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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 3-SULFOLENE
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 3-sulfolene 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 3-sulfolene was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

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

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

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (8); the statistical analysis was performed by Mr. W. W. Belew (6), using methods selected for the Bioassay Program by Dr. J. J. Gart (9).

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

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

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

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SUMMARY

A bioassay of 3-sulfolene for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F1 mice. 3-Sulfolene in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The 78-week period of chemical administration was followed by an observation period of 33 weeks for the high dose female rats and low dose rats of both sexes. The last high dose male rat died in week 60. All treated groups of mice were observed for an additional 13 weeks following chemical administration.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The time-weighted average high and low doses of 3-sulfolene in the chronic study were, respectively, 372 and 197 mg/kg/day for male rats, 240 and 120 mg/kg/day for female rats, 622 and 311 mg/kg/day for male mice and 768 and 384 mg/kg/day for the female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same times that dosed animals were gavaged with the 3-sulfolene mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

There was a significant positive association between the administered dosages of 3-sulfolene and mortality in both sexes of rats and mice. In all groups, except the high dose male rats and the high dose male and female mice, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors.

There were no tumors in either sex of rats or mice for which a significant positive association could be established between chemical administration and incidence.

Under the conditions of this bioassay, there was no evidence for the carcinogenicity of 3-sulfolene to Osborne-Mendel rats or B6C3F1 mice.

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I. INTRODUCTION

3-Sulfolene (NCI No. C04557), a small ring, heterocyclic sulfone, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to 3,4-epoxysulfolane and 1-propanesulfonic acid-3-hydroxy- -sultone, both of which are carcinogenic in mice (Van Duuren et al., 1971).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2,5-dihydrothiophene 1,1-dioxide.* It is also known as 1-thia-3-cyclopentene 1,1-dioxide; butadiene sulfone; or simply sulfolene.

3-Sulfolene is an intermediate in the production of sulfolane, which is used in the petroleum, plastics, and textile industries, and in the synthesis of one or more fungicides or additional chemicals (Rose and Rose, 1966). 3-Sulfolene is also used as a catalyst (Rose and Rose, 1966).

Specific production data for 3-sulfolene are not available; however, the inclusion of this compound in the 1977 Directory of Chemical Producers, U.S.A. (Stanford Research Institute, 1977) implies that it is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually).

The potential for exposure to 3-sulfolene is greatest for workers in 3-sulfolene and sulfolane production facilities but may be considerable for other chemical manufacturing workers, as well.

*The CAS registry number is 77-79-2.

II. MATERIALS AND METHODS

A. Chemicals

3-Sulfolene was purchased from Phillips Petroleum Company and chemical analysis was performed by Hazleton Laboratories America, Inc., Vienna, Virginia. Thin-layer chromatography utilizing seven various concentration combinations of methanol:acetone and/or benzene each revealed only one spot. Ultraviolet analysis showed one peak at λ_{max} = 274 nm. When compared with an ultraviolet standard assumed to have a purity of 100 percent, the purity of the test chemical was indicated to be approximately 92 percent. Chemical analysis performed twelve months later indicated decomposition.

Throughout this report the term 3-sulfolene is used to represent this material.

B. Dosage Preparation

Fresh solutions of 3-sulfolene in Duke's® corn oil (S. F. Sauer Company, Richmond, Virginia) were prepared weekly, sealed and stored in dark bottles at 1°C. These 3-sulfolene solutions were considered generally stable for ten days under the indicated storage conditions. The concentration of 3-sulfolene in corn oil ranged from 10 to 20 percent in rats, and from 2.3 to 8 percent in mice.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. The Osborne-Mendel rat was selected on the basis of a comparative study of the tumorigenic responsiveness to carbon

tetrachloride of five different strains of rats (Reuber and Glover, 1970). The B6C3F1 mouse was selected because it has been used by the NCI for carcinogenesis bioassays and has proved satisfactory in this capacity.

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

D. Animal Maintenance

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

The rats were individually housed in suspended galvanized-steel wire-mesh cages with perforated floors, while mice were housed by sex in groups of 10 in solid-bottom polypropylene cages equipped with filter tops. Sanitized cages with fresh bedding (Sanichips®, Pinewood Sawdust Company, Moonachie, New Jersey) were provided once each

week for mice. Rats received sanitized cages with no bedding with the same frequency. Food hoppers were changed and heat-sterilized once a week for the first 10 weeks and once a month thereafter. Fresh heat-sterilized glass water bottles and sipper tubes were provided three times a week. Food (Wayne Lab-Blox®, Allied Mills, Inc., Chicago, Illinois) and water were available ad libitum.

Rats dosed with 3-sulfolene and their vehicle controls were housed in the same room as other rats intubated with* iodoform (75-47-8) and hexachloroethane (67-72-1). Untreated control rats were housed in a room with other rats intubated with 1,1,2-trichloroethane (79-00-5) and tetrachloroethylene (127-18-4).

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

E. Gastric Intubation

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

*CAS registry numbers are given in parentheses.

mean body weight as a guide for determining the dose. Mean body weights for each group were recorded at weekly intervals for the first 10 weeks and at monthly intervals thereafter. All animals of one sex within a treated group received the same dose. Animals were gavaged with test solutions under a hood to minimize extraneous exposure of other animals and laboratory personnel to the chemical.

F. Selection of Initial Dose Levels

In order to establish the maximum tolerated dosages of 3-sulfolene for administration to treated animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. 3-Sulfolene mixed with corn oil was introduced by gavage to five of the six rat groups at dosages of 56, 100, 178, 316, and 562 mg/kg/day and five of the six mouse groups at dosages of 316, 562, 1000, 1780, and 3160 mg/kg/day. The sixth group of each species served as a control, receiving only the corn oil by gavage. Intubation was performed 5 consecutive days per week for 6 weeks, followed by a 2-week observation period to detect any delayed toxicity.

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

The only deaths observed among treated rats were two females, one receiving 316 mg/kg/day and one receiving 562 mg/kg/day. Mean body weight depression was 17 percent in males treated with 562 mg/kg/day, 18 percent in females treated with 178 mg/kg/day, and 35 percent in females treated with 316 mg/kg/day. The high dosages of 3-sulfolene selected for use in the chronic bioassay were 560 mg/kg/day for male rats and 200 mg/kg/day for female rats.

No mice treated with 562 mg/kg/day or less died during the subchronic test. The only group exhibiting mean body weight depression was the females receiving 316 mg/kg/day; in those groups for which treated mice survived the study (i.e., those receiving 1000 mg/kg/day or less) the mean body weight was greater than the mean body weight of the control group. The high dosage of 3-sulfolene selected for use in the chronic bioassay was 450 mg/kg/day for mice of both sexes.

G. Experimental Design

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

The treated and vehicle control rats shared the same median date of birth and were all approximately 8 weeks old at the time they were placed on test. The untreated controls were approximately 4 weeks younger than the other rats and were started on test approximately 1 week after the other groups.

TABLE 1

DESIGN SUMMARY FOR OSBORNE-MENDEL RATS
3-SULFOLENE GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	3-SULFOLENE DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE DOSAGE[b]
MALE					
UNTREATED CONTROL	20	0	0	110	0
VEHICLE CONTROL	20	0	0	111	0
LOW DOSE	50	280	22		197
		200	10		
		200[c]	36	10	
		0		33	
HIGH DOSE[d]	50	560	22		372[d]
		400	10		
		400[c]	8	2	
		200[c]	14	4	
FEMALE					
UNTREATED CONTROL	20	0	0	110	0
VEHICLE CONTROL	20	0	0	111	0
LOW DOSE	50	100	17		120
		150	15		
		150[c]	36	10	
		0		33	
HIGH DOSE	50	200	17		240
		300	15		
		300[c]	36	10	
		0		33	

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

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

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

[d]Terminated in week 60; therefore, average dosage is calculated over a 60-week period rather than a 78-week period.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
3-SULFOLENE GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	3-SULFOLENE DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE DOSAGE[b]
MALE					
UNTREATED CONTROL	20	0	0	90	0
VEHICLE CONTROL	20	0	0	90	0
LOW DOSE	50	225	2		311
		300	9		
		400	10		
		300	57		
		0		13	
HIGH DOSE	50	450	2		622
		600	9		
		800	10		
		600	57		
		0		13	
FEMALE					
UNTREATED CONTROL	20	0	0	90	0
VEHICLE CONTROL	20	0	0	90	0
LOW DOSE	50	225	2		384
		300	9		
		400	67		
		0		13	
HIGH DOSE	50	450	2		768
		600	9		
		800	67		
		0		13	

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

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

The dosages initially administered were 560 and 280 mg/kg/day for male rats and 200 and 100 mg/kg/day for female rats. Throughout this report the animals initially receiving the higher of the two dosages are referred to as the high dose group and the animals initially receiving the lower of the two dosages are referred to as the low dose group. In week 18 the dosages utilized for high and low dose females were increased to 300 and 150 mg/kg/day, respectively, while the dosages administered to high and low dose males were decreased to 400 and 200 mg/kg/day in week 23. In week 33 administration of 3-sulfolene to all rats ceased for 1 week followed by 4 weeks of compound intubation. This cyclic pattern of dose administration was maintained for the remainder of the bioassay. The dosage administered to the high dose males was decreased to 200 mg/kg/day in week 43. This dosage, too, was administered on a cyclic basis. All high dose male rats surviving until week 60 were sacrificed at that time. All other treated rats were dosed for 78 weeks, followed by an untreated observation period of up to 33 weeks.

The untreated control and all treated mice shared the same median date of birth and were approximately 6 weeks old at the time they were placed on test. The vehicle control mice were approximately 4 weeks older than the other mice and were started on test approximately 4 weeks before the other groups.

The dosages initially administered to both sexes of mice were 450 and 225 mg/kg/day. Throughout this report the animals initially

receiving the former dosage are referred to as the high dose group and those receiving the latter dosage are referred to as the low dose group. The dosages administered were increased twice; the first time, in week 3, to 600 and 300 mg/kg/day and the second time, in week 12, to 800 and 400 mg/kg/day. In week 22 the dosages administered to the high and low dose males were reduced to 600 and 300 mg/kg/day, respectively. These dosages were maintained for the remainder of the 78-week period of chemical administration, and treated mice were observed for a subsequent period of up to 13 weeks.

