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BIOASSAY OF

5-AZACYTIDINE

FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
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Bethesda, Maryland 20014

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CONTRIBUTORS: This report presents the results of the bioassay of 5-azacytidine for possible carcinogenicity conducted for the Carcinogenesis Testing Program, Divison of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. D. P. Griswold[1], J. D. Prejean[1], E. K. Weisburger[2], and J. H. Weisburger[2,3]. Ms. J. Belzer[1] and Mr. I. Brown[1] were responsible for the care and treatment of the laboratory animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation. The neoplasms and chemical-related hyperplastic lesions were reviewed by Dr. J. F. Hardisty[4], who also prepared the interpretive pathology narrative included in this report.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[5]. Statistical analyses were performed by Dr. J. R. Joiner[6], using methods selected for the bioassay program by Dr. J. J. Gart[7]. Chemicals used in this bioassay were analyzed by Drs. P. Lim[8], A. Cheung[8], and R. Yee[8], and the analytical results were reviewed by Dr. S. S. Olin[6].

This report was prepared at Tracor Jitco[6] under the direction of NCI. Those responsible for the report at Tracor Jitco were

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The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[7]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

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SUMMARY

A bioassay of 5-azacytidine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and B6C3F1 mice.

Groups of 35 rats of each sex were administered 5-azacytidine at one of two doses, either 2.6 or 5.2 mg/kg body weight, in buffered saline three times per week for 34 weeks, and were then observed for 46 or 47 weeks. Controls consisted of groups of 15 rats of each sex that received injections of buffered saline (vehicle controls) and 15 rats of each sex that were untreated (untreated controls). All surviving rats were killed at 80 or 81 weeks.

Groups of 35 mice of each sex were administered the chemical at one of two doses, either 2.2 or 4.4 mg/kg body weight, in buffered saline three times per week for 52 weeks, and were then observed for 29 or 30 weeks. Controls consisted of groups of 15 mice of each sex that received injections of buffered saline (vehicle controls) and 15 mice of each sex that were untreated (untreated controls). All surviving mice were killed at 81 or 82 weeks.

5-Azacytidine was toxic to the animals in this bioassay, since mean body weights of both treated rats and treated mice were lower than those of the corresponding vehicle controls, and since none of the high-dose male and female rats and high-dose female mice lived to the end of the bioassay. In treated male and female rats and male mice, survival was inadequate for meaningful statistical analyses of the incidences of tumors.

Only one male and three female high-dose rats had tumors, and none of the tumors in the low-dose group of either sex were present at a significantly increased incidence using any of the statistical tests. Bone-marrow atrophy was present in both treated groups of both sexes of rats.

Only five high-dose male mice and one high-dose female mouse had neoplasms. In low-dose female mice, however, lymphocytic and granulocytic neoplasms of the hematopoietic system occurred in 17 animals, even though only 54% survived until week 81. Granulocytic neoplasms were observed in 10/29 low-dose female mice, but in no other group, and were significant ($P = 0.010$) compared with the vehicle controls. The incidence of combined lymphoma and granulocytic neoplasms was highly significant in the low-dose females (vehicle controls 0/14, low-dose 17/29, $P < 0.001$). No tumors were observed at a significant incidence in male mice. Bone-marrow atrophy was present in high-dose female mice.

It is concluded that under the conditions of this bioassay, the short life span and short duration of treatment of Sprague-Dawley rats of either sex and of male B6C3F1 mice precluded evaluation of the carcinogenicity of 5-azacytidine in these groups; however, the induction of tumors of the hematopoietic system in female B6C3F1 mice was associated with the administration of 5-azacytidine.

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I. INTRODUCTION

5-Azacytidine (CAS 320-67-2; NCI C01569), a synthetic analogue of cytidine, has been used as an investigational anticancer drug in the United States since 1970 (Von Hoff et al., 1976). Chemical synthesis was first reported in 1964 by a Czech group, and later the chemical was isolated as an antibiotic from a culture filtrate of Streptoverticillium ladakanus (Heidelberger, 1973).

The exact mechanism of action of 5-azacytidine is not known. It is rapidly phosphorylated in mammalian tissues and incorporated into both RNA and DNA. By disrupting the processes of translation of nucleic acid sequences into protein, it inhibits the synthesis of protein. Moreover, by inhibiting orotodylic acid decarboxylase, the chemical also affects de novo pyrimidine synthesis. 5-Azacytidine is rapidly cleared from plasma, is concentrated in lymphatic tissues, and is rapidly excreted in the urine as both unchanged drug and metabolites (Carter and Slavik, 1976).

5-Azacytidine was selected for screening in the carcinogenesis program in an attempt to evaluate the carcinogenicity of certain drugs that may be used in humans for prolonged periods.

II. MATERIALS AND METHODS

A. Chemical

5-Azacytidine, which is a common name for 4-amino-1-beta-D-ribofuranosyl-1,3,5-triazine-2(1H)-one, was obtained as a single batch (Lot No. AP-V-128) from Ash-Stevens, Inc., Detroit, Michigan, by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (NCI).

The identity and purity of the chemical were confirmed in analyses at Stanford Research Institute. Elemental analyses (C, H, N, O) were correct for $C_8H_{12}N_4O_5$, the molecular formula of 5-azacytidine. Infrared, ultraviolet, and nuclear magnetic resonance spectra were as expected for this chemical and were identical to spectra of a reference standard. No free 5-azacytosine or D-ribose was detected by paper chromatography. On the basis of these results, the purity was estimated to be > 99%.

The powdered 5-azacytidine was stored at 5°C in small bottles enclosed in sealed plastic bags containing Drierite®.

B. Dosage Preparation

Concentrations of 5-azacytidine of 0.1 or 0.2% for rats and 0.02 or 0.04% for mice were prepared in buffered saline (pH 6.9) for intraperitoneal injection of the chemical. Aqueous solutions

were not stored, because they are unstable at room temperature. The drug and the vehicle were mixed in a 10-ml glass Potter-Elvehjem tissue grinder with a Teflon pestle. Fresh solutions in exact amounts for each administration were prepared preceding injection.

C. Animals

Female Sprague-Dawley rats and male and female Swiss mice were used in subchronic studies.

Sprague-Dawley rats and B6C3F1 mice of both sexes, obtained through contracts of the Division of Cancer Treatment, NCI, were used in chronic studies. The Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, and the B6C3F1 mice were obtained from Charles River Laboratories and from A. R. Schmidt, Madison, Wisconsin. On arrival at the laboratory, the male rats were 30 days old, the female rats were 37 days old, and the mice were all 30 days old. The animals were quarantined for an acclimation period (rats for 5 days, mice for 4-5 days), assigned to control and treated groups, and earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled

rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. There were 15 changes of room air per hour. Air was passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Wayne® Lab Blox (Allied Mills, Inc., Chicago, Ill.) and water were supplied daily and were available ad libitum.

Rats were housed five per cage and mice seven per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The bottoms of the rat cages were lined with Iso-Dri® hardwood chips (Carworth, Edison, N.J.) and cage tops were covered with disposable filter bonnets beginning at week 26; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective treated animals. Animals treated with 5-azacytidine were maintained in the same rooms as animals of the same species being treated with the following chemicals:

RATS

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate (phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate) (estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGDR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)
adriamycin (CAS 23214-92-8)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride) (CAS 136-40-3)
ethionamide (CAS 536-33-4)
L-tryptophan (CAS 73-22-3)

N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate
(phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate)
(estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
(MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGDR)
(CAS 789-61-7)
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emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester)
hydrochloride (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione
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2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)
adriamycin (CAS 23214-92-8)

E. Subchronic Studies

Subchronic studies were conducted in female Sprague-Dawley rats and male and female Swiss mice to estimate the maximum tolerated

doses of 5-azacytidine, on the basis of which low and high doses were determined for administration in the chronic studies. The animals were administered 5-azacytidine by intraperitoneal injection three times per week for 45 days, then observed for an additional 45 days. Five animals of each species were used at each dose, 10 animals were used as untreated controls, and 10 animals were used as vehicle (saline) controls.

