National Cancer Institute
CARCINOGENESIS
Technical Report Series
NO. 78
1978

BIOASSAY OF ICRF-159 FOR POSSIBLE CARCINOGENICITY

CAS No. 21416-87-5

NCI-CG-TR-78

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
National Institutes of Health
Bethesda, Maryland 20014

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DHEW Publication No. (NIH) 78-1328

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FOREWORD: This report presents the results of the bioassay of ICRF-159 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 greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as 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 that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of ICRF-159 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 administration of the chemical and the care of the laboratory animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. R. B. Thompson[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled by EG&G Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed by Mr. A. R. Chamberlin[7], Mr. G. L. Tong[7], and Mr. P. Lim[7], and the results of the analyses were reviewed by Dr. C. W. Jameson[5].

This report was prepared at Tracor Jitco[5] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[6]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings:

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SUMMARY

A bioassay of the experimental anticancer drug ICRF-159 for possible carcinogenicity was conducted by administering the compound by intraperitoneal injection to Sprague-Dawley rats and B6C3F1 mice.

Groups of 35 rats and 35 mice of each sex were injected three times per week with ICRF-159 in buffered saline at one of the following doses, either 48 or 96 mg/kg body weight for the rats and either 40 or 80 mg/kg body weight for the mice. Both rats and mice were dosed for 52 weeks, then observed for 29-34 additional weeks. Untreated-control and vehicle-control groups each consisted of 10 rats and 15 mice of each sex; pooled-control groups consisted of the 10 vehicle controls of each sex of the rats combined with 30 vehicle controls of each sex of rats from similar bioassays of three other chemicals and the 15 vehicle controls of each sex of the mice combined with 30 vehicle controls of each sex of mice from similar bioassays of two other chemicals. All surviving rats were killed at 81-86 weeks; all surviving mice, at 86 weeks.

Mean body weights were depressed in rats and mice administered ICRF-159, and mortality was dose related among male and female rats and male mice. The high mortality among the male rats may have been associated with inflammatory lesions observed in the lungs, the liver, and the pleural and peritoneal cavities. Sufficient numbers of female rats and of both male and female mice were at risk for development of late-appearing tumors. In the male rats, time-adjusted analysis of the incidence of tumors was used for determining statistical significance.

In female rats, the incidence of uterine adenocarcinomas was higher in the low- and high-dose groups ($P < 0.001$) than in the pooled controls (controls 0/38, low-dose 10/33, high-dose 11/32); the incidence was also dose related ($P < 0.001$). In male rats, no tumors occurred in the dosed groups in a significantly increased incidence.

In female mice, the incidence of all hematopoietic neoplasms (histiocytic lymphomas, lymphocytic lymphomas, or lymphocytic leukemias), taken together, was higher in the low-dose group (P = 0.038) and in the high-dose group (P = 0.002) than in the pooled controls (controls 1/45, low-dose 5/31, high-dose 9/34); the incidence was also dose related (P = 0.002). In addition, the incidence of these tumors in the high-dose group was higher (P = 0.026) than that in the vehicle controls (0/15), and the incidence was dose related (P = 0.021) using the vehicle controls. In male mice, lymphocytic neoplasms occurred only in two low-dose and two high-dose animals.

It is concluded that under the conditions of this bioassay, ICRF-159 was carcinogenic for female Sprague-Dawley rats, producing uterine adenocarcinomas, and was also carcinogenic for female B6C3F1 mice, producing lymphomas.

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I. INTRODUCTION

ICRF-159 (CAS 21416-87-5; NSC 129943; NCI C01627) is an experimental anticancer drug, developed by the Imperial Cancer Research Fund in England and tested in Phase II clinical trials in the United States. ICRF-159 has been administered to patients with acute leukemias (Hellmann, 1970; Mathé et al., 1970) and to patients with tumors of the lung, breast, or colon (Carter and Slavik, 1976). Unlike other cytotoxic drugs, ICRF-159 inhibits cell growth only in the stages following DNA synthesis and preceding mitosis (Sharpe and Field, 1970). It is most toxic to cells that divide frequently, since they are likely to be found in these stages. This drug has been shown to inhibit the spontaneous metastasis of an experimental mouse tumor (Salsbury et al., 1970).

ICRF-159 is one in a series of anticancer compounds that were selected for testing in the Carcinogenesis Testing Program to assess the possible carcinogenic effects of drugs that may be administered to humans on a chronic basis.

