCELLULAR CARCINOMA		6 (12%)	3 (6%)
HEMANGIOSARCOMA		1 (2%)	
HEMANGIOSARCOMA, METASTATIC			1 (2%)
URINARY SYSTEM			
#KIDNEY	(18)	(49)	(47)
HEMANGIOSARCOMA			2 (4%)
#URINARY BLADDER	(17)	(47)	(37)
PAPILLOMA, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(18)	(37)	(27)
FOLLICULAR-CELL ADENOMA			1 (4%)
C-CELL CARCINOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(17)	(49)	(48)
INTERSTITIAL-CELL TUMOR		1 (2%)	

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

TABLE B1 (CONTINUED)

	CONTROL(UNTR) 22-2025	LOW DOSE 22-2023	HIGH DOSE 22-2021
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(19)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
*ABDOMINAL WALL	(19)	(50)	(50)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	6	30
MORIBUND SACRIFICE	1	4	13
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	40	7
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2025	LOW DOSE 22-2023	HIGH DOSE 22-2021
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	21	39
TOTAL PRIMARY TUMORS	7	26	52
TOTAL ANIMALS WITH BENIGN TUMORS	4	6	11
TOTAL BENIGN TUMORS	4	6	13
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	16	34
TOTAL MALIGNANT TUMORS	3	20	39
TOTAL ANIMALS WITH SECONDARY TUMORS#		3	4
TOTAL SECONDARY TUMORS		3	6
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 MICHLER'S KETONE

	CONTROL (UNTR) 22-2026	LOW DOSE 22-2024	HIGH DOSE 22-2022
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	1	
ANIMALS NECROPSIED	19	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(49)	(50)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA			2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(47)
HEPATOCELLULAR CARCINOMA, METAST			3 (6%)
HEMANGIOSARCOMA	1 (6%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(49)	(50)
MALIGNANT LYMPHOMA, NOS		4 (8%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (2%)	
LEUKEMIA,NOS		1 (2%)	1 (2%)
#SPLEEN	(19)	(46)	(47)
SARCOMA, NOS		1 (2%)	
HEMANGIOSARCOMA	2 (11%)		
#LYMPH NODE	(15)	(36)	(33)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
#MESENTERIC L. NODE	(15)	(36)	(33)
HEMANGIOSARCOMA	1 (7%)		
#SMALL INTESTINE	(18)	(48)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#KIDNEY	(19)	(49)	(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 (UNTR) 22-2026	LOW DOSE 22-2024	HIGH DOSE 22-2022
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(50)
HEPATOCELLULAR ADENOMA		25 (51%)	11 (22%)
HEPATOCELLULAR CARCINOMA		16 (33%)	38 (76%)
#BILE DUCT	(19)	(49)	(50)
BILE DUCT CARCINOMA			2 (4%)
#SMALL INTESTINE	(18)	(48)	(48)
ADENOMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(19)	(49)	(49)
TUBULAR-CELL ADENOCARCINOMA	1 (5%)		
#URINARY BLADDER	(17)	(46)	(41)
PAPILLARY ADENOMA		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(44)	(40)
PHEOCHROMOCYTOMA			1 (3%)
#THYROID	(17)	(37)	(37)
FOLLICULAR-CELL ADENOMA			1 (3%)
C-CELL CARCINOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
#OVARY	(18)	(46)	(41)
ADENOCARCINOMA, NOS	1 (6%)		
HAMARTOMA			1 (2%)
NERVOUS SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2026	LOW DOSE 22-2024	HIGH DOSE 22-2022
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(19)	(49)	(50)
ADENOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	6	8
MORIBUND SACRIFICE		1	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	42	33
ANIMAL MISSING	1	1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2026	LOW DOSE 22-2024	HIGH DOSE 22-2022
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	43	49
TOTAL PRIMARY TUMORS	7	52	61
TOTAL ANIMALS WITH BENIGN TUMORS		25	16
TOTAL BENIGN TUMORS		27	16
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	22	40
TOTAL MALIGNANT TUMORS	7	25	45
TOTAL ANIMALS WITH SECONDARY TUMORS#			3
TOTAL SECONDARY TUMORS			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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH MICHLER'S KETONE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH MICHLER'S KETONE