In rats, administration of the five doses originally selected (0.13, 0.33, 0.65, 1.3, and 2.6 mg/kg body weight) resulted in no deaths and in no weight depression exceeding the 15% guideline, when treated animals were compared with untreated controls. A second study was performed using doses of 2.6, 5.2, 10.4, and 20.8 mg/kg. At 20.8 mg/kg, three animals died in week 4 and all died by week 6. Two animals receiving 10.4 mg/kg died, one in week 6 and one in week 8. At doses of 2.6 or 5.2 mg/kg, no deaths occurred, no lesions were observed, and weight depression did not exceed the 15% guideline. The low and high doses for rats were set at 2.6 and 5.2 mg/kg for the chronic studies.

In mice, the subchronic study was initially conducted in males at doses of 0.22, 0.6, 1.1, 2.2, and 4.4 mg/kg. No deaths attributable to drug toxicity and no weight depression exceeding the 15% guideline resulted. A second study was performed in females, using doses of 4.4, 8.8, 17.6, and 35.2 mg/kg. All animals

receiving 17.6 or 35.2 mg/kg died by week 6, and 3/5 at 8.8 mg/kg died by week 8; however, there were no deaths at 4.4 mg/kg. Weights of animals surviving to the end of the study were similar to those of controls, and no lesions were seen at necropsy. The low and high doses for mice were set at 2.2 and 4.4 mg/kg for the chronic studies.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2. Treatment of the rats was terminated at week 34, due to excessive mortality.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and those that were moribund were killed and necropsied. Rats and mice were weighed individually each week for 2 months and every 2 weeks for the remainder of the study. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were routinely examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph

Table 1. Design of Chronic Studies of 5-Azacytidine in Rats

Sex and Treatment Group	Initial No. of Animals[a]	5-Azacytidine Dose[b] (mg/kg)	Time on Study: Treated[c] (weeks)	Time on Study: Untreated (weeks)
Male				
Untreated-Control	15	0		81
Vehicle-Control	15	0[d]	34	46-47
Low-Dose	35	2.6	34	46
High-Dose	35	5.2	34	10[e]
Female				
Untreated-Control	15	0		81
Vehicle-Control	15	0[d]	34	47
Low-Dose	35	2.6	34	46
High-Dose	35	5.2	34	11[e]

[a]Male rats and their controls were 35 days of age when placed on study; females were 42 days of age.

[b]5-Azacytidine was administered in buffered saline by intraperitoneal injection three times per week at a volume of 0.25 ml/100 g body weight. Doses were based on individual weights.

[c]Treatment terminated at 34 weeks rather than at 52 weeks because of high mortality in the high-dose groups.

[d]Vehicle controls received only buffered saline solution, at the same volume as treated rats.

[e]The remaining high-dose males were killed at week 44 and high-dose females at week 45.

Table 2. Design of Chronic Studies of 5-Azacytidine in Mice

Sex and Treatment Group	Initial No. of Animals[a]	5-Azacytidine Dose[b] (mg/kg)	Time on Study: Treated (weeks)	Time on Study: Untreated (weeks)
Male				
Untreated-Control	15	0		81-82
Vehicle-Control	15	0[c]	52	29-30
Low-Dose	35	2.2	52	29
High-Dose	35	4.4	52	29
Female				
Untreated-Control	15	0		82
Vehicle-Control	15	0[c]	52	29-30
Low-Dose	35	2.2	52	29
High-Dose	35	4.4	52	9[d]

[a]All animals were 38 days of age when placed on study.

[b]5-Azacytidine was administered in buffered saline by intraperitoneal injection three times per week at a volume of 1 ml/100 g body weight. Doses were based on the mean weight of the animals in each cage.

[c]Vehicle controls received only buffered saline solution, at the same volume as treated mice.

[d]The remaining high-dose female mice were killed at week 61.

nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals may have been judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design,

clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for 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 is 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) was used to compare the tumor incidence of a control group with 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) 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), was also used. Under the assumption of a linear trend, this test determines 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 is 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 an animal died naturally or was 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 treated 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% 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 ($P < 0.025$ 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. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Among males, the mean body weights were lower in the treated groups than in the controls starting at about week 4 of the study, with progressively greater differences occurring as the study progressed, up to approximately week 34 (figure 1). When treatment was discontined at week 34, the mean body weights of the low-dose group markedly increased, as did those of the four remaining high-dose animals. Among females, only the high-dose group had slightly lower mean body weights compared with the controls. 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 other signs of toxicity were recorded for the rats during the bioassay.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered 5-azacytidine by intraperitoneal injection at the doses of this experiment, together with those of the controls, are shown in figure 2.

In each sex, the result of the Tarone test for positive dose-related trend in mortality over the period is significant ($P <$