II. MATERIALS AND METHODS

A. Chemical

The chemical (±)bis-4,4′-(1-methyl-1,2-ethanediyl)-2,6-piperazinedione, commonly called ICRF-159, was obtained for the chronic study in a single batch (Lot No. PD/AS 5014/71) from the Imperial Chemical Industries, LTD., Cheshire, England. The purity of this batch was determined to be 98-99% by non-aqueous potentiometric titration, chromatography, and elemental analysis (C,H,N,O) at the Stanford Research Institute. The melting point was 229-233°C with decomposition, similar to the value of 223°C reported elsewhere (Wasserman et al., 1973). Thin-layer chromatography, comparing the test material with a mixture of hydrolysis products, indicated 1-2% hydrolysis. Nuclear magnetic resonance, infrared, and ultraviolet spectra were consistent with the structure.

The chemical was stored in the presence of a desiccant (Drierite®) at 5°C.

B. Dosage Preparation

Suspensions of ICRF-159 were prepared fresh each day that the chemical was administered. The chemical was suspended in a buffered saline vehicle by mixing in a Potter-Elvehjem tissue

grinder. The buffered saline vehicle (pH = 6.9) contained 0.85% NaCl, 0.40% NaH_2PO_4, and 0.65% Na_2HPO_4.

C. Animals

For the subchronic studies, female Sprague-Dawley rats and male Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

For the chronic tests, Sprague-Dawley rats and B6C3F1 mice of each sex were obtained from Charles River Breeding Laboratories. All animals were supplied through a contract with the Division of Cancer Treatment, National Cancer Institute. Male rats were received at the test laboratory at 29 days of age, female rats at 36 days of age, and male and female mice at 30 days of age. On arrival at the laboratory, all animals were quarantined for approximately 1 week. Animals with no visible signs of disease were assigned to control or dosed groups and earmarked for individual identification.

D. Animal Maintenance

The 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%. The air was changed 15 times per hour and passed through both intake and exhaust fiberglass

roughing filters. In addition to natural light, illumination was provided by fluorescent lighting for 9 hours each day. Wayne® Lab Blox animal feed (Allied Mills, Inc., Chicago, Ill.) and water were made available ad libitum and replenished daily.

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 rat cages were provided with Iso-Dri® hardwood chip bedding (Carworth, Edison, N.J.), and the cage tops were covered with disposable filter bonnets, beginning at week 25; 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 dosed animals. Animals administered ICRF-159 were maintained in the same room in which the following chemicals were on test:

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)
5-azacytidine (CAS 320-67-2)
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 [IPD] (CAS 3458-22-8)
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 hydrochloride) (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)

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)
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)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
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tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

E. Subchronic Studies

Subchronic studies were conducted using female Sprague-Dawley rats and male Swiss mice to estimate the maximum tolerated doses of ICRF-159, on the basis of which "low" and "high" doses were determined for administration in the chronic studies. The rats were administered doses of 1.2, 3, 6, 12, 24, 48, 96, 192, or 384 mg/kg body weight; the mice were administered doses of 2, 5, 10, 20, 40, 80, 160, 320, or 640 mg/kg body weight. Dosed animals were injected intraperitoneally with ICRF-159 three times per

week for 45 days, then observed for an additional 45 days. Five animals of each species were injected with the chemical at each dose, 10 animals of each species were injected with the vehicle alone (vehicle controls), and 10 animals of each species left untreated (untreated controls).

In rats, death occurred by week 3 in 4/5 animals administered 384 mg/kg and by week 7 in 1/5 animals administered 192 mg/kg. No animals died at any lower doses. At the end of the administration period, body weight gains were not affected in animals administered 1.2 and 3 mg/kg. Weight gains in animals at higher doses were variable, ranging from 52-86% of control values and showing no dose-related trend. At the highest dose administered, the mean weight gain was 78% of that of controls. All animals having low weight gains at the end of the administration period had mean weight gains comparable to those of controls by the end of the study. No gross abnormalities were noted at necropsy. The low and high doses for the chronic studies using rats were set at 48 mg/kg and 96 mg/kg.

In mice, death occurred by week 3 in 2/5 animals administered 5 mg/kg and within the first 3 weeks of the study in all animals administered 160 mg/kg and above. Mean body weight gains in the surviving dosed animals were not affected except at a dose of 80 mg/kg, where the weight gains in the dosed animals were 40% lower

than that in the controls at the end of the administration period. No gross abnormalities were found at necropsy. The low and high doses for the chronic studies using mice were set 40 mg/kg and 80 mg/kg.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2.