H. Clinical and Histopathologic Examinations

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

During the course of this bioassay several pathology protocols were in effect, each for different periods of time. The minimum protocol required that, if possible, certain tissues were to be taken and examined histopathologically from all control animals, from any animal in which a tumor was observed during gross examination, and from at least 10 grossly normal males and 10 grossly normal females

from each treated group. In addition, any tissues showing gross abnormalities were to be taken and examined histopathologically. Under later protocols, some tissues were taken from additional dosed animals. The number of animals in each group from which a tissue was examined is indicated in Appendices A through D.

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

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

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

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

Mean group body weight depression was observed for high dose male rats when compared to controls; this was not the case, however, for the other treated rat groups (Figure 1). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

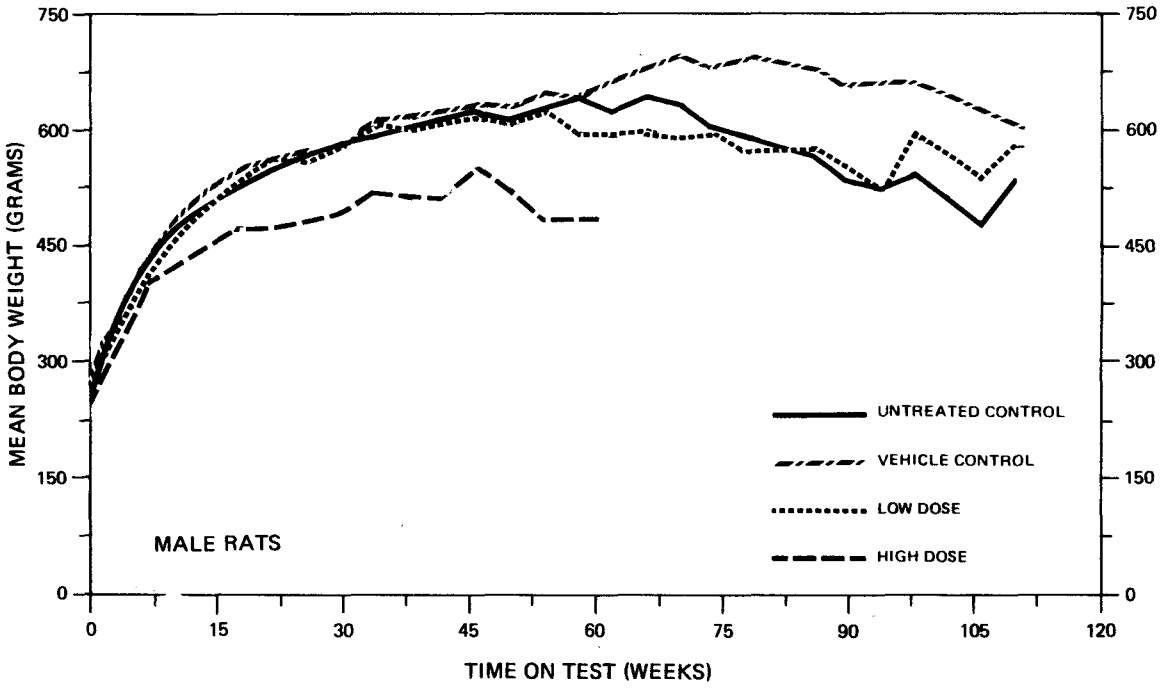
No characteristic clinical signs were observed during the first 5 weeks of the study. Beginning in week 6 and until the end of compound administration in week 78, a hunched appearance was noted in an increasing number of treated rats. Abdominal urine stains were also observed, particularly among treated females.

Respiratory signs characterized by labored respiration, wheezing or nasal discharge were observed at a low incidence during the study, increasing in all groups as the animals aged. Other signs commonly observed in aging rats and noted at a comparable frequency in treated and control rats included body sores, alopecia, reddened or squinted eyes, roughened or stained fur, tissue masses, and palpable nodules.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 3-sulfolene-dosed groups are shown in Figure 2.

For male rats the Tarone test indicated a significant ($P < 0.001$) positive association between dosage and mortality when dosed groups



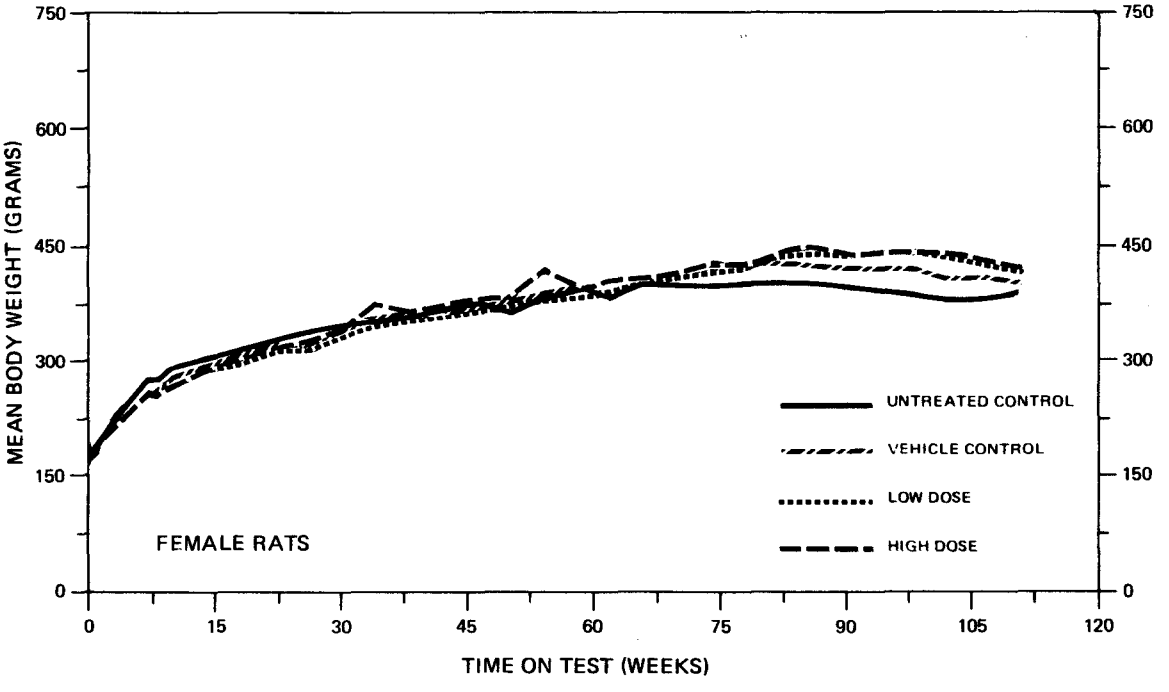


FIGURE 1
GROWTH CURVES FOR 3-SULFOLENE CHRONIC STUDY RATS

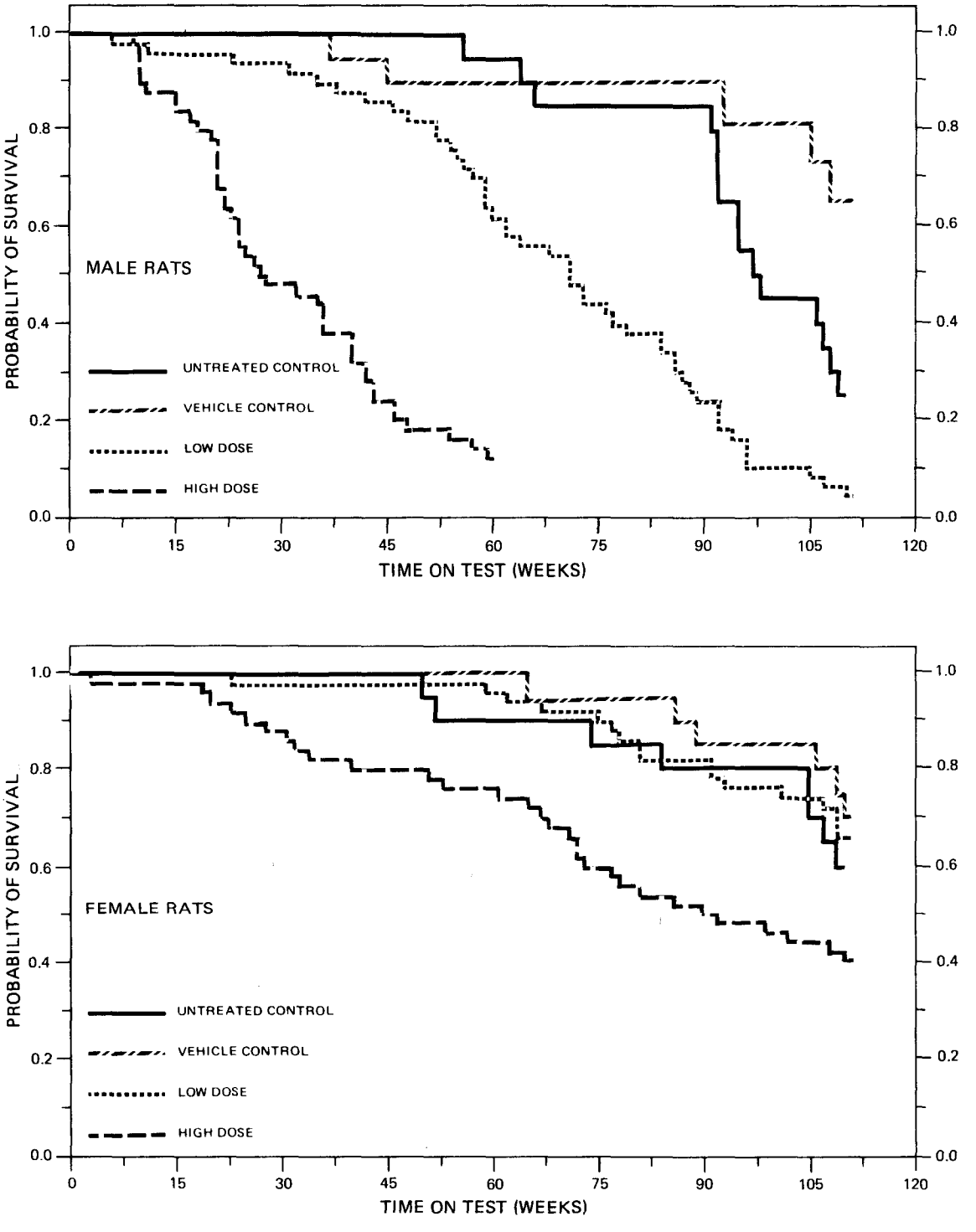


FIGURE 2
SURVIVAL COMPARISONS OF 3-SULFOLENE CHRONIC STUDY RATS

were compared to the vehicle controls. Due to the accelerated mortality in the high dose group the departure from linear trend was also significant ($P < 0.001$). In the high dose group 50 percent (25/50) were dead after only 27 weeks, with the six rats still surviving sacrificed at week 60. Survival was somewhat better in the low dose treated group where the median survival was 71 weeks. In the vehicle control group seven males were sacrificed in week 60, with 55 percent (11/20) of the rats surviving on test at least 90 weeks. In the untreated control group 85 percent (17/20) survived on test at least 90 weeks. In the high dose group survival was not adequate.