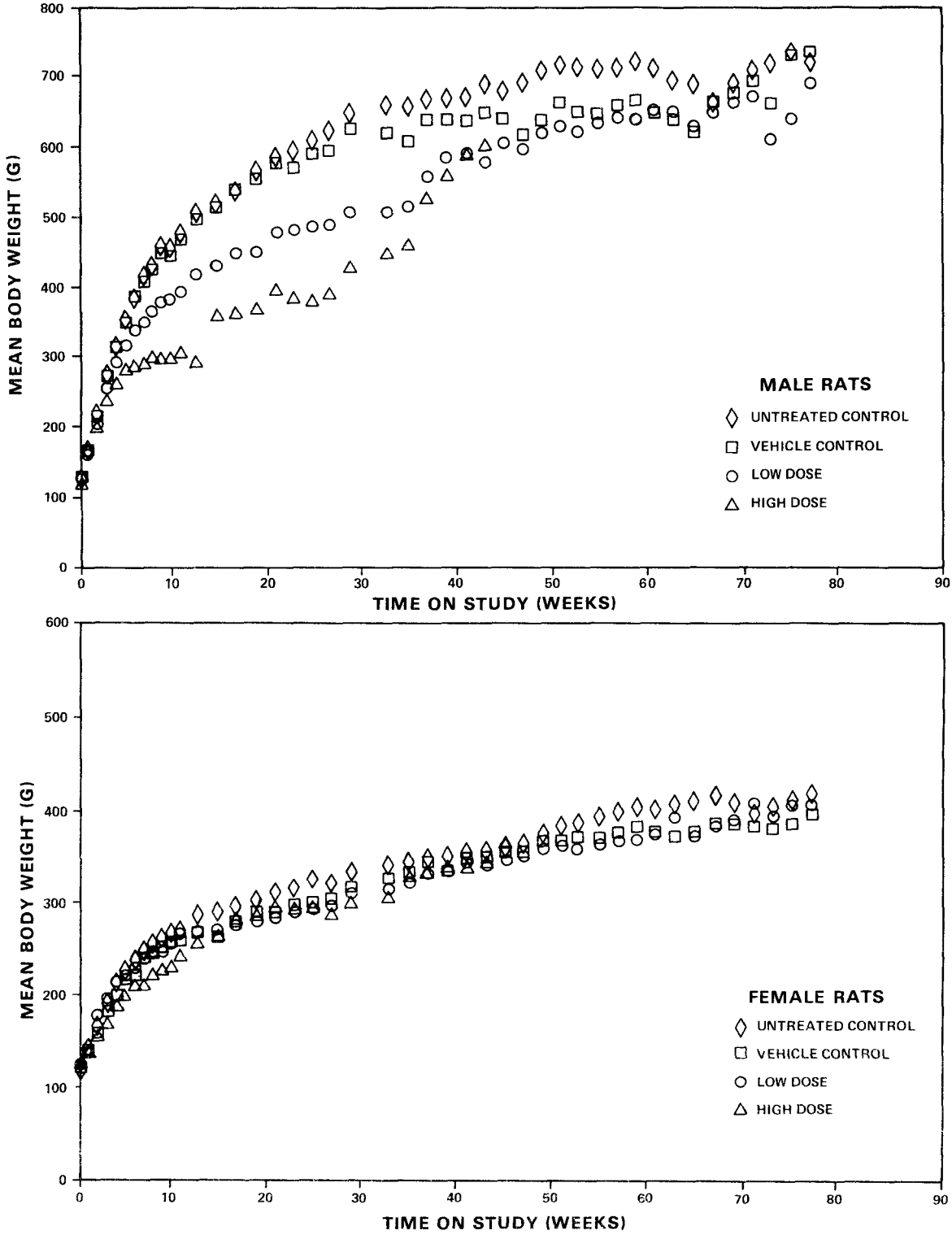

Figure 1. Growth Curves For Rats Treated With 5-Azacytidine

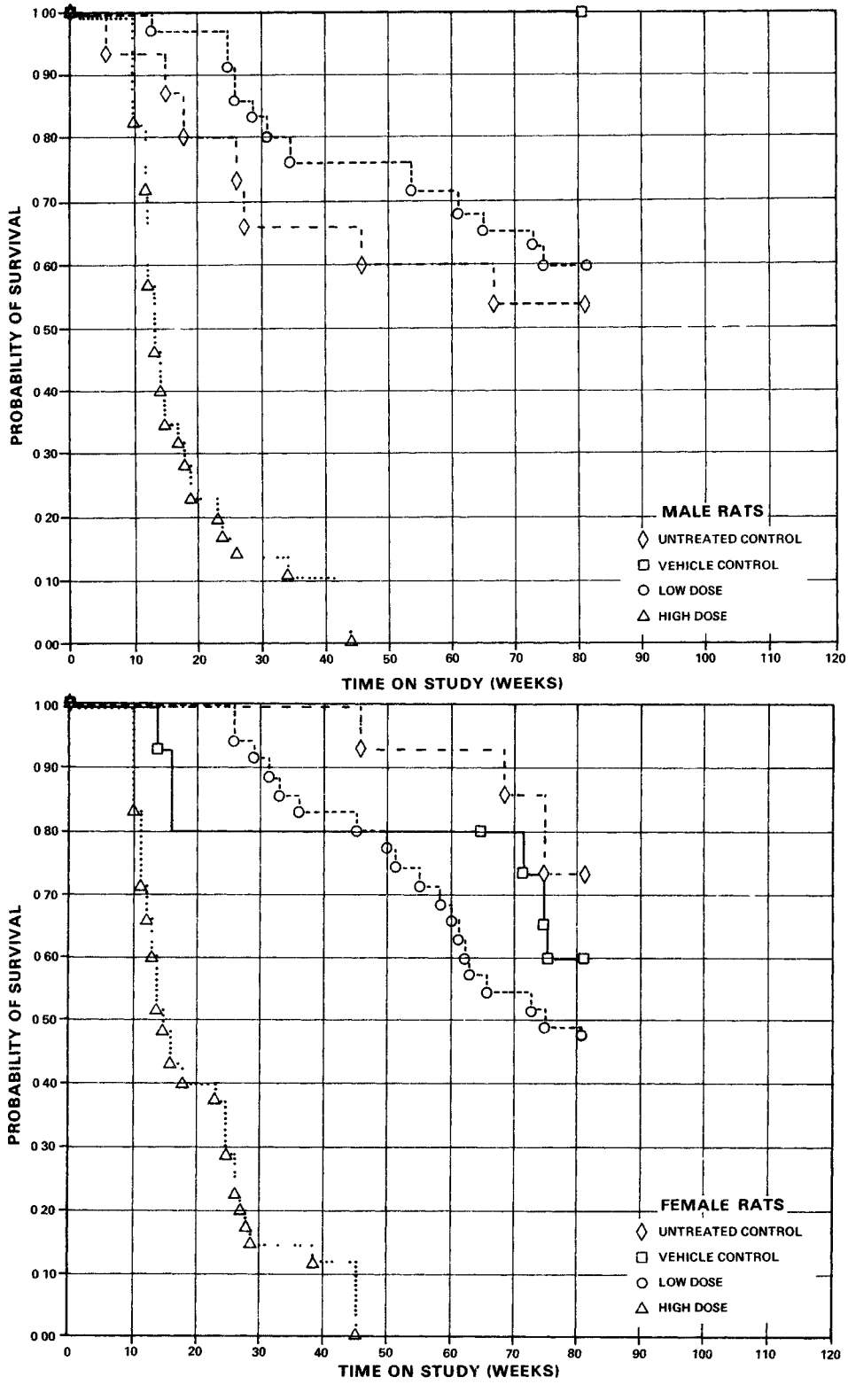

Figure 2. Survival Curves For Rats Treated With 5-Azacytidine

0.001), and a departure from linear trend is present ($P < 0.001$), due to the steep increase in mortality in the high-dose rats, of which all died before the end of the study. The median time on study was only 13 weeks for these rats, and the high mortality at the high dose may have reduced the occurrence of late-developing tumors. In the low-dose rats, 21/35 (60%) of the males and 17/35 (49%) of the females lived to the end of the study at week 81. Time-adjusted analysis was performed on female rats that lived at least 26 weeks, which is the earliest time of an observed tumor. There were 15 such animals in the untreated controls, 1[illegible] in the vehicle controls, 31 in the low-dose group, and 10 in the high-dose group.

C. Pathology (Rats)

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.

A variety of neoplasms were seen with approximately equal frequency in the control and treated rats. Neoplasms were seen more frequently in the female rats than in the males. The most frequently observed neoplasms in the females involved the pituitary gland and the mammary gland. Chromophobe adenomas of the pituitary were observed frequently in the control females

only. Benign and malignant mammary gland neoplasms were seen frequently in both control and low-dose female rats. The low incidence of neoplasia in the male and female high-dose groups was probably due to the unusually high number of early deaths that occurred in both high-dose groups.

Several inflammatory and degenerative lesions occurred with approximately equal frequency in treated and control animals. Hepatocellular degeneration and necrosis, usually centrilobular, were present in several of the treated animals and were the only nonneoplastic lesions that appeared to be directly related to exposure to the chemical. None of the control rats had similar hepatic lesions. These lesions were present in 1/31 low-dose and 5/31 high-dose females. Necrotizing liver lesions were also present in 5/31 low-dose and 15/33 high-dose male rats. All of the animals with liver necrosis died prior to the end of this study. Bone-marrow atrophy was present in 5/30 low-dose and 26/33 high-dose males and in 2/30 low-dose and 15/29 high-dose females, but its significance in relation to the early deaths could not be evaluated, due to the lack of clinical pathology data.

In the judgment of the pathologists, the results of this study indicate that the administration of 5-azacytidine at the doses used in this study caused hepatotoxicity and bone-marrow atrophy

in Sprague-Dawley rats. No carcinogenic effect was observed during the shortened life spans of the animals.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals with an incidence of at least 5% in either the vehicle-control or low-dose groups. Due to the shortened survival in the high-dose groups, results for these groups are not analyzed in the tables.

Although there was no incidence of chromophobe adenoma of the pituitary in the low-dose female rats (0/30), this tumor is listed in table E2 because of the incidence observed in the vehicle-control group (4/15 [27%]); this incidence gave a significant result in the negative direction. The absence of tumors in the treated group may be accounted for by the high mortality observed in this group. A time-adjusted analysis, eliminating animals that died before week 52 on study, indicated that the difference between the incidences in the treated (0/25) and control (4/14) groups was still significant in the negative direction.

There is no incidence of tumors at any specific site in either sex that is statistically significant in the positive direction.

The absence of such tumors, however, may be due to the abnormally short treatment periods and life spans in the treated animals, rather than to absence of carcinogenicity of the test chemical in the rats.