	CONTROL (UNTR) 11-1025	LOW DOSE 11-1023	HIGH DOSE 11-1021
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
ABSCESS, NOS			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
CYST, NOS	1 (5%)		
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(48)	(47)
INFLAMMATION, NOS		2 (4%)	
#LUNG	(19)	(50)	(48)
ATELECTASIS	1 (5%)		1 (2%)
CONGESTION, NOS		1 (2%)	
EDEMA, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
BRONCHOPNEUMONIA SUPPURATIVE		2 (4%)	
PNEUMONIA, CHRONIC MURINE	13 (68%)	24 (48%)	14 (29%)
FIBROSIS, FOCAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(48)
THROMBOSIS, NOS		1 (2%)	
#HEART/ATRIUM	(20)	(50)	(48)
THROMBUS, ORGANIZED			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1025	LOW DOSE 11-1023	HIGH DOSE 11-1021
#MYOCARDIUM	(20)	(50)	(48)
FIBROSIS		2 (4%)	3 (6%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY	1 (5%)	6 (12%)	
LIPOIDOSIS			1 (2%)
BASOPHILIC CYTO CHANGE		9 (18%)	1 (2%)
FOCAL CELLULAR CHANGE		1 (2%)	1 (2%)
CLEAR-CELL CHANGE		3 (6%)	
HYPERPLASIA, NODULAR		1 (2%)	
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
NECROSIS, NOS			1 (2%)
#LIVER/HEPATOCYTES	(20)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
#PANCREAS	(20)	(47)	(47)
FIBROSIS, FOCAL		1 (2%)	
#PANCREATIC ACINUS	(20)	(47)	(47)
ATROPHY, NOS		2 (4%)	2 (4%)
#STOMACH	(20)	(47)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
#STOMACH WALL	(20)	(47)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	
#SMALL INTESTINE	(20)	(46)	(49)
HYPERPLASIA, LYMPHOID	1 (5%)		1 (2%)
#LARGE INTESTINE	(20)	(46)	(48)
MINERALIZATION			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
INFLAMMATION, CHRONIC	15 (75%)	35 (71%)	36 (72%)

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

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 11-1025	LOW DOSE 11-1023	HIGH DOSE 11-1021
#URINARY BLADDER	(15)	(26)	(33)
CALCULUS, NOS			1 (3%)
HEMORRHAGE		1 (4%)	
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(39)	(42)
HEMORRHAGIC CYST	1 (6%)		
#ADRENAL	(18)	(47)	(47)
HEMORRHAGIC CYST			1 (2%)
LIPOIDOSIS			2 (4%)
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL MEDULLA	(18)	(47)	(47)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(16)	(33)	(43)
FOLLICULAR CYST, NOS			2 (5%)
HYPERPLASIA, FOLLICULAR-CELL		1 (3%)	
#PANCREATIC ISLETS	(20)	(47)	(47)
HYPERPLASIA, NOS		2 (4%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(28)	(28)
HEMORRHAGE		1 (4%)	
HYPERPLASIA, ADENOMATOUS		1 (4%)	1 (4%)
#TESTIS	(20)	(49)	(48)
ATROPHY, NOS		1 (2%)	3 (6%)
ASPERMATOGENESIS			1 (2%)
#TESTIS/TUBULE	(20)	(49)	(48)
CALCIFICATION, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(49)
HEMORRHAGE			1 (2%)

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1025	LOW DOSE 11-1023	HIGH DOSE 11-1021
ABSCESS, NOS			1 (2%)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(51)
CATARACT			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTC/NECROPSY/HISTO PERF			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 MICHLER'S KETONE