For female rats the Tarone test also showed a significant ($P = 0.002$) positive association between dosage and mortality when dosed groups were compared to the vehicle controls. Adequate numbers of animals were at risk from late-developing tumors with 52 percent (26/50) of the high dose, 82 percent (41/50) of the low dose, 85 percent (17/20) of the vehicle control, and 80 percent (16/20) of the untreated control group surviving on test at least 90 weeks.

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).

Pituitary tumors, which normally occur in elevated incidences in this strain of rats, were observed in 22/50 (44 percent) of the low dose females and in 6/50 (12 percent) of the high dose females.

Although the incidence of this tumor in the low dose females was not appreciably different from the incidences observed in control females (i.e., 8/20 [40 percent] in the untreated control and 7/20 [35 percent] in the vehicle control), the incidence among the high dose females was lower than expected. In addition, the incidences of mammary fibroadenomas were 17/50 (34 percent) and 9/50 (18 percent) in the low and high dose female rats, respectively. These neoplasms occur frequently in this strain in incidences approximating 30 percent. As observed, the incidence in the low dose females approximated that expected while the incidence among the high dose females was lower than expected. These instances of commonly observed tumors, occurring in unexpectedly low incidences are probably reflections of the poor survival among the high dose female rats.

The toxicity of 3-sulfolene leading to early mortality was reflected morphologically primarily in the circulatory, urinary, biliary, and genital systems. There was an increased incidence of commonly occurring nonneoplastic lesions including metastatic calcification of the lung, testes and stomach; vascular calcification in the aorta, pulmonary artery, mesenteric artery, and pancreatic artery; periarteristis of the pancreatic artery; hepatocellular fatty change; bile duct hyperplasia; intrahepatic cholangiectasia; portal bile stasis; chronic renal inflammation; cystitis; parathyroid hyperplasia; prostatitis; hypospermatogenesis; and testicular atrophy. The severity and incidence of these lesions was greatly increased and

their occurrence at an earlier age indicated the chemical was involved in this exacerbation of "spontaneous" lesions.

The early onset and increased incidence of chronic renal inflammation, with equivocal evidence of toxic tubular nephrosis apparently resulted in secondary parathyroid hyperplasia with subsequent metastatic calcification of numerous tissues.

In conclusion, administration of 3-sulfolene via gastric intubation resulted in early mortality, which was associated with the occurrence of a variety of nonneoplastic lesions. Neoplasms that were observed occurred in incidences that were within or below the range of spontaneous incidence commonly observed in Osborne-Mendel rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis 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 3-sulfolene-dosed groups and where such tumors were observed in at least 5 percent of the group. Because of the high early mortality noted in rats of both sexes, these analyses were based on those rats surviving at least 52 weeks.

For female rats the Cochran-Armitage test indicated a significant ($P = 0.029$) negative association between dose and the incidence of pituitary chromophobe adenomas. The Fisher exact tests, however, were not significant.

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH 3-SULFOLENE AND SURVIVING AT LEAST 52 WEEKS[a]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibrosarcoma[b]	2/18(0.11)	2/41(0.05)	0/9(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	0.439	0.000
Lower Limit	---	0.035	0.000
Upper Limit	---	5.743	6.011
Weeks to First Observed Tumor	108	60	---
Pituitary: Chromophobe Adenoma[b]	2/18(0.11)	3/41(0.07)	1/9(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	0.659	1.000
Lower Limit	---	0.085	0.018
Upper Limit	---	7.463	15.823
Weeks to First Observed Tumor	105	96	60

[a]Treated groups received time-weighted average doses of 197 or 372 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 3-SULFOLENE AND SURVIVING AT LEAST 52 WEEKS[a]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	0/20(0.00)	3/49(0.06)	0/39(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	Infinite	---
Lower Limit	---	0.255	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	111	---
Pituitary: Chromophobe Adenoma[b]	7/20(0.35)	22/49(0.45)	6/39(0.15)
P Values[c]	P = 0.029(N)	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.033	---	---
Relative Risk (Vehicle Control)[d]	---	1.283	0.440
Lower Limit	---	0.657	0.147
Upper Limit	---	3.044	1.342
Weeks to First Observed Tumor	89	77	72
Thyroid: Follicular-Cell Carcinoma[b]	1/20(0.05)	3/49(0.06)	1/39(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	1.224	0.513
Lower Limit	---	0.108	0.007
Upper Limit	---	62.958	39.256
Weeks to First Observed Tumor	111	109	92

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma[b]	2/20(0.10)	4/49(0.08)	2/39(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	0.816	0.513
Lower Limit	---	0.131	0.040
Upper Limit	---	8.603	6.709
Weeks to First Observed Tumor	111	109	92
Pancreatic Islets: Islet-Cell Adenoma or Islet-Cell Carcinoma[b]	0/20(0.00)	1/49(0.02)	3/39(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.023	0.321
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	111	102
Mammary Gland: Fibroadenoma[b]	6/20(0.30)	17/49(0.35)	9/39(0.23)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	1.156	0.769
Lower Limit	---	0.530	0.295
Upper Limit	---	3.142	2.306
Weeks to First Observed Tumor	106	75	102

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Adenoma NOS or Adenocarcinoma NOS[b]	1/20(0.05)	5/49(0.10)	5/39(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	2.041	2.564
Lower Limit	---	0.254	0.320
Upper Limit	---	94.440	117.857
Weeks to First Observed Tumor	111	93	102
Mammary Gland: Adenocarcinoma NOS, Adenoma NOS, or Fibroadenoma[b]	7/20(0.35)	20/49(0.41)	11/39(0.28)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	1.166	0.806
Lower Limit	---	0.587	0.351
Upper Limit	---	2.810	2.117
Weeks to First Observed Tumor	106	75	102
Ovary: Granulosa-Cell Tumor[b]	1/20(0.05)	1/48(0.02)	4/39(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	0.417	2.051
Lower Limit	---	0.006	0.225
Upper Limit	---	32.058	98.244
Weeks to First Observed Tumor	111	111	71

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stomal Polyp[b]	1/20(0.05)	4/48(0.08)	0/39(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	1.667	0.000
Lower Limit	---	0.192	0.000
Upper Limit	---	80.315	9.531
Weeks to First Observed Tumor	111	111	---

[a]Treated groups received time-weighted average doses of 120 or 240 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 $P < 0.05$.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between the administration of 3-sulfolene and tumor incidence. Thus, there was no evidence that 3-sulfolene was a carcinogen in Osborne-Mendel rats.

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

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Mean body weight depression was not observed when comparing treated mice with controls (Figure 3). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

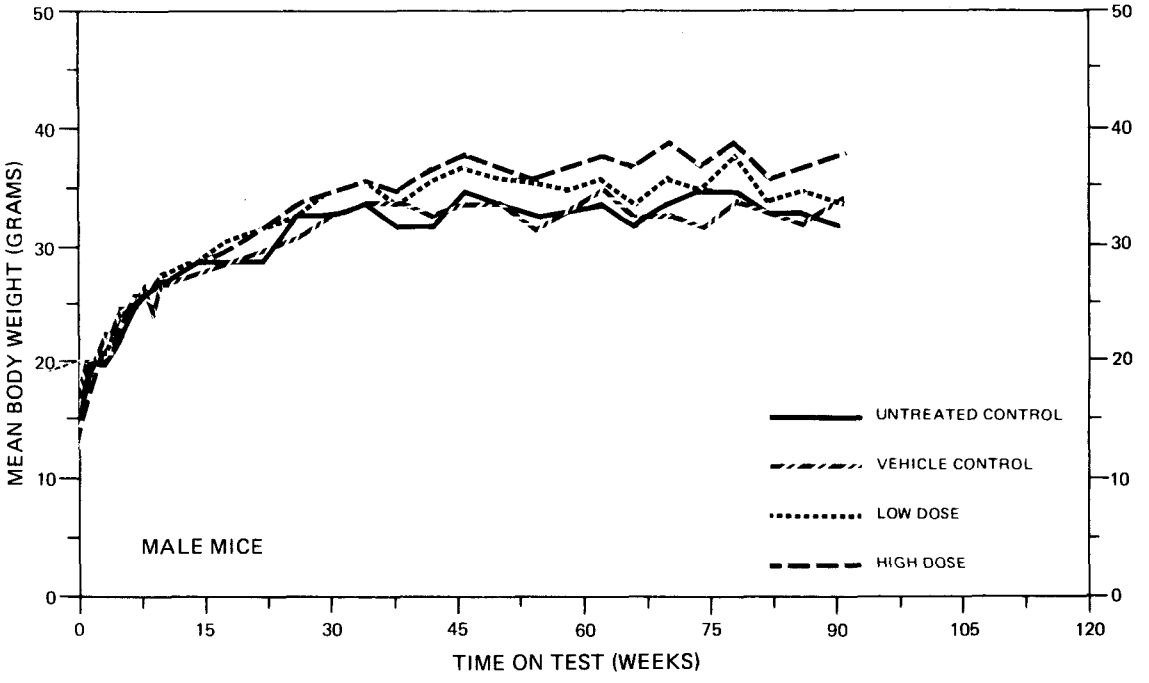
There was no definitive evidence of compound effect on physical appearance and behavior; however, a rapid decline in survival among the high dose mice, beginning in week 18 of the study, was considered to be a result of compound toxicity.

Signs commonly observed in group-housed laboratory mice were observed at a comparable rate in treated and control mice. These signs included sores on the body, localized alopecia, a hunched appearance, genital irritation, roughened or stained fur, bloated appearance, and palpable nodules or tissue masses.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 3-sulfolene-dosed groups are shown in Figure 4.