In each of the 95% confidence intervals of relative risk, shown in the tables, except for the comparison involving the pituitary tumors in female rats, the value of one is included; this indicates the absence of positive significant results. It should also be noted that these intervals have upper limits greater than one, indicating the theoretical possibility of the induction of tumors by 5-azacytidine, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights of the treated mice were dose related throughout the period of the study, and were lower than those of the vehicle controls (figure 3). When treatment was stopped at week 52, some increases in the mean body weights of the remaining animals occurred. A greater difference occurred between body weights of treated and control females than between those of treated and control males. 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 significant signs of toxicity were recorded for the mice during the bioassay.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered 5-azacytidine by intraperitoneal injection at the doses of this experiment, together with those of the controls, are shown in figure 4.

In male mice, the result of the Tarone test for positive dose-related trend in mortality over the period is significant ($P < 0.001$), with 20% of the high-dose group, 37% of the low-dose

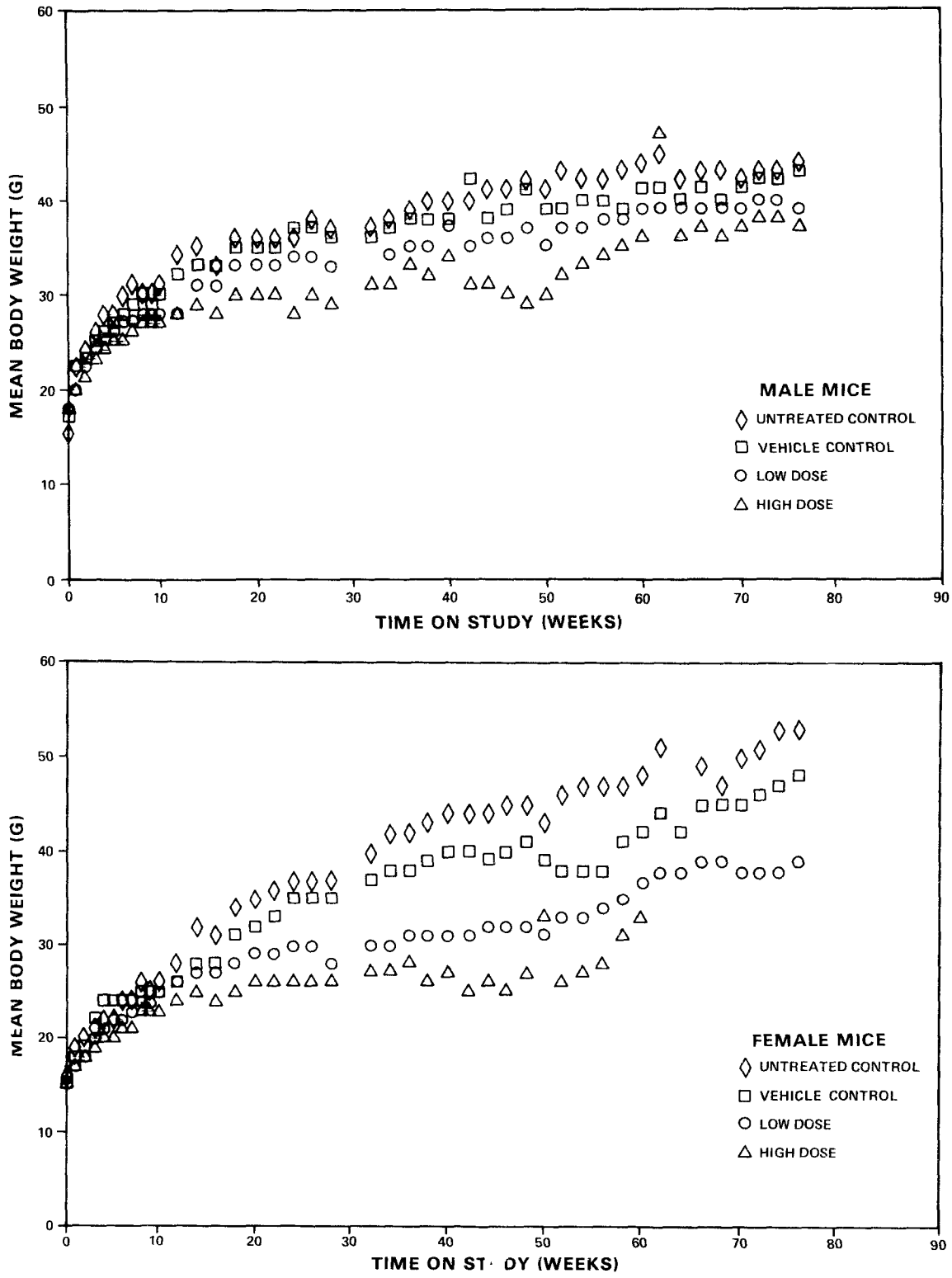

Figure 3. Growth Curves For Mice Treated With 5-Azacytidine

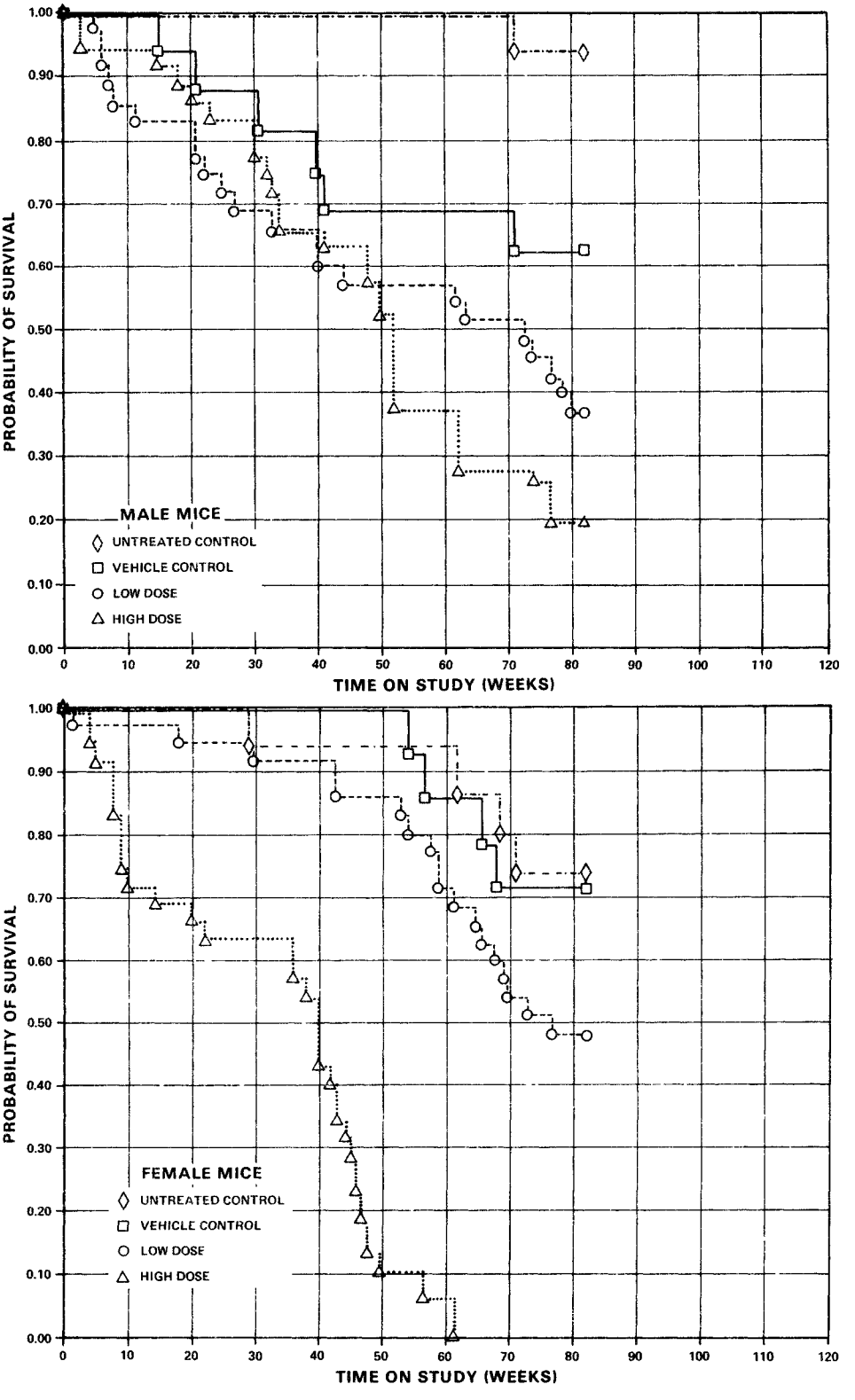

Figure 4. Survival Curves For Mice Treated With 5-Azacytidine

group, 63% of the vehicle controls, and 93% of the untreated controls living to the end of the study. The early deaths of the treated mice may have reduced the occurrence of late-developing tumors.