	CONTROL (UNTR) 11-1026	LOW DOSE 11-1024	HIGH DOSE 11-1022
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	20	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(49)
INFLAMMATION, ACUTE SUPPURATIVE	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(48)
ATELECTASIS			1 (2%)
HEMORRHAGE	2 (10%)		
PNEUMONIA, CHRONIC MURINE	14 (70%)	17 (37%)	1 (2%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
#HEART/VENTRICLE	(20)	(42)	(44)
INFARCT, NOS		1 (2%)	
#MYOCARDIUM	(20)	(42)	(44)
FIBROSIS	1 (5%)		
FIBROSIS, FOCAL			1 (2%)
*CORONARY ARTERY	(20)	(49)	(49)
HYPERTROPHY, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(49)
NECROSIS, NOS		1 (2%)	2 (4%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1026	LOW DOSE 11-1024	HIGH DOSE 11-1022
NECROSIS, DIFFUSE			1 (2%)
LIPOIDOSIS	1 (5%)		
BASOPHILIC CYTO CHANGE	7 (35%)		1 (2%)
CLEAR-CELL CHANGE			1 (2%)
#BILE DUCT	(20)	(47)	(49)
FIBROSIS		1 (2%)	
#PANCREAS	(20)	(44)	(40)
ATROPHY, NOS		1 (2%)	
#PANCREATIC DUCT	(20)	(44)	(40)
HYPERPLASIA, NOS		1 (2%)	
#PANCREATIC ACINUS	(20)	(44)	(40)
ATROPHY, NOS			2 (5%)
#STOMACH	(20)	(47)	(45)
ULCER, NOS			1 (2%)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL			1 (2%)
#SMALL INTESTINE	(20)	(46)	(46)
HYPERPLASIA, LYMPHOID			3 (7%)
#LARGE INTESTINE	(20)	(44)	(46)
HYPERPLASIA, LYMPHOID			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(46)	(47)
INFLAMMATION, INTERSTITIAL			1 (2%)
INFLAMMATION, CHRONIC	5 (25%)	31 (67%)	24 (51%)
NEPHROPATHY			10 (21%)
NEPHROPATHY, TOXIC			2 (4%)
#KIDNEY/TUBULE	(20)	(46)	(47)
NEPHROSIS, CHOLEMIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(44)	(31)
CYST, NOS	1 (6%)	5 (11%)	2 (6%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1026	LOW DOSE 11-1024	HIGH DOSE 11-1022
HEMORRHAGIC CYST	2 (13%)		
#ADRENAL	(20)	(46)	(41)
HEMORRHAGIC CYST			4 (10%)
METAMORPHOSIS FATTY	1 (5%)		
LIPOIDOSIS		1 (2%)	
LYMPHOCYTOSIS		1 (2%)	
#ADRENAL CORTEX	(20)	(46)	(41)
HYPERPLASIA, NODULAR	1 (5%)		
HYPERPLASIA, FOCAL	1 (5%)		
#THYROID	(16)	(27)	(37)
HYPERPLASIA, C-CELL			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(49)
CYST, NOS		1 (2%)	
*MAMMARY DUCT	(20)	(49)	(49)
HYPERPLASIA, NOS	1 (5%)		
*CLITORAL GLAND	(20)	(49)	(49)
ABSCESS, NOS		1 (2%)	
#UTERUS	(20)	(44)	(38)
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)
#OVARY	(20)	(44)	(35)
CYST, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(20)	(49)	(49)
CATARACT			1 (2%)
*EYE/LACRIMAL GLAND	(20)	(49)	(49)
INFLAMMATION, NOS			1 (2%)

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1026	LOW DOSE 11-1024	HIGH DOSE 11-1022
HYPERPLASIA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(49)	(49)
INFLAMMATION, CHRONIC	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY		1	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 MICHLER'S KETONE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH MICHLER'S KETONE

	CONTROL (UNTR) 22-2025	LOW DOSE 22-2023	HIGH DOSE 22-2021
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	19	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
HEMORRHAGE		1 (2%)	6 (12%)
HEMATOMA, NOS			1 (2%)
INFLAMMATION, NOS			2 (4%)
NECROSIS, NOS			4 (8%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(49)
HEMORRHAGE			1 (2%)
ABSCESS, NOS			1 (2%)
PNEUMONIA, CHRONIC MURINE	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(49)	(44)
CONGESTION, NOS			1 (2%)
FIBROSIS		1 (2%)	
NECROSIS, NOS			1 (2%)
HYPERPLASIA, RETICULUM CELL	3 (17%)		1 (2%)
HYPERPLASIA, LYMPHOID	3 (17%)	2 (4%)	4 (9%)
#LYMPH NODE	(17)	(35)	(9)
HEMORRHAGIC CYST		1 (3%)	
FIBROSIS		1 (3%)	
HYPERPLASIA, RETICULUM CELL			1 (11%)
HYPERPLASIA, LYMPHOID		1 (3%)	
#MESENTERIC L. NODE	(17)	(35)	(9)
HEMORRHAGE	1 (6%)		