Since the numbers of rats and mice in the vehicle-control groups were small, pooled vehicle-control groups also were used for statistical comparisons. The groups of 10 vehicle-control rats and 15 vehicle-control mice of each sex from the current bioassay of ICRF-159 were combined with corresponding groups of 10 vehicle-control rats of each sex from similar bioassays of procarbazine, thio-TEPA, and 3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] and of 15 vehicle-control mice of each sex from similar bioassays of thio-TEPA and IPD to give pooled groups of 40 vehicle-control rats and 45 vehicle-control mice of each sex. The vehicle-control animals that were used in the pooled-control groups were of the same strains (Sprague-Dawley rats and B6C3F1 mice) and from the same supplier, and they were examined by the same pathologists; furthermore, the different control groups received the same vehicle for injection

Table 1. Design of Chronic Studies of ICRF-159 in Rats

Sex and Test Group	Initial No. of Animals[a]	ICRF-159 Doses (mg/kg)[b]	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Untreated-Control	10	0		85
Vehicle-Control	10	0[c]	52	33
Low-Dose	35	48	52	32
High-Dose	35	96	52	2[illegible]
Female				
Untreated Control	10	0		85-86
Vehicle-Control	10	0[c]	52	33
Low-Dose	35	48	52	32-33
High-Dose	35	96	52	29

[a]Male rats were 35 days of age and female rats were 42 days of age when placed on study.

[b]ICRF-159 was administered by intraperitoneal injection three times per week in buffered saline, at a volume of 0.25 ml/100 g body weight. Doses were based on individual weights.

[c]Vehicle-control groups received only buffered saline solution, at the same volume as dosed groups.

Table 2. Design of Chronic Studies of ICRF-159 in Mice

Sex and Test Group	Initial No. of Animals[a]	ICRF-159 Doses (mg/kg)[b]	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Untreated-Control	15	0		86
Vehicle-Control	15	0[c]	52	34
Low-Dose	35	40	52	34
High-Dose	35	80	52	34
Female				
Untreated Control	15	0		86
Vehicle-Control	15	0[c]	52	34
Low-Dose	35	40	52	34
High-Dose	35	80	52	34

[a]All mice were 35 days of age when placed on study.

[b]ICRF-159 was administered by intraperitoneal injection three times per week in buffered saline, 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-control groups received only buffered saline solution, at the same volume as dosed groups.

and were placed on study at starting times differing by no more than 3 weeks.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and those animals appearing moribund were killed and necropsied. Rats were weighed individually each week for 2 months and every 2 weeks thereafter. 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 examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph 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 whenever possible. 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 were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals for 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 dosed animals at each dose level. When results for a number of dosed 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 relation-

ship. 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 dosed group compared with 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 dosed 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 dosed 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 dosed 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 dosed 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 signifi-

cant 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)

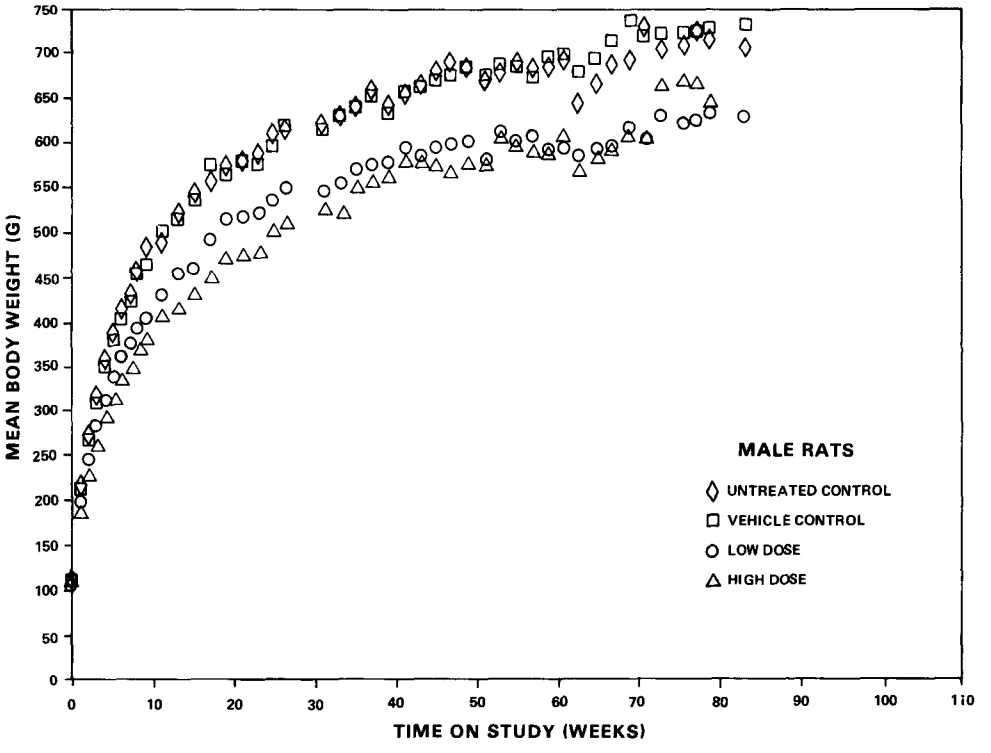
Mean body weights of both male and female dosed rats were lower than those of either vehicle or matched controls, with the weights of the high-dose rats being slightly lower than those of the low- dose rats (figure 1). Fluctuation in the growth curves may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation.