In female mice, the result of the Tarone test is also significant ($P < 0.001$), and a departure from linear trend is observed ($P < 0.001$), because of the steep increase in mortality among the high-dose mice, of which all died before the end of the study. These early deaths of the high-dose female mice may have suppressed the occurrence of late-developing tumors. Of the low-dose female mice, 49% survived until termination of the study at week 82. In male mice, a time-adjusted analysis was performed on those animals living at least 52 weeks. There were 15 such animals in the untreated controls, 11 in the vehicle controls, and 18 in both the low- and high-dose groups.

C. Pathology (Mice)

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

A variety of neoplasms were present in both treated and control groups. With the exception of the hematopoietic system, neoplasms occurred infrequently and were distributed equally

among the organ systems and groups studied. Several neoplasms of the hematopoietic system involving multiple organs and tissues of the spleen, lymph node, liver, and lung were present in both the male and female mice. Both lymphocytic and granulocytic neoplasms were present in the tissues examined. With the exception of one untreated-control female mouse with malignant lymphoma, all of the hematopoietic neoplasms occurred in treated animals. Hematopoietic neoplasms were diagnosed most frequently in the low-dose groups (male 4/31, female 17/29), and the lower incidence of hematopoietic neoplasms in the high-dose groups (male 3/32, female 0/30) may be the result of the high number of early deaths in these groups. In general, the hematopoietic neoplasms present in the low-dose groups were poorly differentiated, and precise classification of cell type was extremely difficult. The neoplasms observed histologically were classified into the following cell types: malignant lymphoma, lymphocytic type; malignant lymphoma, histiocytic type; malignant lymphoma, undifferentiated type; granulocytic sarcoma; and granulocytic leukemia.

Several inflammatory, degenerative, and proliferative lesions commonly seen in B6C3F1 hybrid mice occurred with approximately equal frequency in treated and control animals. The most common of these lesions were interstitial pneumonia with perivascular

and peribronchiolar lymphocytic hyperplasia and extramedullary hematopoiesis in the spleen. Necrosis of the liver was occasionally observed. Mice that died prior to termination of the study had no consistent lesions, except bone-marrow atrophy in high-dose females, that would account for the early deaths.

In the judgment of the pathologists, the results of this study indicate that the administration of 5-azacytidine at the doses used had a carcinogenic effect upon the hematopoietic system in the B6C3F1 hybrid female mouse. This carcinogenic effect was manifested by the apparent induction of lymphocytic and granulocytic neoplasms in the low-dose female mice. There appeared to be only a slight increase in the number of hematopoietic neoplasms in the high-dose groups; however, the high number of early deaths in this group may have accounted for this observation.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals and with an incidence of at least 5% in one or more than one group.

In male mice, there is no incidence of tumors at any specific site that is statistically significant. When time-adjusted

analyses, eliminating animals that died before week 52 of the study, were performed on the incidences of lymphoma of the hematopoietic system, the incidences (vehicle controls 0/11, low-dose 4/18 [22%], high-dose 3/18 [17%]) still remain not significant.

In female mice, the analyses of the incidences of lymphoma show that the Fisher exact comparison of incidences in the low-dose (7/27 [24%]) and vehicle-control (0/14) groups has a probability level of 0.048; however, this probability is above the 0.025 level required by the Bonferroni inequality criterion when the multiple comparison test is applied. Granulocytic leukemia or sarcoma of the hematopoietic system was found exclusively in the low-dose female mice. The Fisher exact test shows that the incidence in the low-dose group (10/29 [34%]) is significantly higher ($P = 0.010$) than that in the vehicle controls (0/14).

The analyses of the combination of lymphoma, granulocytic leukemia, and sarcoma in female mice indicates that the incidence of these tumors in the low-dose group (17/29) differs significantly ($P < 0.001$) from that in the vehicle-control group (0/14), in which no such tumors were seen. The untreated-control group, not shown in the table, was observed to have a 1/15 (7%) incidence of lymphoma. The early mortality in the high-dose group may account for the absence of these tumors in this group. The

incidence of these types of tumors compiled to date in the bioassay studies at this laboratory is 49/265 (19%) in female mice, compared with 17/29 (59%) observed in the low-dose female mice in this study; however, the laboratory results to date show only 1/265 granulocytic sarcoma in vehicle controls compared with 9/29 (31%) in the study. The statistical conclusion is that there is an association between the incidence of hematopoietic tumors and the administration of the low dose of the chemical to female mice.

V. DISCUSSION

The test chemical, 5-azacytidine, was toxic to both the rats and the mice in this bioassay, as demonstrated by (1) the lower mean body weights of the treated animals compared with the controls and (2) the high mortality which occurred during the study. The mean body weights of the treated male rats were much lower than those of the controls during the treatment period of 34 weeks. Because of the high mortality, treatment was discontinued; the low-dose males then gained weight rapidly. Nearly all high-dose animals had died by this time; the median time on study for all high-dose rats was only 13 weeks. Among the low-dose rats, 60% of the male and 49% of the females survived until termination of the study at week 81. Both the low survival and the short duration of treatment prevented evaluation of carcinogenicity of this compound in rats.

Treated mice had lower mean body weights than controls throughout the study, and survival was dose related in both male and female mice. All high-dose females died prior to termination of the study, and only 7 high-dose males, 13 low-dose males, and 17 low-dose females survived to the end of the study at week 81.

Only one male and three female high-dose rats had tumors, and none of the incidences of the tumors in the low-dose group of

either sex were significantly increased using any of the statistical tests. Bone-marrow atrophy was observed in 17% of the low-dose and 79% of the high-dose male rats, in 7% of the low-dose and 52% of the high-dose female rats, and in only 2% of the combined controls. Centrilobular degeneration and necrosis of hepatocytes were observed in 16% of the low-dose and 45% of the high-dose male rats, in 3% of the low-dose and 16% of the high-dose female rats, and in none of the combined controls.

Only five high-dose male mice and one high-dose female mouse had neoplasms. In low-dose female mice, neoplasms of the hematopoietic system including malignant lymphoma, granulocytic sarcoma, and granulocytic leukemia occurred in 17 aniamls, even though only 54% survived until week 81. Malignant lymphoma occurred in greater numbers in the low-dose female mice (7/29) than in the vehicle controls (0/14). The probability level for direct comparison with the vehicle controls was $P = 0.048$; however, this is above the 0.025 level required by the Bonferroni inequality criterion for significance in multiple comparisons. Granulocytic leukemia or sarcoma was observed in 10/29 low-dose female mice, but in no other group, and 7/29 of the low-dose females died prior to the first observation of this tumor at week 53. The incidence was significant when compared with that of the vehicle-control group ($P = 0.010$). The incidence of combined lymphoma

and granulocytic neoplasms was highly significant in the low-dose females (vehicle controls 0/14, low-dose 17/29, $P < 0.001$). Early deaths in the high-dose animals may have precluded the observation of neoplasms, and thus, the low incidence of tumors in the high-dose group does not contradict the association with treatment of the tumors observed in the low-dose females. No tumors were observed at a significant incidence in male mice. Hepatocellular necrosis similar to that seen in the rats was occasionally observed, but the occurrence of this lesion does not explain the early deaths. Bone-marrow atrophy was present in 10/29 (34%) of the high-dose female mice.