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

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 22-2025	LOW DOSE 22-2023	HIGH DOSE 22-2021
HYPERPLASIA, RETICULUM CELL		1 (3%)	
#THYMUS	(1)		(1)
HYPERPLASIA, LYMPHOID	1 (100%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(18)	(46)	(43)
INFLAMMATION, FOCAL			1 (2%)
FIBROSIS, FOCAL			1 (2%)
NECROSIS, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(15)	(39)	(13)
HEMORRHAGIC CYST			1 (8%)
FIBROSIS			1 (8%)
HYPERPLASIA, LYMPHOID	1 (7%)		
#LIVER	(19)	(49)	(48)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS			2 (4%)
INFLAMMATION, ACUTE		1 (2%)	
NECROSIS, NOS		1 (2%)	2 (4%)
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (5%)	1 (2%)	1 (2%)
BASOPHILIC CYTO CHANGE	1 (5%)	1 (2%)	2 (4%)
FOCAL CELLULAR CHANGE		2 (4%)	
ANGIECTASIS			2 (4%)
#HEPATIC CAPSULE	(19)	(49)	(48)
INFLAMMATION, NOS			1 (2%)
#LIVER/CENTRILOBULAR	(19)	(49)	(48)
DEGENERATION, NOS		1 (2%)	1 (2%)
NECROSIS, NOS			2 (4%)
METAMORPHOSIS FATTY			1 (2%)
#LIVER/HEPATOCYTES	(19)	(49)	(48)
ATYPIA, NOS			17 (35%)
#SMALL INTESTINE	(19)	(50)	(45)
INFLAMMATION, GRANULOMATOUS		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2025	LOW DOSE 22-2023	HIGH DOSE 22-2021
HYPERPLASIA, LYMPHOID		2 (4%)	3 (7%)
#LARGE INTESTINE	(19)	(50)	(44)
NEMATODIASIS			2 (5%)
URINARY SYSTEM			
#KIDNEY	(18)	(49)	(47)
HYDRONEPHROSIS	1 (6%)		
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
INFLAMMATION, CHRONIC	1 (6%)	5 (10%)	5 (11%)
INFLAMMATION, GRANULOMATOUS	1 (6%)		
CALCINOSIS, NOS			1 (2%)
#KIDNEY/MEDULLA	(18)	(49)	(47)
CYST, NOS		1 (2%)	
#URINARY BLADDER	(17)	(47)	(37)
CALCULUS, NOS	1 (6%)	1 (2%)	
INFLAMMATION, CHRONIC	1 (6%)		
HYPERPLASIA, EPITHELIAL		1 (2%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#PROSTATE	(16)	(48)	(44)
HYPERPLASIA, CYSTIC			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(19)	(48)	(47)
HEMORRHAGE			1 (2%)
CORPORA AMYLACEA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2025	LOW DOSE 22-2023	HIGH DOSE 22-2021
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(19)	(50)	(50)
INFLAMMATION, GRANULOMATOUS	1 (5%)		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
THORACIC CAVITY			
HEMOTHORAX			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	17	1
AUTOLYSIS/NO NECROPSY	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 MICHLER'S KETONE

	CONTROL (UNTR) 22-2026	LOW DOSE 22-2024	HIGH DOSE 22-2022
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	1	
ANIMALS NECROPSIED	19	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(47)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, GRANULOMATOUS	1 (6%)		
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(46)	(47)
CONGESTION, NOS		1 (2%)	
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, RETICULUM CELL	1 (5%)	1 (2%)	
HYPERPLASIA, LYMPHOID	2 (11%)	3 (7%)	4 (9%)
HEMATOPOIESIS	1 (5%)	1 (2%)	
#LYMPH NODE	(15)	(36)	(33)
HYPERPLASIA, RETICULUM CELL			2 (6%)
#PANCREATIC L.NODE	(15)	(36)	(33)
CYST, NOS			1 (3%)
#MESENTERIC L. NODE	(15)	(36)	(33)
INFLAMMATION, GRANULOMATOUS		1 (3%)	
HYPERPLASIA, RETICULUM CELL	3 (20%)		1 (3%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2026	LOW DOSE 22-2024	HIGH DOSE 22-2022
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(14)	(39)	(27)
HYPERPLASIA, LYMPHOID	1 (7%)		1 (4%)
#LIVER	(19)	(49)	(50)
CYST, NOS		1 (2%)	
MULTIPLE CYSTS			1 (2%)
HEMORRHAGE		1 (2%)	
GRANULOMA, NOS	1 (5%)		
NECROSIS, NOS		1 (2%)	2 (4%)
NECROSIS, FOCAL		1 (2%)	
FOCAL CELLULAR CHANGE		1 (2%)	
HYPERPLASIA, RETICULUM CELL	1 (5%)		
HEMATOPOIESIS	1 (5%)		
#HEPATIC CAPSULE	(19)	(49)	(50)
INFLAMMATION, FOCAL			1 (2%)
#LIVER/HEPATOCYTES	(19)	(49)	(50)
ATYPIA, NOS		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
#BILE DUCT	(19)	(49)	(50)
HYPERPLASIA, NOS			1 (2%)
#SMALL INTESTINE	(18)	(48)	(48)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
NEMATODIASIS			1 (2%)
HYPERPLASIA, LYMPHOID	3 (17%)	2 (4%)	
#ILEUM	(18)	(48)	(48)
HYPERPLASIA, LYMPHOID	1 (6%)		
#LARGE INTESTINE	(18)	(46)	(48)
NEMATODIASIS	1 (6%)		1 (2%)
#COLON	(18)	(46)	(48)
HYPERPLASIA, LYMPHOID	1 (6%)		
URINARY SYSTEM			
#KIDNEY	(19)	(49)	(49)
INFLAMMATION, INTERSTITIAL			1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2026	LOW DOSE 22-2024	HIGH DOSE 22-2022
INFLAMMATION, CHRONIC	1 (5%)	2 (4%)	3 (6%)
INFLAMMATION, GRANULOMATOUS	1 (5%)		
#KIDNEY/TUBULE	(19)	(49)	(49)
NECROSIS, NOS			1 (2%)
#KIDNEY/PELVIS	(19)	(49)	(49)
INFLAMMATION, NOS	1 (5%)		
#URINARY BLADDER	(17)	(46)	(41)
INFLAMMATION, GRANULOMATOUS	1 (6%)		
HYPERPLASIA, PAPILLARY			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL MEDULLA	(17)	(44)	(40)
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(17)	(37)	(37)
CYSTIC FOLLICLES			1 (3%)
HYPERPLASIA, FOLLICULAR-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(18)	(46)	(42)
HYDROMETRA		1 (2%)	2 (5%)
#UTERUS/ENDOMETRIUM	(18)	(46)	(42)
CYST, NOS	1 (6%)		2 (5%)
INFLAMMATION, NOS	1 (6%)		
INFLAMMATION, SUPPURATIVE	1 (6%)	1 (2%)	
INFLAMMATION, HEMORRHAGIC			1 (2%)
INFLAMMATION, VESICULAR	1 (6%)	1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	1 (6%)		1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV	3 (17%)		
HYPERPLASIA, NOS	1 (6%)	7 (15%)	1 (2%)
HYPERPLASIA, CYSTIC	6 (33%)	8 (17%)	
#OVARY	(18)	(46)	(41)
CYST, NOS	2 (11%)	5 (11%)	
FOLLICULAR CYST, NOS		1 (2%)	
HEMORRHAGIC CYST	1 (6%)		
INFLAMMATION, GRANULOMATOUS	1 (6%)		