For male mice the Tarone test indicated a significant ($P < 0.001$) positive association between dosage and mortality when comparing the dosed group to the vehicle control. Due to the accelerated mortality in the high dose group, the departure from linear trend was also significant ($P < 0.001$). In the high dose treated group 52 percent (26/50) had died after only 21 weeks, with 24 percent (12/50) surviving



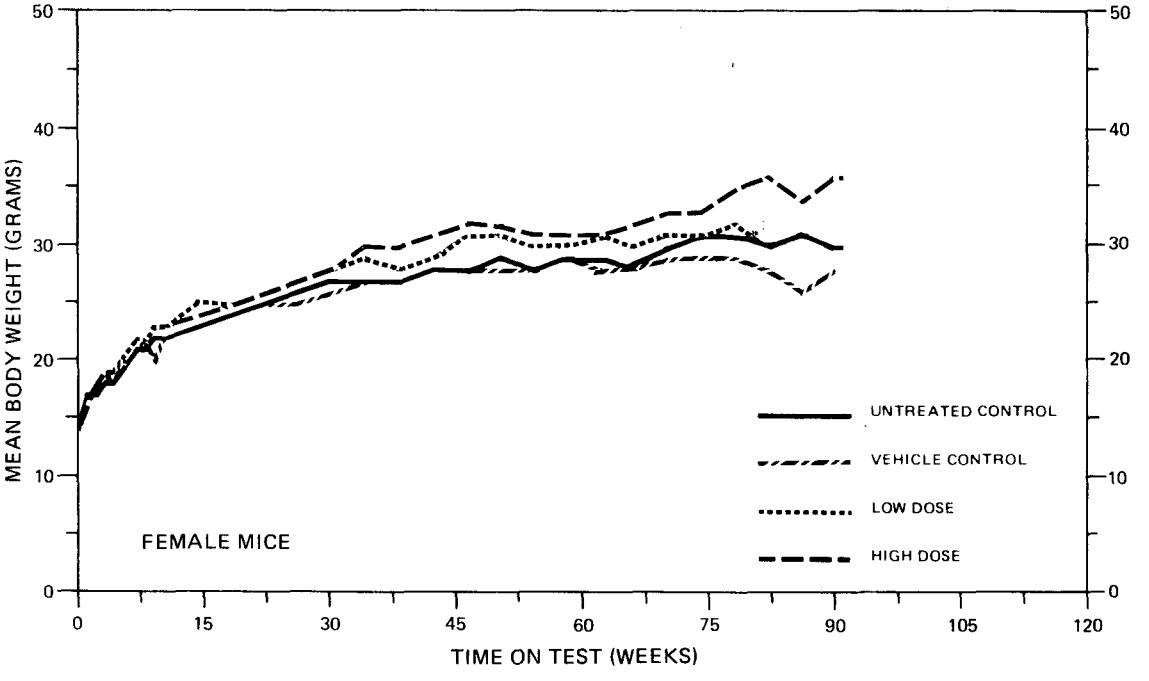
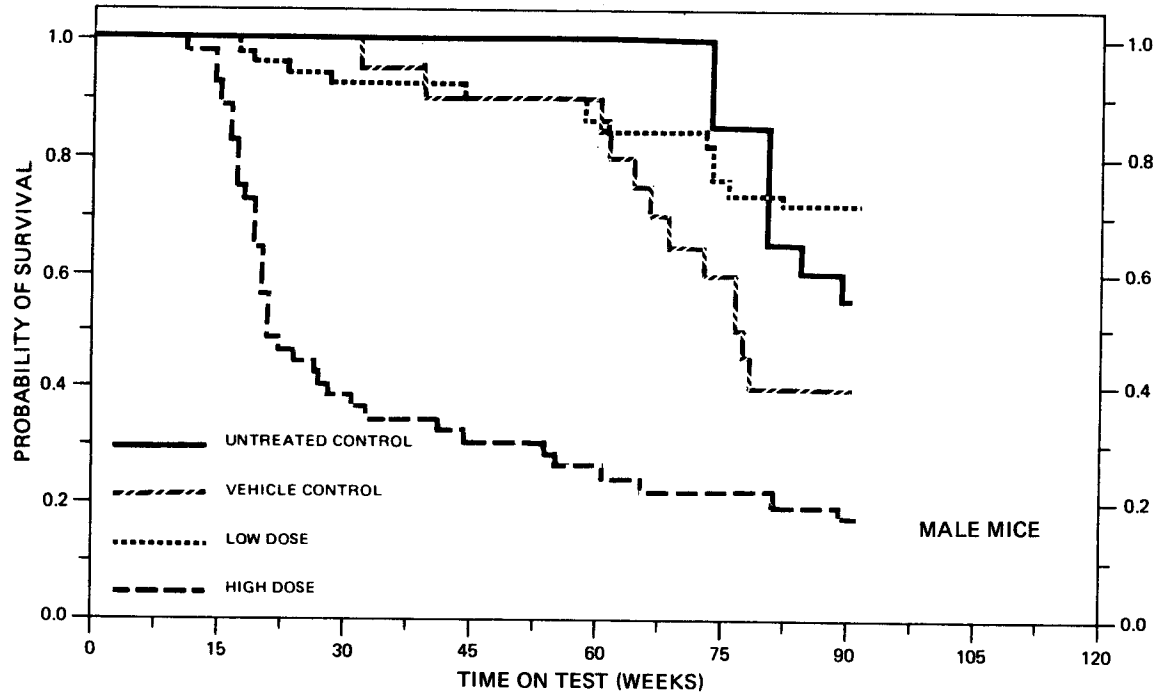


FIGURE 3
GROWTH CURVES FOR 3-SULFOLENE CHRONIC STUDY MICE



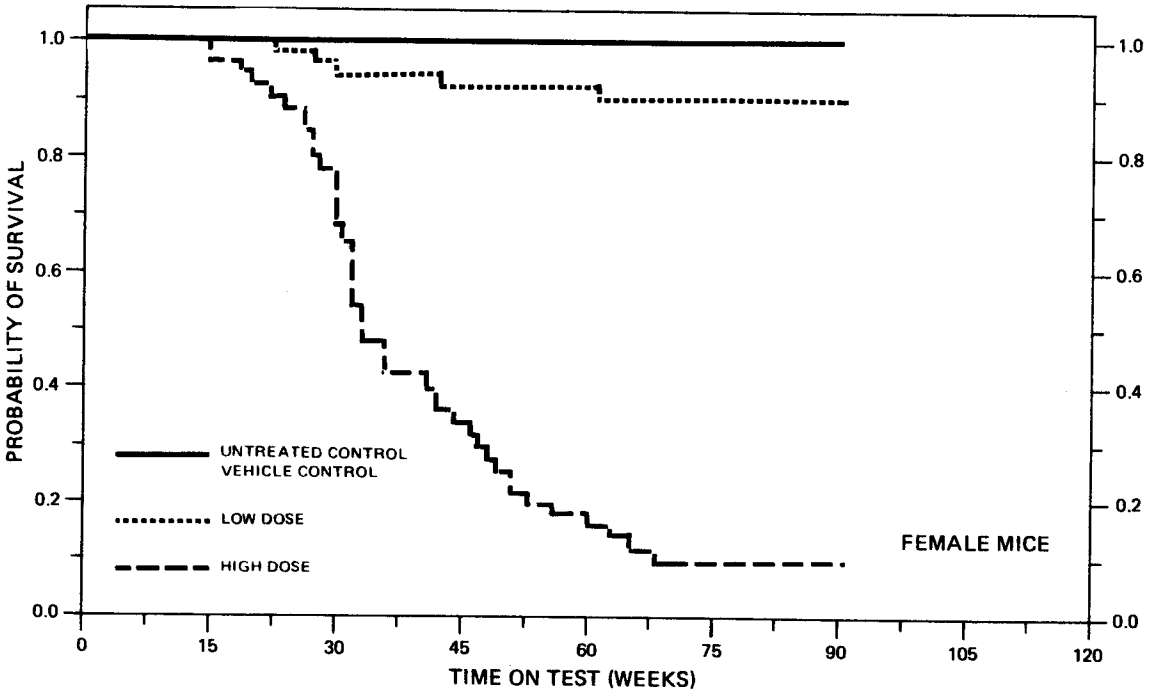


FIGURE 4
SURVIVAL COMPARISONS OF 3-SULFOLENE CHRONIC STUDY MICE

on test at least 65 weeks. Of the high dose mice that died before week 66, only two were reported to have lesions of any type; thus early mortality was not tumor-related. Survival was better in the low dose and control groups with 76 percent (38/50) of the low dose treated group, 60 percent (12/20) of the vehicle controls, and 85 percent (17/20) of the untreated controls surviving on test at least 75 weeks. In the low dose, vehicle control and untreated control groups, adequate numbers of mice were at risk from late-developing tumors. In the high dose group, however, survival was not adequate.

For female mice the Tarone test also showed a significant ($P < 0.001$) positive association between dosage and mortality when comparing the dosed groups to the vehicle control. The accelerated mortality in the high dose group resulted in a significant ($P < 0.001$) parture from linear trend. In the high dose treated group the median survival was 33 weeks with only 22 percent (11/50) of the animals surviving on test at least 52 weeks. Of the high dose females that died before week 52 only two were reported to have lesions of any type; thus early mortality was not tumor-related. Survival was good in the other groups with 90 percent (45/50) of the low dose group, 85 percent (17/20) of the vehicle controls, and 95 percent (19/20) of the untreated controls alive on test at the end of the study. In the low dose group and in both control groups, adequate numbers of mice were at risk from late-developing tumors. In the high dose group, however, survival was not adequate.

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).

Administration of 3-sulfolene resulted in high mortality in high dose male and female mice. Only 10 percent of the high dose females and 16 percent of the high dose males survived the experiment. Therefore, the low dose groups may be the only groups about which a conclusion of carcinogenicity can be made.

Hepatocellular carcinomas occurred in 2/20 (10 percent) untreated control, 1/20 (5 percent) vehicle control, 11/50 (22 percent) low dose, and 5/49 (10 percent) high dose males, and 1/49 (2 percent) low dose females. However, early deaths occurred in the high dose male and female groups from weeks 15 through 30 of the experiment, and only eight high dose males and five high dose females remained alive at the termination of the test (91 weeks). Survival in the low dose male and female groups was 36/50 and 45/50, respectively. The incidence of hepatocellular carcinomas in the low dose male group was considered to be within the normal range of such lesions seen in other studies conducted at this laboratory.