No other long-term studies of 5-azacytidine have been reported; however, humans treated with the drug have developed leucopenia (lowered total white cell count) and, occasionally, hepatic toxicity (Von Hoff et al., 1976).

It is concluded that under the conditions of this bioassay, the short life span and short duration of treatment of Sprague-Dawley rats of either sex and of male B6C3F1 mice precluded evaluation of the carcinogenicity of 5-azacytidine in these groups; however, the induction of tumors of the hematopoietic system in female B6C3F1 mice was associated with the administration of 5-azacytidine.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Berenblum, I., ed., Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of the UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

Carter, S. K. and Slavik, S., Investigational drugs under study by the United States National Cancer Institute. Cancer Treatment Reviews, 1976, pp. 451-468.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B 34(2):187-220, 1972.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970, pp. 48-52.

Gart, J. J. The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Statist. Inst. 39(2):148-169, 1971.

Heildelberger, C., Pyrimidine and pyrimidine nucleoside antimetabolites, In: Cancer Medicine, Holland, J. E. and Frei, E., III, eds., Lea and Febiger, Philadelphia, 1973, pp. 784-786.

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Am. Statist. Assoc. 53:457-481, 1958.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. and Biomed. Res. 7:230-248, 1974.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo (a) pyrene and ferric oxide. Cancer Res. 32: 1073-1081, 1972.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62(3):679-682, 1975.

Von Hoff, D. D., Slavik, M., and Muggia, F. M., 5-Azacytidine - A new anticancer drug with effectiveness in acute myelogenous leukemia. In: Advances in Pharmacology and Chemotherapy, Report of the Division of Cancer Treatment, NCI, August 1976.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	14	15	32	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	32	33
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(15)	(32)	(33)
PAPILLOMA, NOS			1 (3%)	
SQUAMOUS CELL CARCINOMA			1 (3%)	
*SUBCUT TISSUE	(14)	(15)	(32)	(33)
UNDIFFERENTIATED CARCINOMA			1 (3%)	
BASAL-CELL CARCINOMA			1 (3%)	
SARCOMA, NOS			1 (3%)	
FIBROSARCOMA	1 (7%)			
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(15)	(32)	(33)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (3%)	
GRANULOCYTIC LEUKEMIA			2 (6%)	
GRANULOCYTIC SARCOMA			1 (3%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NONE				

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
#PITUITARY	(13)	(15)	(29)	(31)
CHROMOPHOBE CARCINOMA		1 (7%)	1 (3%)	
#ADRENAL	(14)	(15)	(31)	(33)
PHEOCHROMOCYTOMA			1 (3%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(14)	(15)	(32)	(33)
ADENOCARCINOMA, NOS			1 (3%)	
FIBROADENOMA				1 (3%)
#TESTIS	(14)	(15)	(28)	(32)
INTERSTITIAL-CELL TUMOR			2 (7%)	
NERVOUS SYSTEM				
#BRAIN	(14)	(15)	(30)	(32)
ASTROCYTOMA	1 (7%)			
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 A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@	4		11	20
MORIBUND SACRIFICE	3		3	15
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	8	15	21	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	1	11	1
TOTAL PRIMARY TUMORS	2	1	14	1
TOTAL ANIMALS WITH BENIGN TUMORS			4	1
TOTAL BENIGN TUMORS			4	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	1	9	
TOTAL MALIGNANT TUMORS	2	1	10	
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 A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	15	15	31	31
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	31	31
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(15)	(15)	(31)	(31)
FIBROMA		1 (7%)		
RESPIRATORY SYSTEM				
#LUNG	(15)	(14)	(30)	(30)
ADENOCARCINOMA, NOS, METASTATIC	1 (7%)		2 (7%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(15)	(31)	(31)
LYMPHOCYTIC LEUKEMIA			1 (3%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
#KIDNEY	(15)	(13)	(31)	(31)
TUBULAR-CELL ADENOCARCINOMA	1 (7%)			
ENDOCRINE SYSTEM				
#PITUITARY	(14)	(15)	(30)	(27)
CHROMOPHOBE ADENOMA	5 (36%)	4 (27%)		
CHROMOPHOBE CARCINOMA		1 (7%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(15)	(31)	(31)
ADENOCARCINOMA, NOS	4 (27%)		9 (29%)	1 (3%)
PAPILLARY ADENOCARCINOMA			1 (3%)	1 (3%)
FIBROADENOMA	2 (13%)	3 (20%)	6 (19%)	1 (3%)
#UTERUS	(15)	(15)	(30)	(29)
LEIOMYOSARCOMA			1 (3%)	
ENDOMETRIAL STROMAL POLYP			1 (3%)	
NERVOUS SYSTEM				
#BRAIN	(14)	(14)	(30)	(30)
CHROMOPHOBE CARCINOMA, METASTATI		1 (7%)		
SPECIAL SENSE ORGANS				
*EAR CANAL	(15)	(15)	(31)	(31)
KERATOACANTHOMA			1 (3%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MESENTERY	(15)	(15)	(31)	(31)
LIPOMA	1 (7%)			
ALL OTHER SYSTEMS				
NONE				

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@	1	1	10	22
MORIBUND SACRIFICE	3	5	8	13
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	11	9	17	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	7	15	3
TOTAL PRIMARY TUMORS	13	9	20	3
TOTAL ANIMALS WITH BENIGN TUMORS	8	6	7	1
TOTAL BENIGN TUMORS	8	8	8	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	1	11	2
TOTAL MALIGNANT TUMORS	5	1	12	2
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	2	
TOTAL SECONDARY TUMORS	1	1	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	16	35	35
ANIMALS NECROPSIED	15	13	31	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	30	30
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(29)	(30)
UNDIFFERENTIATED CARCINOMA METAS	1 (7%)			
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (8%)		1 (3%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(13)	(31)	(32)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			4 (13%)	2 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (3%)
#MEDIASTINAL L.NODE	(15)	(3)	(23)	(20)
UNDIFFERENTIATED CARCINOMA METAS	1 (7%)			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(30)	(30)
UNDIFFERENTIATED CARCINOMA	1 (7%)			
HEPATOCELLULAR ADENOMA	1 (7%)	1 (8%)	1 (3%)	1 (3%)
URINARY SYSTEM				
NONE				

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
#THYROID	(11)	(7)	(24)	(20)
ADENOMA, NOS			1 (4%)	
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(15)	(13)	(31)	(32)
KERATOACANTHOMA				1 (3%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	16	35	35
NATURAL DEATH@		5	18	15
MORIBUND SACRIFICE	1	1	4	13
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	14	10	13	7
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	2	6	5
TOTAL PRIMARY TUMORS	2	2	6	6
TOTAL ANIMALS WITH BENIGN TUMORS	1	2	2	2
TOTAL BENIGN TUMORS	1	2	2	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	1		4	3
TOTAL MALIGNANT TUMORS	1		4	3
TOTAL ANIMALS WITH SECONDARY TUMORS#	1			
TOTAL SECONDARY TUMORS	2			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* 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 GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	14	35	35
ANIMALS NECROPSIED	15	14	29	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	29	30
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(15)	(14)	(29)	(30)
BASAL-CELL CARCINOMA				1 (3%)
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(28)	(30)
UNDIFFERENTIATED CARCINOMA METAS	1 (7%)			
BASAL-CELL CARCINOMA, METASTATIC			1 (4%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (8%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (8%)		
SARCOMA, NOS, METASTATIC			1 (4%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(14)	(29)	(30)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (3%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)		4 (14%)	
GRANULOCYTIC LEUKEMIA			1 (3%)	
GRANULOCYTIC SARCOMA			9 (31%)	
#LIVER	(15)	(13)	(29)	(30)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)	
#KIDNEY	(15)	(13)	(29)	(29)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)	
CIRCULATORY SYSTEM				
#HEART	(14)	(12)	(28)	(30)
SARCOMA, NOS			1 (4%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(29)	(30)
HEPATOCELLULAR ADENOMA	1 (7%)			
LEIOMYOSARCOMA, METASTATIC			1 (3%)	
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#ADRENAL	(14)	(13)	(27)	(29)
LEIOMYOSARCOMA, METASTATIC			1 (4%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(14)	(29)	(30)
ADENOCARCINOMA, NOS			2 (7%)	
#UTERUS	(15)	(13)	(28)	(27)
SARCOMA, NOS			1 (4%)	
LEIOMYOSARCOMA			2 (7%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(15)	(14)	(29)	(30)
BASAL-CELL CARCINOMA			1 (3%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(14)	(29)	(30)
LEIOMYOSARCOMA, METASTATIC			1 (3%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*PLEURA	(15)	(14)	(29)	(30)
LEIOMYOSARCOMA, METASTATIC			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	14	35	35
NATURAL DEATH@	1	1	14	11
MORIBUND SACRIFICE	3	3	4	24
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	11	10	17	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	2	22	1
TOTAL PRIMARY TUMORS	2	2	24	1
TOTAL ANIMALS WITH BENIGN TUMORS	1	1		
TOTAL BENIGN TUMORS	1	1		
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	1	22	1
TOTAL MALIGNANT TUMORS	1	1	24	1
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		3	
TOTAL SECONDARY TUMORS	1		6	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS				
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN				