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2026	LOW DOSE 22-2024	HIGH DOSE 22-2022
#RIGHT OVARY	(18)	(46)	(41)
ABSCESS, NOS	1 (6%)		
#LEFT OVARY	(18)	(46)	(41)
CYST, NOS	1 (6%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(19)	(49)	(50)
GRANULOMA, NOS	1 (5%)		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	1
ANIMAL MISSING/NO NECROPSY	1	1	
AUTC/NECROPSY/HISTO PERF		1	

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

Review of the Bioassay of Michler's Ketone* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 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, and State health officials. 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 Michler's Ketone for carcinogenicity.

A representative of DuPont Haskel Laboratories made a public statement regarding the bioassay of Michler's Ketone. He said that the Michler's Ketone used in the bioassay may not have been representative of the technical grade product, despite the fact that it was obtained from DuPont. The presumption for atypicallity is based on negative results in the Ames assay when pure Michler's Ketone and the technical Michler's Ketone were tested, whereas the Michler's Ketone used in the NCI bioassay was positive. The representative suggested that the carcinogenic activity in the NCI bioassay was due to an impurity in the Michler's Ketone. He recommended that the NCI bioassay report be withheld until "some definitive test" could be undertaken.

A Clearinghouse member said that he was reluctant to withhold the report since the Michler's Ketone tested, which was obtained from DuPont, was found to be carcinogenic. He suggested that the report reference the concern expressed by DuPont. A Program staff member noted that the NCI tested material was already several years old when it was subjected to the Ames assay. A Subgroup member pointed out that the purity of the NCI tested material was only 80%.

The reviewer said that Michler's Ketone was carcinogenic in treated rats and mice. After briefly describing the experimental design, he commented on the poor survival in the high dose treatment groups in both

species, indicating that the maximum tolerated doses may have been exceeded. He added that the significance of the study may have been compromised by the toxicity effect. The reviewer said that the Michler's Ketone may pose a carcinogenic risk to humans.

A Program staff member noted that there were deaths as early as 60 weeks among treated animals and that the weight loss may have been attributed to cancer preceding death. It thus was difficult to evaluate the significance of the weight loss in terms of toxicity.

There was no objection to a motion that the report on the bioassay of Michler's Ketone be accepted with the modification regarding the concern expressed by DuPont.

Members Present were:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Kenneth Wilcox, Michigan State Health Department

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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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