The incidence of inflammatory, degenerative, and proliferative lesions was also reduced in the high dose mice of both sexes due to early deaths. There were no pathologic alterations attributed to administration of the chemical.

In conclusion, the administration of the high doses of 3-sulfolene increased mortality in mice of both sexes, thus potential carcinogenic effect could not be evaluated in these groups. Survival of animals receiving low doses was believed to be sufficient to conclude that there was no tumorigenic effect at that concentration.

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 3-sulfolene-dosed groups and where such tumors were observed in at least 5 percent of the group. Because of high early mortality noted in mice of both sexes, these analyses were based on those mice surviving at least 52 weeks or, in the event that the tumor of interest was observed earlier than 52 weeks, these surviving at least until the first tumor of interest was observed.

In male mice an increased incidence of hepatocellular carcinomas was observed in the treated groups compared to the controls. The Cochran-Armitage test indicated a significant ($P = 0.040$) positive association between dose and incidence. The Fisher exact tests, however, were not significant at these low sample sizes.

No other tests from sites in either male or female mice were significant. Based upon these results there was no statistical evidence of the carcinogenicity of 3-sulfolene in mice.

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH 3-SULFOLENE AND SURVIVING AT LEAST 52 WEEKS[a]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibrosarcoma[b]	2/18(0.11)	0/45(0.00)	0/16(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.341	3.593
Weeks to First Observed Tumor	77	---	---
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b,e]	0/18(0.00)	4/50(0.08)	2/42(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.350	0.132
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	17	91
Hematopoietic System: Malignant Lymphoma[b]	1/18(0.06)	6/45(0.13)	2/15(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	2.400	2.400
Lower Limit	---	0.329	0.138
Upper Limit	---	107.714	131.036
Weeks to First Observed Tumor	76	72	78

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	1/18(0.06)	11/45(0.24)	5/15(0.33)
P Values[c]	P = 0.040	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	4.400	6.000
Lower Limit	---	0.732	0.782
Upper Limit	---	184.020	256.215
Weeks to First Observed Tumor	72	73	81

[a]Treated groups received time-weighted average doses of 311 or 622 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]For sites where the first tumor of interest was observed earlier than 52 weeks, the analyses were based upon all animals that survived until or past the date that the first tumor was observed.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH 3-SULFOLENE AND SURVIVING AT LEAST 52 WEEKS[a]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	1/18(0.06)	5/45(0.11)	1/11(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	2.000	1.636
Lower Limit	---	0.252	0.022
Upper Limit	---	92.361	116.035
Weeks to First Observed Tumor	90	91	91
Hematopoietic System: Malignant Lymphoma[b]	1/18(0.06)	3/45(0.07)	0/11(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	1.200	0.000
Lower Limit	---	0.107	0.000
Upper Limit	---	61.573	28.506
Weeks to First Observed Tumor	90	91	---
Pituitary: Chromophobe Adenoma[b]	0/18(0.00)	2/39(0.05)	0/6(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	Infinite	---
Lower Limit	---	0.143	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	91	---

TABLE 6 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 384 or 768 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.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In all of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that all of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by 3-sulfolene that could not be established under the conditions of this test.

V. DISCUSSION

There was a significant positive association between the administered dosages of 3-sulfolene and mortality in both sexes of rats and mice. In all groups, except the high dose male rats and the high dose male and female mice, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors.

Among rats evidence of the toxicity of 3-sulfolene that led to accelerated mortality was morphologically reflected primarily in the circulatory, urinary, biliary, and reproductive systems. There were no significant positive associations between administration of the compound and the incidences of any tumor in either sex.

When hepatocellular carcinomas were statistically analyzed in male mice surviving at least 52 weeks the incidences utilized were 1/18 (6 percent), 11/45 (24 percent), and 5/15 (33 percent) in the vehicle control, low dose, and high dose groups. Although there was a significant positive association between dosage and the incidences of this neoplasm in males, this finding was not supported by significant results from any of the Fisher exact comparisons using these low sample sizes. There were no other tumors in mice of either sex for which a significant positive association between chemical administration and incidence could be established.

Under the conditions of this bioassay 3-sulfolene administered by gavage was not carcinogenic to Osborne-Mendel rats or B6C3F1 mice.

These findings are supported by a previous study (Van Duuren et al., 1971) in which 3-sulfolene was tested for carcinogenicity in female ICR/Ha Swiss mice, along with a number of other small ring compounds including several monoepoxides and one acetal. In contrast to two structurally similar compounds, 3,4-epoxysulfolane and 1-propanesulfonic acid-3-hydroxy-Y-sultone, which induced a variety of benign and malignant tumors under similar testing conditions, weekly subcutaneous injections of 3-sulfolene in 30 mice for up to 93 weeks produced no tumors of any nature.

VI. BIBLIOGRAPHY

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

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

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

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

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

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

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

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

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

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

Rose, A. and E. Rose, The Condensed Chemical Dictionary, Seventh edition. Van Nostrand Reinhold Company, New York, 1966.

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

Stanford Research Institute, 1977 Directory of Chemical Producers, U.S.A. Menlo Park, California, 1977.

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

Van Duuren, B.L., S. Melchionne, R. Blair, B.M. Goldschmidt, and C. Katz, "Carcinogenicity of Isoesters of Epoxides and Lactones: Aziridine Ethanol, Propane Sultone, and Related Compounds." Journal of the National Cancer Institute 46(1):143-149, 1971.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 3-SULFOLENE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 3-SULFOLENE

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
BASAL-CELL CARCINOMA				1 (2%)
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
FIBROMA	1 (5%)	1 (5%)	1 (2%)	
FIBROSARCOMA		2 (10%)	2 (4%)	
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)	
HEMATOPOIETIC SYSTEM				
*ABDOMINAL CAVITY	(20)	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
#SPLEEN	(19)	(20)	(50)	(50)
HEMANGIOMA		1 (5%)		
HEMANGIOSARCOMA	2 (11%)		1 (2%)	
#MESENTERIC L. NODE	(19)	(18)	(45)	(30)
HEMANGIOMA		1 (6%)		
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(14)	(11)	(27)	(1)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (9%)		

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
#LIVER	(20)	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA			1 (2%)	
#PANCREAS	(19)	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (5%)		
#STOMACH	(20)	(20)	(50)	(50)
LEIOMYOSARCOMA		1 (5%)		
URINARY SYSTEM				
#KIDNEY	(19)	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (5%)		
MIXED TUMOR, MALIGNANT	1 (5%)			
ENDOCRINE SYSTEM				
#PITUITARY	(19)	(19)	(50)	(48)
CHROMOPHOBE ADENOMA	3 (16%)	2 (11%)	3 (6%)	1 (2%)
CHROMOPHOBE CARCINOMA	1 (5%)			
#ADRENAL	(19)	(20)	(50)	(50)
PHEOCHROMOCYTOMA		1 (5%)		
#THYROID	(19)	(20)	(50)	(49)
FOLLICULAR-CELL ADENOMA		1 (5%)		
FOLLICULAR-CELL CARCINOMA	1 (5%)	1 (5%)	2 (4%)	
C-CELL CARCINOMA			1 (2%)	
#PANCREATIC ISLETS	(19)	(20)	(50)	(50)
ISLET-CELL ADENOMA	2 (11%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)	
PAPILLARY CYSTADENOCARCINOMA,NOS			1 (2%)	
MEDULLARY CARCINOMA		1 (5%)		
FIBROADENOMA	1 (5%)		1 (2%)	
NERVOUS SYSTEM				
#BRAIN	(19)	(20)	(50)	(50)
CHROMOPHOBE CARCINOMA, METASTATI	1 (5%)			

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(20)	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (5%)		
BODY CAVITIES				
*PERITONEUM	(20)	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (5%)		
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(20)	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT			1 (2%)	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	14	5	47	43
MORIBUND SACRIFICE			1	1
SCHEDULED SACRIFICE		7		
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	6	8	2	6
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	9	13	1
TOTAL PRIMARY TUMORS	12	13	17	2
TOTAL ANIMALS WITH BENIGN TUMORS	6	7	4	1
TOTAL BENIGN TUMORS	7	7	5	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	4	10	1
TOTAL MALIGNANT TUMORS	5	6	12	1
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1		
TOTAL SECONDARY TUMORS	1	4		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 3-SULFOLENE

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
FIBROMA			3 (6%)	
FIBROSARCOMA	1 (5%)			
FIBROUS HISTIOCYTOMA, MALIGNANT				1 (2%)
HEMANGIOSARCOMA			1 (2%)	1 (2%)
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(50)
BILE DUCT CARCINOMA, METASTATIC			1 (2%)	
FIBROUS HISTIOCYTOMA, METASTATIC				1 (2%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(20)	(50)	(49)
BILE DUCT CARCINOMA, METASTATIC			1 (2%)	
CIRCULATORY SYSTEM				
*MESENTERIC ARTERY	(20)	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, METASTATIC				1 (2%)
DIGESTIVE SYSTEM				
#LIVER	(20)	(20)	(50)	(50)
NEOPLASTIC NODULE	1 (5%)		2 (4%)	
FIBROUS HISTIOCYTOMA, METASTATIC				1 (2%)
*BILE DUCT	(20)	(20)	(50)	(50)
BILE DUCT CARCINOMA			1 (2%)	