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS GIVEN INTRAPERITONEAL INJECTIONS

OF 5-AZACYTIDINE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	14	15	32	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	32	33
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(12)	(15)	(28)	(33)
INFLAMMATION, ACUTE/CHRONIC		1 (7%)	4 (14%)	
#LUNG/BRONCHIOLE	(14)	(13)	(32)	(32)
HYPERPLASIA, LYMPHOID			1 (3%)	
#LUNG	(14)	(13)	(32)	(32)
INFLAMMATION, INTERSTITIAL		1 (8%)	4 (13%)	1 (3%)
BRONCHOPNEUMONIA SUPPURATIVE	3 (21%)		5 (16%)	
BRONCHOPNEUMONIA, CHRONIC			2 (6%)	
BRONCHOPNEUMONIA CHRONIC SUPPURA			4 (13%)	1 (3%)
HYPERPLASIA, LYMPHOID		1 (8%)	2 (6%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(13)	(15)	(30)	(33)
ATROPHY, NOS		2 (13%)	5 (17%)	26 (79%)
#SPLEEN	(14)	(15)	(31)	(32)
INFLAMMATION, NECROTIZING				1 (3%)
NECROSIS, NOS				3 (9%)
#LYMPH NODE	(7)	(13)	(22)	(28)
INFLAMMATION, NECROTIZING				1 (4%)
CIRCULATORY SYSTEM				
#HEART/ATRIUM	(14)	(13)	(32)	(32)
THROMBOSIS, NOS				1 (3%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(14)	(13)	(32)	(32)
INFLAMMATION, NECROTIZING				1 (3%)
DEGENERATION, NOS				1 (3%)
DIGESTIVE SYSTEM				
#LIVER	(14)	(14)	(31)	(33)
CONGESTION, PASSIVE				1 (3%)
CONGESTION, CHRONIC PASSIVE				1 (3%)
INFLAMMATION, NECROTIZING				1 (3%)
NECROSIS, COAGULATIVE				2 (6%)
NECROSIS, CENTRAL			5 (16%)	3 (9%)
#LIVER/CENTRILOBULAR	(14)	(14)	(31)	(33)
NECROSIS, NOS				1 (3%)
NECROSIS, COAGULATIVE				8 (24%)
*BILE DUCT	(14)	(15)	(32)	(33)
INFLAMMATION, CHRONIC			1 (3%)	
#GASTRIC SUBMUCOSA	(14)	(15)	(31)	(33)
ABSCESS, CHRONIC				1 (3%)
URINARY SYSTEM				
#KIDNEY	(14)	(15)	(31)	(32)
HYDRONEPHROSIS	1 (7%)			
INFLAMMATION, INTERSTITIAL			2 (6%)	
INFLAMMATION, SUPPURATIVE				1 (3%)
GLOMERULONEPHRITIS, MEMBRANOUS	3 (21%)	1 (7%)	1 (3%)	
PYELONEPHRITIS, ACUTE/CHRONIC				1 (3%)
INFLAMMATION, CHRONIC			1 (3%)	
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#PROSTATE	(14)	(15)	(28)	(30)
INFLAMMATION, SUPPURATIVE				1 (3%)
NERVOUS SYSTEM				
NONE				

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	7	12	4	4
AUTOLYSIS/NO NECROPSY	1		3	2

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	15	15	31	31
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	31	31
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(14)	(15)	(29)	(28)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)	
#LUNG	(15)	(14)	(30)	(30)
INFLAMMATION, INTERSTITIAL		1 (7%)	1 (3%)	2 (7%)
BRONCHOPNEUMONIA SUPPURATIVE	1 (7%)		4 (13%)	
BRONCHOPNEUMONIA NECROTIZING				1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (7%)			
HYPERPLASIA, LYMPHOID		1 (7%)		1 (3%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(15)	(15)	(30)	(29)
ATROPHY, NOS			2 (7%)	15 (52%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER/CENTRILOBULAR	(14)	(14)	(31)	(31)
NECROSIS, NOS			1 (3%)	
NECROSIS, COAGULATIVE				4 (13%)
CYTOLOGIC DEGENERATION				1 (3%)
URINARY SYSTEM				
NONE				

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(15)	(31)	(31)
HYPERPLASIA, CYSTIC	1 (7%)			
#UTERUS/ENDOMETRIUM	(15)	(15)	(30)	(29)
INFLAMMATION, SUPPURATIVE	1 (7%)	2 (13%)	3 (10%)	
INFLAMMATION, CHRONIC SUPPURATIV			3 (10%)	
#OVARY	(10)	(13)	(23)	(27)
INFLAMMATION, SUPPURATIVE			1 (4%)	
INFLAMMATION, CHRONIC SUPPURATIV			3 (13%)	1 (4%)
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 C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	4	7	9	10
AUTO/NECROPSY/HISTO PERF				2
AUTOLYSIS/NO NECROPSY			4	4

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	16	35	35
ANIMALS NECROPSIED	15	13	31	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	30	30
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(13)	(31)	(32)
EPIDERMAL INCLUSION CYST	1 (7%)			
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(29)	(30)
INFLAMMATION, INTERSTITIAL		1 (8%)	4 (14%)	
BRONCHOPNEUMONIA SUPPURATIVE			1 (3%)	
PERIVASCULITIS			1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM				1 (3%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(15)	(13)	(29)	(25)
ATROPHY, NOS		1 (8%)	3 (10%)	
#SPLEEN	(15)	(13)	(26)	(28)
HYPERPLASIA, HEMATOPOIETIC			2 (8%)	
HYPERPLASIA, RETICULUM CELL			1 (4%)	1 (4%)
HEMATOPOIESIS		1 (8%)		
#MESENTERIC L. NODE	(15)	(3)	(23)	(20)
INFLAMMATION, SUPPURATIVE			1 (4%)	
INFLAMMATION, HEMORRHAGIC			1 (4%)	
CIRCULATORY SYSTEM				
NONE				

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(30)	(30)
PERIVASCULITIS			1 (3%)	
NECROSIS, NOS			1 (3%)	
NECROSIS, COAGULATIVE			1 (3%)	
NECROSIS, CENTRAL			1 (3%)	
HYPERPLASIA, NODULAR	2 (13%)			
HYPERPLASIA, HEMATOPOIETIC			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(15)	(13)	(28)	(29)
PYELONGPHRITIS, ACUTE/CHRONIC			1 (4%)	
INFLAMMATION, CHRONIC		1 (8%)		
#U. BLADDER/SUBMUCOSA	(14)	(12)	(26)	(21)
HEMORRHAGE		1 (8%)		
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*SEMINAL VESICLE	(15)	(13)	(31)	(32)
INFLAMMATION, SUPPURATIVE		1 (8%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES				
*PERITONEUM	(15)	(13)	(31)	(32)
INFLAMMATION, CHRONIC		1 (8%)		1 (3%)
*MESENTERY	(15)	(13)	(31)	(32)
NECROSIS, FAT				1 (3%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	10	9	13	22
AUTO/NECROPSY/NO HISTO			1	2
AUTOLYSIS/NO NECROPSY		3	4	3