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
#PANCREAS	(20)	(20)	(50)	(50)
BILE DUCT CARCINOMA, METASTATIC			1 (2%)	
FIBROUS HISTIOCYTOMA, METASTATIC				1 (2%)
#STOMACH	(20)	(20)	(50)	(50)
BILE DUCT CARCINOMA, METASTATIC			1 (2%)	
#SMALL INTESTINE	(20)	(20)	(50)	(50)
BILE DUCT CARCINOMA, METASTATIC			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, METASTATIC				1 (2%)
LIPOMA			1 (2%)	1 (2%)
HAMARTOMA+	1 (5%)		1 (2%)	1 (2%)
#URINARY BLADDER	(20)	(20)	(50)	(49)
FIBROUS HISTIOCYTOMA, METASTATIC				1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(20)	(20)	(50)	(50)
CHROMOPHOBE ADENOMA	8 (40%)	7 (35%)	22 (44%)	6 (12%)
#ADRENAL	(20)	(20)	(50)	(50)
CORTICAL CARCINOMA		1 (5%)		
PHEOCHROMOCYTOMA		1 (5%)		
#THYROID	(19)	(20)	(50)	(50)
FOLLICULAR-CELL ADENOMA		1 (5%)	1 (2%)	1 (2%)
FOLLICULAR-CELL CARCINOMA		1 (5%)	3 (6%)	1 (2%)
C-CELL ADENOMA	2 (11%)		2 (4%)	
#PANCREATIC ISLETS	(20)	(20)	(50)	(50)
ISLET-CELL ADENOMA	1 (5%)		1 (2%)	2 (4%)
ISLET-CELL CARCINOMA				1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
ADENOMA, NOS		1 (5%)		1 (2%)
ADENOCARCINOMA, NOS			5 (10%)	4 (8%)
FIBROADENOMA	3 (15%)	6 (30%)	17 (34%)	9 (18%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
*VAGINA	(20)	(20)	(50)	(50)
ENDOMETRIAL STROMAL SARCOMA, MET		1 (5%)		
#UTERUS	(20)	(20)	(49)	(50)
ADENOCARCINOMA, NOS		1 (5%)		
ENDOMETRIAL STROMAL POLYP	1 (5%)	1 (5%)	4 (8%)	
ENDOMETRIAL STROMAL SARCOMA		1 (5%)		
#OVARY	(20)	(20)	(49)	(50)
BILE DUCT CARCINOMA, METASTATIC			1 (2%)	
GRANULOSA-CELL TUMOR		1 (5%)	1 (2%)	4 (8%)
SERTOLI-CELL TUMOR			1 (2%)	
FIBROUS HISTIOCYTOMA, METASTATIC				1 (2%)
NERVOUS SYSTEM				
#CEREBRUM	(20)	(20)	(50)	(50)
OLIGODENDROGLIOMA			1 (2%)	
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(20)	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT			1 (2%)	

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	8	6	16	28
MORIBUND SACRIFICE			1	2
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	12	14	33	20
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	14	39	21
TOTAL PRIMARY TUMORS	18	22	68	33
TOTAL ANIMALS WITH BENIGN TUMORS	13	11	36	15
TOTAL BENIGN TUMORS	16	17	53	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	4	11	8
TOTAL MALIGNANT TUMORS	1	4	12	8
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1	1
TOTAL SECONDARY TUMORS		1	6	7
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	1	3	4
TOTAL UNCERTAIN TUMORS	1	1	3	4
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 3-SULFOLENE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 3-SULFOLENE

	CONTROL (UNTR) 02-M101	CONTROL (VEH) 02-M091	LOW DOSE 02-M102	HIGH DOSE 02-M103
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	49
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
SEBACEOUS ADENOMA				1 (2%)
FIBROSARCOMA	1 (5%)	1 (5%)		
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
FIBROSARCOMA		2 (10%)		
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		4 (8%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)			
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(20)	(20)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (10%)		3 (6%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	1 (2%)
#SPLEEN	(20)	(20)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)			
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (5%)	2 (4%)	
#MESENTERIC L. NODE	(19)	(20)	(50)	(36)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (5%)		
#LIVER	(20)	(20)	(50)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)			
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (5%)		
#PROSTATE	(20)	(18)	(49)	(43)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (6%)		

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 02-M101	CONTROL (VEH) 02-M091	LOW DOSE 02-M102	HIGH DOSE 02-M103
*SEMINAL VESICLE	(20)	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (5%)		
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
*LIVER	(20)	(20)	(50)	(49)
HEPATOCELLULAR CARCINOMA	2 (10%)	1 (5%)	11 (22%)	5 (10%)
HEMANGIOSARCOMA			1 (2%)	
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
*THYROID	(19)	(19)	(47)	(45)
FOLLICULAR-CELL ADENOMA			1 (2%)	
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 02-M101	CONTROL (VEH) 02-M091	LOW DOSE 02-M102	HIGH DOSE 02-M103
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	9	11	12	41
MORIBUND SACRIFICE		1	2	
SCHEDULED SACRIFICE				1
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	11	8	36	8
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	5	20	8
TOTAL PRIMARY TUMORS	11	9	23	10
TOTAL ANIMALS WITH BENIGN TUMORS	1		5	2
TOTAL BENIGN TUMORS	1		5	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	5	15	7
TOTAL MALIGNANT TUMORS	10	9	18	7
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS				
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN				

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 3-SULFOLENE

	CONTROL(UNTR) 02-F101	CONTROL(VEH) 02-F091	LOW DOSE 02-F104	HIGH DOSE 02-F105
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	49	48
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(20)	(20)	(49)	(49)
OSTEOSARCOMA		1 (5%)		
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(49)	(47)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (5%)	4 (8%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(20)	(20)	(49)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)			
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		2 (4%)	
#SPLEEN	(20)	(19)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)			
#MESENTERIC L. NODE	(19)	(20)	(47)	(44)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (5%)		
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(20)	(19)	(49)	(48)
HEPATOCELLULAR CARCINOMA			1 (2%)	
URINARY SYSTEM				
NONE				

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 02-F101	CONTROL (VEH) 02-F091	LOW DOSE 02-F104	HIGH DOSE 02-F105
ENDOCRINE SYSTEM				
#PITUITARY	(18)	(20)	(42)	(25)
CHROMOPHOBE ADENOMA			2 (5%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(49)	(49)
ADENOCARCINOMA, NOS			1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(20)	(49)	(45)
ADENOCARCINOMA, NOS		1 (5%)		
#OVARY	(20)	(20)	(49)	(44)
GRANULOSA-CELL TUMOR			1 (2%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 02-F101	CONTROL (VEH) 02-F091	LOW DOSE 02-F104	HIGH DOSE 02-F105
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@			5	45
MORIBUND SACRIFICE				
SCHEDULED SACRIFICE	1			
ACCIDENTALLY KILLED		3		
TERMINAL SACRIFICE	19	17	45	5
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	4	10	1
TOTAL PRIMARY TUMORS	4	4	13	1
TOTAL ANIMALS WITH BENIGN TUMORS		1	6	1
TOTAL BENIGN TUMORS		1	6	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	3	6	
TOTAL MALIGNANT TUMORS	4	3	6	
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1	
TOTAL UNCERTAIN TUMORS			1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS				
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN				

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 3-SULFOLENE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 3-SULFOLENE

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (5%)			
INFLAMMATION, NOS	1 (5%)	1 (5%)		
ULCER, FOCAL				1 (2%)
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (5%)			
RESPIRATORY SYSTEM				
#TRACHEA	(19)	(20)	(50)	(49)
ULCER, NOS			1 (2%)	
INFLAMMATION, CHRONIC		2 (10%)	1 (2%)	
#LUNG/BRONCHUS	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE SUPPURATIVE				1 (2%)
#LUNG/BRONCHIOLE	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
#LUNG	(20)	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE				2 (4%)
INFLAMMATION, ACUTE			1 (2%)	
ABSCESS, NOS			1 (2%)	2 (4%)
PNEUMONIA, CHRONIC MURINE	16 (80%)	15 (75%)	33 (66%)	22 (44%)
INFLAMMATION, GRANULOMATOUS		1 (5%)		
PERIARTERITIS			1 (2%)	
CALCIFICATION, DYSTROPHIC			1 (2%)	
CALCIFICATION, METASTATIC		1 (5%)		
#LUNG/ALVEOLI	(20)	(20)	(50)	(50)
CALCIFICATION, NOS			5 (10%)	1 (2%)
CALCIFICATION, METASTATIC			2 (4%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(20)	(20)	(49)	(50)
HYPERPLASIA, HEMATOPOIETIC		1 (5%)	1 (2%)	
#SPLEEN	(19)	(20)	(50)	(50)
INFLAMMATION, GRANULOMATOUS			1 (2%)	
HEMATOPOIESIS	1 (5%)	1 (5%)	1 (2%)	
#LYMPH NODE	(19)	(18)	(45)	(30)
HYPERPLASIA, NOS		1 (6%)		
#THYMUS	(16)	(10)	(23)	(24)
ATROPHY, NOS		1 (10%)		
CIRCULATORY SYSTEM				
#HEART	(20)	(20)	(50)	(50)
THROMBUS, ORGANIZED			1 (2%)	
CALCIUM DEPOSIT	2 (10%)			
CALCIFICATION, NOS			3 (6%)	
CALCIFICATION, METASTATIC		1 (5%)	3 (6%)	
#MYOCARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
FIBROSIS	1 (5%)		13 (26%)	
FIBROSIS, FOCAL				1 (2%)
NECROSIS, NOS			1 (2%)	
CALCIFICATION, NOS			4 (8%)	1 (2%)
CALCIFICATION, METASTATIC			1 (2%)	
#ENDOCARDIUM	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)			
*ARTERY	(20)	(20)	(50)	(50)
MINERALIZATION		1 (5%)		
INFLAMMATION, NOS		1 (5%)		
MEDIAL CALCIFICATION			2 (4%)	
NECROSIS, NOS		1 (5%)		
HYPERPLASIA, HEMATOPOIETIC		1 (5%)		
*AORTA	(20)	(20)	(50)	(50)
PERIARTERITIS			1 (2%)	
MEDIAL CALCIFICATION	3 (15%)	1 (5%)	10 (20%)	1 (2%)
CALCIFICATION, METASTATIC			1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
*CORONARY ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION	2 (10%)		3 (6%)	1 (2%)
CALCIFICATION, METASTATIC			1 (2%)	
*PULMONARY ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION			14 (28%)	1 (2%)
CALCIFICATION, METASTATIC			1 (2%)	
*MESENTERIC ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION	1 (5%)	1 (5%)	9 (18%)	
CALCIFICATION, NOS			1 (2%)	
CALCIFICATION, METASTATIC			3 (6%)	
*TESTICULAR ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION			2 (4%)	
CALCIFICATION, NOS			1 (2%)	
DIGESTIVE SYSTEM				
#LIVER	(20)	(20)	(50)	(50)
BILE STASIS			18 (36%)	31 (62%)
INFLAMMATION, NOS	1 (5%)			
INFLAMMATION, FOCAL			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE				1 (2%)
INFLAMMATION, ACUTE NECROTIZING				1 (2%)
GRANULOMA, NOS			1 (2%)	
PERIARTERITIS			1 (2%)	
DEGENERATION, NOS			1 (2%)	
PELIOSIS HEPATIS		3 (15%)	4 (8%)	
METAMORPHOSIS FATTY	1 (5%)	1 (5%)		
FOCAL CELLULAR CHANGE			1 (2%)	
ANGIECTASIS				1 (2%)
#HEPATIC LOBULE	(20)	(20)	(50)	(50)
INFLAMMATION, NECROTIZING				1 (2%)
DEGENERATION, NOS			1 (2%)	
METAMORPHOSIS FATTY			1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(20)	(50)	(50)
DEGENERATION, NOS				1 (2%)
METAMORPHOSIS FATTY			5 (10%)	1 (2%)
#LIVER/PERIPORTAL	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (5%)		
FIBROSIS		1 (5%)	1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
*BILE DUCT	(20)	(20)	(50)	(50)
DILATATION, NOS				1 (2%)
BILE STASIS				1 (2%)
INFLAMMATION, CHRONIC			9 (18%)	12 (24%)
HYPERPLASIA, NOS		5 (25%)	24 (48%)	28 (56%)
#PANCREAS	(19)	(20)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (5%)		
FIBROSIS				1 (2%)
PERIARTERITIS		1 (5%)	7 (14%)	
ATROPHY, NOS				1 (2%)
#PANCREATIC DUCT	(19)	(20)	(50)	(50)
INFLAMMATION, CHRONIC				1 (2%)
FIBROSIS				1 (2%)
#STOMACH	(20)	(20)	(50)	(50)
CALCIUM DEPOSIT	3 (15%)			
CALCIFICATION, NOS			2 (4%)	1 (2%)
CALCIFICATION, FOCAL			1 (2%)	
CALCIFICATION, METASTATIC		1 (5%)	10 (20%)	1 (2%)
#LARGE INTESTINE	(20)	(18)	(49)	(50)
PARASITISM		2 (11%)	2 (4%)	
URINARY SYSTEM				
#KIDNEY	(19)	(20)	(50)	(50)
HYDRONEPHROSIS			1 (2%)	1 (2%)
HEMORRHAGE				1 (2%)
PYELONEPHRITIS, NOS	2 (11%)			4 (8%)
PYELONEPHRITIS, ACUTE				1 (2%)
ABSCESS, NOS			1 (2%)	
INFLAMMATION, CHRONIC	13 (68%)	14 (70%)	43 (86%)	23 (46%)
PYELONEPHRITIS, CHRONIC			1 (2%)	
CALCIUM DEPOSIT	1 (5%)			
CALCIFICATION, NOS		3 (15%)	7 (14%)	3 (6%)
CALCIFICATION, METASTATIC		1 (5%)		1 (2%)
#KIDNEY/CAPSULE	(19)	(20)	(50)	(50)
HEMORRHAGE				1 (2%)
*URETER	(20)	(20)	(50)	(50)
CALCIFICATION, NOS			4 (8%)	
CALCIFICATION, METASTATIC			1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
#URINARY BLADDER	(19)	(19)	(50)	(49)
CALCULUS, NOS				2 (4%)
INFLAMMATION, NOS	1 (5%)			
ULCER, NOS				1 (2%)
INFLAMMATION, ACUTE				3 (6%)
ULCER, ACUTE				4 (8%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	1 (2%)
INFLAMMATION, CHRONIC				1 (2%)
CALCIFICATION, NOS			1 (2%)	
HYPERPLASIA, EPITHELIAL			2 (4%)	
ENDOCRINE SYSTEM				
#PITUITARY	(19)	(19)	(50)	(48)
HYPERPLASIA, CHROMOPHOBE-CELL		1 (5%)		
#ADRENAL	(19)	(20)	(50)	(50)
CALCIFICATION, METASTATIC			2 (4%)	
#ADRENAL CORTEX	(19)	(20)	(50)	(50)
DEGENERATION, NOS		7 (35%)	20 (40%)	8 (16%)
ANGIECTASIS		1 (5%)	2 (4%)	
#THYROID	(19)	(20)	(50)	(49)
FOLLICULAR CYST, NOS	1 (5%)			
#PARATHYROID	(19)	(17)	(50)	(37)
HYPERPLASIA, NOS	1 (5%)	2 (12%)	15 (30%)	6 (16%)
REPRODUCTIVE SYSTEM				
#PROSTATE	(19)	(16)	(39)	(27)
INFLAMMATION, NOS	2 (11%)			
INFLAMMATION, SUPPURATIVE			1 (3%)	
INFLAMMATION, ACUTE			5 (13%)	4 (15%)
INFLAMMATION, ACUTE FOCAL			2 (5%)	
INFLAMMATION, ACUTE/CHRONIC				2 (7%)
INFLAMMATION, CHRONIC		1 (6%)	1 (3%)	2 (7%)
*SEMINAL VESICLE	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
INFLAMMATION, ACUTE/CHRONIC				1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
#TESTIS	(19)	(20)	(50)	(50)
PERIARTERITIS		1 (5%)		
CALCIFICATION, NOS			2 (4%)	1 (2%)
CALCIFICATION, METASTATIC			1 (2%)	
ATROPHY, NOS	8 (42%)	1 (5%)	9 (18%)	2 (4%)
HYPOSPERMATOGENESIS		4 (20%)	11 (22%)	7 (14%)
#TESTIS/TUBULE	(19)	(20)	(50)	(50)
CALCIFICATION, NOS			1 (2%)	
*EPIDIDYMIS	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE FOCAL				1 (2%)
PERIARTERITIS		1 (5%)		
NECROSIS, FAT		1 (5%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE/CORNEA	(20)	(20)	(50)	(50)
ULCER, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
*BONE	(20)	(20)	(50)	(50)
FIBROUS OSTEODYSTROPHY		1 (5%)	3 (6%)	
*STERNUM	(20)	(20)	(50)	(50)
EXOSTOSIS			1 (2%)	
*SKELETAL MUSCLE	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)			
INFLAMMATION, ACUTE				1 (2%)
BODY CAVITIES				
*PERITONEUM	(20)	(20)	(50)	(50)
INFLAMMATION, FOCAL				1 (2%)
INFLAMMATION, ACUTE FOCAL				1 (2%)

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-141M	CONTROL (VEH) 01-101M	LOW DOSE 01-102M	HIGH DOSE 01-103M
*PLEURA	(20)	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE				1 (2%)
INFLAMMATION, ACUTE				1 (2%)
*PERICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE				1 (2%)
*EPICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
*MESENTERY	(20)	(20)	(50)	(50)
PERIARTERITIS	2 (10%)	3 (15%)	8 (16%)	
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
INFLAMMATION, CHRONIC FOCAL				1
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED			1	2

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 3-SULFOLENE

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
CYST, NOS			2 (4%)	
INFLAMMATION, NOS	1 (5%)			
ULCER, NOS		1 (5%)		
INFLAMMATION, SUPPURATIVE			1 (2%)	
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
HEMORRHAGE				1 (2%)
GRANULOMA, FOREIGN BODY				1 (2%)
CALCIFICATION, NOS				1 (2%)
RESPIRATORY SYSTEM				
*NASAL CAVITY	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
#TRACHEA	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE SUPPURATIVE				2 (4%)
#LUNG	(20)	(20)	(50)	(50)
PNEUMONIA, ASPIRATION			2 (4%)	1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)	2 (4%)
INFLAMMATION, ACUTE		1 (5%)		
INFLAMMATION, ACUTE SUPPURATIVE			3 (6%)	1 (2%)
ABSCESS, NOS		1 (5%)	3 (6%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	19 (95%)	18 (90%)	39 (78%)	23 (46%)
INFLAMMATION, PYOGRANULOMATOUS				1 (2%)
CALCIFICATION, METASTATIC		1 (5%)		
#LUNG/ALVEOLI	(20)	(20)	(50)	(50)
CALCIFICATION, METASTATIC				1 (2%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(20)	(20)	(50)	(50)
HYPERPLASIA, HEMATOPOIETIC		4 (20%)	2 (4%)	2 (4%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
#SPLEEN	(20)	(20)	(50)	(49)
HEMATOPOIESIS	3 (15%)	2 (10%)	4 (8%)	2 (4%)
#MANDIBULAR L. NODE	(20)	(20)	(50)	(50)
INFLAMMATION, NOS		1 (5%)		
INFLAMMATION, ACUTE		1 (5%)		
#CERVICAL LYMPH NODE	(20)	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID		1 (5%)		
CIRCULATORY SYSTEM				
#HEART	(20)	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
#MYOCARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
FIBROSIS		1 (5%)	2 (4%)	1 (2%)
CALCIFICATION, METASTATIC				1 (2%)
#ENDOCARDIUM	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)			1 (2%)
*ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION		1 (5%)		
CALCIFICATION, METASTATIC		1 (5%)		
*AORTA	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION	1 (5%)			1 (2%)
*CORONARY ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION				1 (2%)
*PULMONARY ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION				1 (2%)
*MESENTERIC ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION			1 (2%)	2 (4%)
DIGESTIVE SYSTEM				
#LIVER	(20)	(20)	(50)	(50)
BILE STASIS				1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
#LIVER (CON'T)				
THROMBOSIS, NOS				1 (2%)
INFLAMMATION, NOS	1 (5%)			
PELIOSIS HEPATIS				1 (2%)
NECROSIS, FOCAL				1 (2%)
METAMORPHOSIS FATTY	2 (10%)			
FOCAL CELLULAR CHANGE		1 (5%)	1 (2%)	1 (2%)
ANGIECTASIS			1 (2%)	1 (2%)
#HEPATIC LOBULE	(20)	(20)	(50)	(50)
METAMORPHOSIS FATTY			1 (2%)	1 (2%)
#LIVER/CENTRILOBULAR	(20)	(20)	(50)	(50)
INFLAMMATION, NECROTIZING				1 (2%)
METAMORPHOSIS FATTY			1 (2%)	2 (4%)
#LIVER/HEPATOCYTES	(20)	(20)	(50)	(50)
FOCAL CELLULAR CHANGE				1 (2%)
*BILE DUCT	(20)	(20)	(50)	(50)
DILATATION, NOS			2 (4%)	1 (2%)
INFLAMMATION, CHRONIC			1 (2%)	1 (2%)
HYPERPLASIA, NOS	2 (10%)	6 (30%)	14 (28%)	8 (16%)
ANGIECTASIS		1 (5%)		
#PANCREAS	(20)	(20)	(50)	(50)
PERIARTERITIS		1 (5%)	2 (4%)	2 (4%)
ATROPHY, NOS				1 (2%)
#STOMACH	(20)	(20)	(50)	(50)
ULCER, FOCAL	3 (15%)		3 (6%)	
CALCIFICATION, NOS				1 (2%)
CALCIFICATION, METASTATIC		1 (5%)		1 (2%)
HYPERKERATOSIS				1 (2%)
#GASTRIC MUCOSA	(20)	(20)	(50)	(50)
ULCER, NOS				2 (4%)
ULCER, FOCAL				1 (2%)
#SMALL INTESTINE	(20)	(20)	(50)	(50)
ULCER, NOS		1 (5%)		
#LARGE INTESTINE	(20)	(20)	(50)	(50)
PARASITISM		1 (5%)	4 (8%)	
#COLON	(20)	(20)	(50)	(50)
PARASITISM		1 (5%)		