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	14	35	35
ANIMALS NECROPSIED	15	14	29	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	29	30
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(28)	(30)
INFLAMMATION, INTERSTITIAL	2 (13%)	1 (8%)	1 (4%)	
HYPERPLASIA, LYMPHOID	3 (20%)		2 (7%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(13)	(13)	(28)	(29)
ATROPHY, NOS				10 (34%)
#SPLEEN	(12)	(13)	(29)	(29)
HYPERPLASIA, HEMATOPOIETIC		1 (8%)	1 (3%)	1 (3%)
HYPERPLASIA, RETICULUM CELL			1 (3%)	
HYPERPLASIA, LYMPHOID			2 (7%)	
#MESENTERIC L. NODE	(10)	(9)	(24)	(23)
HYPERPLASIA, RETICULUM CELL			1 (4%)	
#THYMUS	(7)	(8)	(10)	
HYPERPLASIA, HEMATOPOIETIC			1 (10%)	
CIRCULATORY SYSTEM				
#HEART	(14)	(12)	(28)	(30)
PERIARTERITIS	1 (7%)			
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(29)	(30)
NECROSIS, FOCAL				1 (3%)
NECROSIS, COAGULATIVE				1 (3%)
HYPERPLASIA, FOCAL			1 (3%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*BILE DUCT	(15)	(14)	(29)	(30)
INFLAMMATION, ACUTE/CHRONIC	1 (7%)			
#PANCREAS	(13)	(13)	(25)	(27)
INFLAMMATION, CHRONIC				1 (4%)
NECROSIS, FOCAL				1 (4%)
URINARY SYSTEM				
#KIDNEY	(15)	(13)	(29)	(29)
AMYLOIDOSIS				1 (3%)
#KIDNEY/GLOMERULUS	(15)	(13)	(29)	(29)
NECROSIS, FOCAL				1 (3%)
ENDOCRINE SYSTEM				
#THYROID	(13)	(9)	(13)	(21)
NECROSIS, FOCAL				1 (5%)
#PARATHYROID	(4)	(2)	(5)	(7)
PERIARTERITIS	1 (25%)			
REPRODUCTIVE SYSTEM				
#UTERUS	(15)	(13)	(28)	(27)
HYPERPLASIA, RETICULUM CELL			1 (4%)	
#UTERUS/ENDOMETRIUM	(15)	(13)	(28)	(27)
INFLAMMATION, NECROTIZING		1 (8%)		
HYPERPLASIA, CYSTIC	1 (7%)			
#UTERUS/MYOMETRIUM	(15)	(13)	(28)	(27)
INFLAMMATION, NECROTIZING		1 (8%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(14)	(29)	(30)
INFLAMMATION, CHRONIC				1 (3%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	7	9	5	15
AUTO/NECROPSY/NO HISTO		1		
AUTOLYSIS/NO NECROPSY			6	5

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS TREATED WITH 5-AZACYTIDINE

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Treated with 5-Azacytidine[a]

Topography: Morphology	Vehicle Control	Low Dose
Hematopoietic System: Lymphoma, Granulocytic Leukemia, or Sarcoma[b]	0/15 (0)	4/32 (13)
P Values[c,d]	--	N.S.
Relative Risk (Vehicle Control)[e]		Infinite
Lower Limit		0.463
Upper Limit		Infinite
Weeks to First Observed Tumor	--	73
Testis: Interstitial-cell Tumor[b]	0/15 (0)	2/28 (7)
P Values[c,d]	--	N.S.
Relative Risk (Vehicle Control)[e]		Infinite
Lower Limit		0.168
Upper Limit		Infinite
Weeks to First observed Tumor	--	80

[a]Treated groups received doses of 2.6 or 5.2 mg/kg by injection three times per week for 34 weeks.

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

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Treated with 5-Azacytidine[e]

(continued)

[c]Beneath the incidence of tumors in the treated group is the probability level for the Fisher exact test for the comparison of that group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Treated with 5-Azacytidine[a]

Topography: Morphology	Vehicle Control	Low Dose
Pituitary: Chromophobe Adenoma[b]	4/15 (27)	0/30 (0)
P Values[c,d]	--	P = 0.009(N)
Relative Risk (Vehicle Control)[e]		0.000
Lower Limit		0.000
Upper Limit		0.521
Weeks to First Observed Tumor	65	--
Mammary Gland: Papillary Adenocarcinoma, Fibroadenoma, or Adenocarcinoma, NOS (not otherwise specified)[b]	3/15 (20)	13/31 (42)
P Values[c,d]	--	N.S.
Relative Risk (Vehicle Control)[e]		2.097
Lower Limit		0.716
Upper Limit		10.013
Weeks to First Observed Tumor	76	26

[a]Treated groups received doses of 2.6 or 5.2 mg/kg by injection three times per week for 34 weeks.

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Treated with 5-Azacytidine[a]

(continued)

[c]Beneath the incidence of tumors in the treated group is the probability level for the Fisher exact test for the comparison of that group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE TREATED WITH 5-AZACYTIDINE

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Treated with 5-Azacytidine[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	0/13 (0)	4/31 (13)	3/32 (9)
P Values[c,d]	--	N.S.	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	Infinite
Lower Limit		0.421	0.265
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	73	74

[a]Treated groups received doses of 2.2 or 4.4 mg/kg by injection three times per week for 52 weeks.

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

[c]Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]Since survival in the high-dose group was short, the tests for dose-related trend are not reported.

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Treated with 5-Azacytidine[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Multiple Sites:			
Lymphoma[b]	0/14 (0)	7/29 (24)	0/30 (0)
P Values[c,d]	--	P = 0.048	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		1.010	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	43	--
Hematopoietic System:			
Granulocytic Leukemia or Sarcoma[b]	0/14 (0)	10/29 (34)	0/30 (0)
P Values[c,d]	--	P = 0.010	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		1.554	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	53	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Treated with 5-Azacytidine[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma, Granulocytic Leukemia, or Sarcoma[b]	0/14 (0)	17/29 (59)	0/30 (0)
P Values[c,d]	--	P < 0.001	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		2.848	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	43	--
Mammary Gland: Adenocarcinoma[b]	0/14 (0)	2/29 (7)	0/30 (0)
P Values[c,d]	--	N.S.	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		0.153	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	61	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Treated with 5-Azacytidine[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Uterus: Sarcoma, NOS, or Leiomyosarcoma[b]	0/13 (0)	3/28 (11)	0/27 (0)
P Values[c,d]	--	N.S.	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		0.300	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	81	--

[a]Treated groups received doses of 2.2 or 4.4 mg/kg by injection three times per week for 52 weeks.

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

[c]Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]Since survival in the high-dose group was short, the tests for dose-related trend are not reported.

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

Review of the Bioassay of 5-Azacytidine*for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

November 28, 1977

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in organic chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which 5-Azacytidine was reviewed.

The primary reviewer commented that the severe toxicity of the drug resulted in so many deaths that meaningful statistical analyses could not be done on the treated rats or male mice. In female mice, tumors of the hematopoietic system were associated with treatment. Bone marrow atrophy was a significant finding in the treated rats. The reviewer agreed with the staff's conclusion that 5-Azacytidine induced lymphomas and granulocytic leukemias in the treated female mice. The secondary reviewer also agreed with the conclusion given in the report. A discussion ensued as to the possible mechanism by which 5-Azacytidine exerts its effect.

Despite the inadequacies of the study, a motion was made that there was sufficient evidence to conclude that 5-Azacytidine induced hematopoietic neoplasms. The motion was seconded and approved by all present except Mr. Garfinkel, who opposed it based on the small animal groups and high early mortality.

Members present were:

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

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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