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
#CECUM	(20)	(20)	(50)	(50)
PARASITISM			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(50)
MINERALIZATION		2 (10%)		
HYDRONEPHROSIS		1 (5%)		
PYELONEPHRITIS, NOS		1 (5%)	1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	6 (30%)	4 (20%)	17 (34%)	25 (50%)
NEPHROPATHY		1 (5%)		
CALCIUM DEPOSIT	1 (5%)			
CALCIFICATION, NOS		7 (35%)	3 (6%)	2 (4%)
#URINARY BLADDER	(20)	(20)	(50)	(49)
ULCER, ACUTE			1 (2%)	
ULCER, CHRONIC			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(20)	(20)	(50)	(50)
HYPERPLASIA, CHROMOPHOBE-CELL		2 (10%)	2 (4%)	4 (8%)
#ADRENAL	(20)	(20)	(50)	(50)
CALCIUM DEPOSIT	1 (5%)			
HYPOPLASIA, NOS			1 (2%)	
#ADRENAL CORTEX	(20)	(20)	(50)	(50)
DEGENERATION, NOS		6 (30%)	11 (22%)	11 (22%)
ANGIECTASIS	5 (25%)	8 (40%)	11 (22%)	11 (22%)
#THYROID	(19)	(20)	(50)	(50)
FOLLICULAR CYST, NOS	2 (11%)			2 (4%)
#PARATHYROID	(20)	(18)	(49)	(37)
HYPERPLASIA, NOS				7 (19%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
GALACTOCELE			1 (2%)	
NECROSIS, FAT	1 (5%)			

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
*VAGINA	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)			
INFLAMMATION, ACUTE SUPPURATIVE				1 (2%)
#UTERUS	(20)	(20)	(49)	(50)
HYDROMETRA				1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(20)	(49)	(50)
INFLAMMATION, NOS	2 (10%)	1 (5%)		
INFLAMMATION, ACUTE			5 (10%)	1 (2%)
HYPERPLASIA, CYSTIC			2 (4%)	
#OVARY/OVIDUCT	(20)	(20)	(49)	(50)
INFLAMMATION, ACUTE			2 (4%)	
#OVARY	(20)	(20)	(49)	(50)
CYST, NOS	1 (5%)			
FOLLICULAR CYST, NOS		1 (5%)		
NERVOUS SYSTEM				
#BRAIN/MENINGES	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
#BRAIN	(20)	(20)	(50)	(50)
HEMORRHAGE				1 (2%)
SPECIAL SENSE ORGANS				
*EYE/CRYSTALLINE LENS	(20)	(20)	(50)	(50)
DISLOCATION COMPLETE				1 (2%)
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(20)	(20)	(50)	(50)
INFLAMMATION, PYOGRANULOMATOUS				1 (2%)
PERIARTERITIS		1 (5%)		
*MUSCLE OF NECK	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
BODY CAVITIES				
*ABDOMINAL CAVITY	(20)	(20)	(50)	(50)
HEMORRHAGE				1 (2%)

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 01-141F	CONTROL (VEH) 01-101F	LOW DOSE 01-104F	HIGH DOSE 01-105F
*PLEURA	(20)	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)	
INFLAMMATION, ACUTE				5 (10%)
INFLAMMATION, CHRONIC				1 (2%)
FIBROSIS				1 (2%)
*PERICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)	1 (2%)
INFLAMMATION, CHRONIC				1 (2%)
*MESENTERY	(20)	(20)	(50)	(50)
PERIARTERITIS			2 (4%)	2 (4%)
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
INFLAMMATION, NOS			1	
INFLAMMATION, CHRONIC				1
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED			1	2

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 3-SULFOLENE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 3-SULFOLENE

	CONTROL (UNTR) 02-M101	CONTROL (VEH) 02-M091	LOW DOSE 02-M102	HIGH DOSE 02-M103
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	49
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
INFLAMMATION, NOS		1 (5%)		
ACANTHOSIS		1 (5%)		
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
ABSCESS, NOS	2 (10%)	1 (5%)		
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(48)
PNEUMONIA, CHRONIC MURINE			6 (12%)	1 (2%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(20)	(50)	(48)
AMYLOIDOSIS	2 (10%)	5 (25%)		
#CERVICAL LYMPH NODE	(19)	(20)	(50)	(36)
HYPERPLASIA, RETICULUM CELL			1 (2%)	
#MESENTERIC L. NODE	(19)	(20)	(50)	(36)
INFLAMMATION, NOS			1 (2%)	
ANGIECTASIS		1 (5%)		
CIRCULATORY SYSTEM				
#ENDOCARDIUM	(20)	(20)	(50)	(49)
HYPERPLASIA, NOS		1 (5%)		
DIGESTIVE SYSTEM				
#LIVER	(20)	(20)	(50)	(49)
INFLAMMATION, NOS	2 (10%)			

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 02-M101	CONTROL (VEH) 02-M091	LOW DOSE 02-M102	HIGH DOSE 02-M103
PELIOSIS HEPATIS				2 (4%)
INFARCT, NOS			1 (2%)	
HYPERPLASIA, NODULAR		1 (5%)		
ANGIECTASIS	1 (5%)		1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(50)	(49)
HYDRONEPHROSIS	3 (15%)	7 (35%)		
CYST, NOS				1 (2%)
PYELONEPHRITIS, NOS		1 (5%)		
INFLAMMATION, CHRONIC	14 (70%)	9 (45%)	3 (6%)	2 (4%)
AMYLOIDOSIS	4 (20%)	5 (25%)		1 (2%)
METAMORPHOSIS FATTY	1 (5%)			
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
#BRAIN/MENINGES	(20)	(20)	(50)	(49)
INFLAMMATION, NOS			1 (2%)	
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 02-M101	CONTROL (VEH) 02-M091	LOW DOSE 02-M102	HIGH DOSE 02-M103
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	2	5	24	38
AUTO/NECROPSY/NO HISTO				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 3-SULFOLENE

	CONTROL (UNTR) 02-F101	CONTROL (VEH) 02-F091	LOW DOSE 02-F104	HIGH DOSE 02-F105
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	49	48
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(49)	(47)
PNEUMONIA, CHRONIC MURINE		2 (10%)	7 (14%)	1 (2%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(19)	(49)	(48)
HYPERPLASIA, LYMPHOID	1 (5%)			
#BRONCHIAL LYMPH NODE	(19)	(20)	(47)	(44)
INFLAMMATION, NOS			1 (2%)	
#MESENTERIC L. NODE	(19)	(20)	(47)	(44)
INFLAMMATION, NOS			1 (2%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(20)	(19)	(49)	(48)
INFLAMMATION, NOS	1 (5%)			
PELIOSIS HEPATIS			1 (2%)	3 (6%)
ANGIECTASIS			1 (2%)	
#STOMACH	(20)	(20)	(48)	(48)
HYPERKERATOSIS	1 (5%)			
ACANTHOSIS	1 (5%)			

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 02-F101	CONTROL (VEH) 02-F091	LOW DOSE 02-F104	HIGH DOSE 02-F105
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(18)	(20)	(42)	(25)
ANGIECTASIS	1 (6%)			
#THYROID	(18)	(20)	(39)	(39)
FOLLICULAR CYST, NOS			1 (3%)	
REPRODUCTIVE SYSTEM				
#UTERUS	(20)	(20)	(49)	(45)
HYDROMETRA	4 (20%)		10 (20%)	3 (7%)
INFLAMMATION, NOS	2 (10%)	1 (5%)	1 (2%)	1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(20)	(49)	(45)
HYPERPLASIA, CYSTIC	5 (25%)	10 (50%)	9 (18%)	4 (9%)
#OVARY	(20)	(20)	(49)	(44)
CYST, NOS	3 (15%)	6 (30%)	16 (33%)	2 (5%)
INFLAMMATION, NOS	2 (10%)	1 (5%)	1 (2%)	1 (2%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(20)	(20)	(49)	(49)
HYPERPLASIA, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				

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

TABLE D2 (CONCLUDED)

	CONTROL(UNTR) 02-F101	CONTROL(VEH) 02-F091	LOW DOSE 02-F104	HIGH DOSE 02-F105
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	4	4	10	40
AUTO/NECROPSY/NO HISTO				1
AUTOLYSIS/NO NECROPSY			1	1

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

Review of the Bioassay of 3-Sulfolene* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

March 7, 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 3-Sulfolene for carcinogenicity.

The primary reviewer noted that 3-Sulfolene is an intermediate of Sulfolene which is used in petroleums, plastics, and textiles and in the synthesis of some fungicides. He briefly described the experimental design and conditions under which 3-Sulfolene was tested. The poor survival among the high dose male rats precluded an evaluation of the carcinogenicity of 3-Sulfolene in this group. Under the conditions of test, no carcinogenic effect was observed in the low dose males or either treatment group of female rats. Excessive mortality also resulted among the high dose male and female treated mice. An increased incidence of hepatocellular carcinomas was observed among the male mice, although it was not statistically significant when compared to historical controls. The primary reviewer said that the study was complicated by excessive mortality and changes in dosages during the course of the chronic phase. As a result, he concluded that the study was inadequate to assess the carcinogenicity of 3-Sulfolene.

The secondary reviewer said that consideration should be given to a retest of 3-Sulfolene based on the incidence of hepatocellular carcinomas among treated male mice. He concluded that the studies' shortcomings were so severe that it was not possible to draw any conclusion on the carcinogenicity of 3-Sulfolene.

A discussion ensued as to whether an assessment of the carcinogenicity of 3-Sulfolene could be based on the low dose treated animals. It was finally agreed that the report sufficiently defined the limitations of the study. The primary reviewer therefore moved that the report be accepted as written. The motion was seconded and approved unanimously.

Members present were

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

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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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