There were no other clinical signs recorded which were indicative of drug-related toxicity in the rats.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered ICRF-159 by intraperitoneal injection at the doses of this bioassay, together with those of the untreated and vehicle controls, are shown in figure 2.

The result of the Tarone test for positive dose-related trend in mortality over the period of the bioassay is significant ($P < 0.001$) in each sex. In male rats, 10/10 (100%) of the untreated controls, 9/10 (90%) of the vehicle controls, 24/36 (67%) of the low-dose group, and 8/35 (23%) of the high-dose group lived at



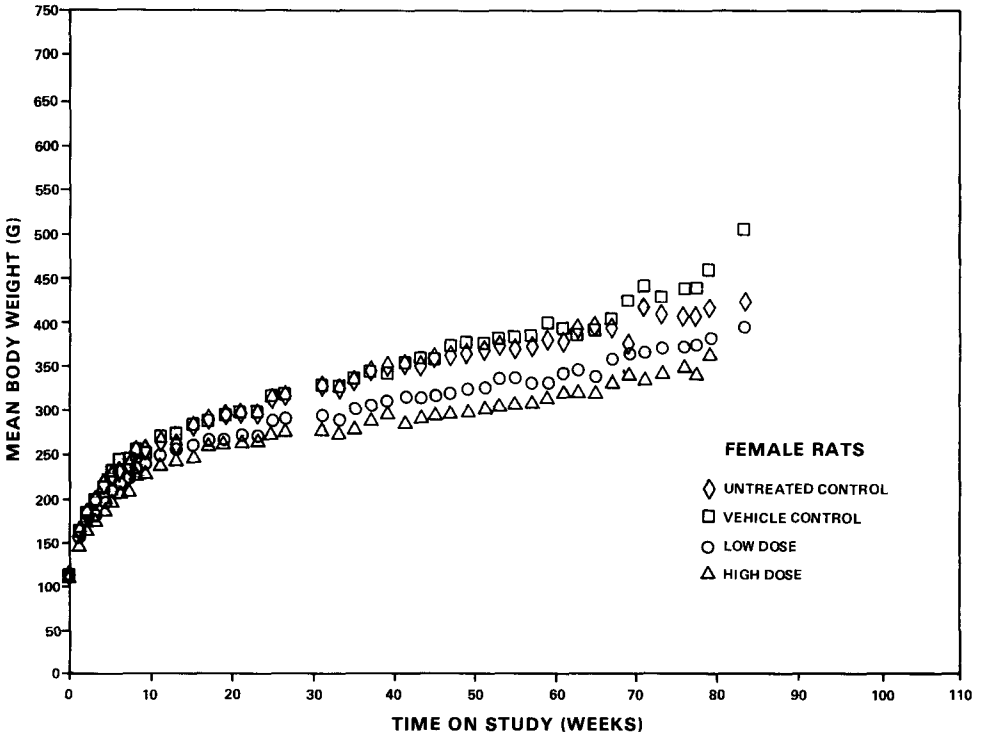


Figure 1. Growth Curves For Rats Treated With ICRF-159

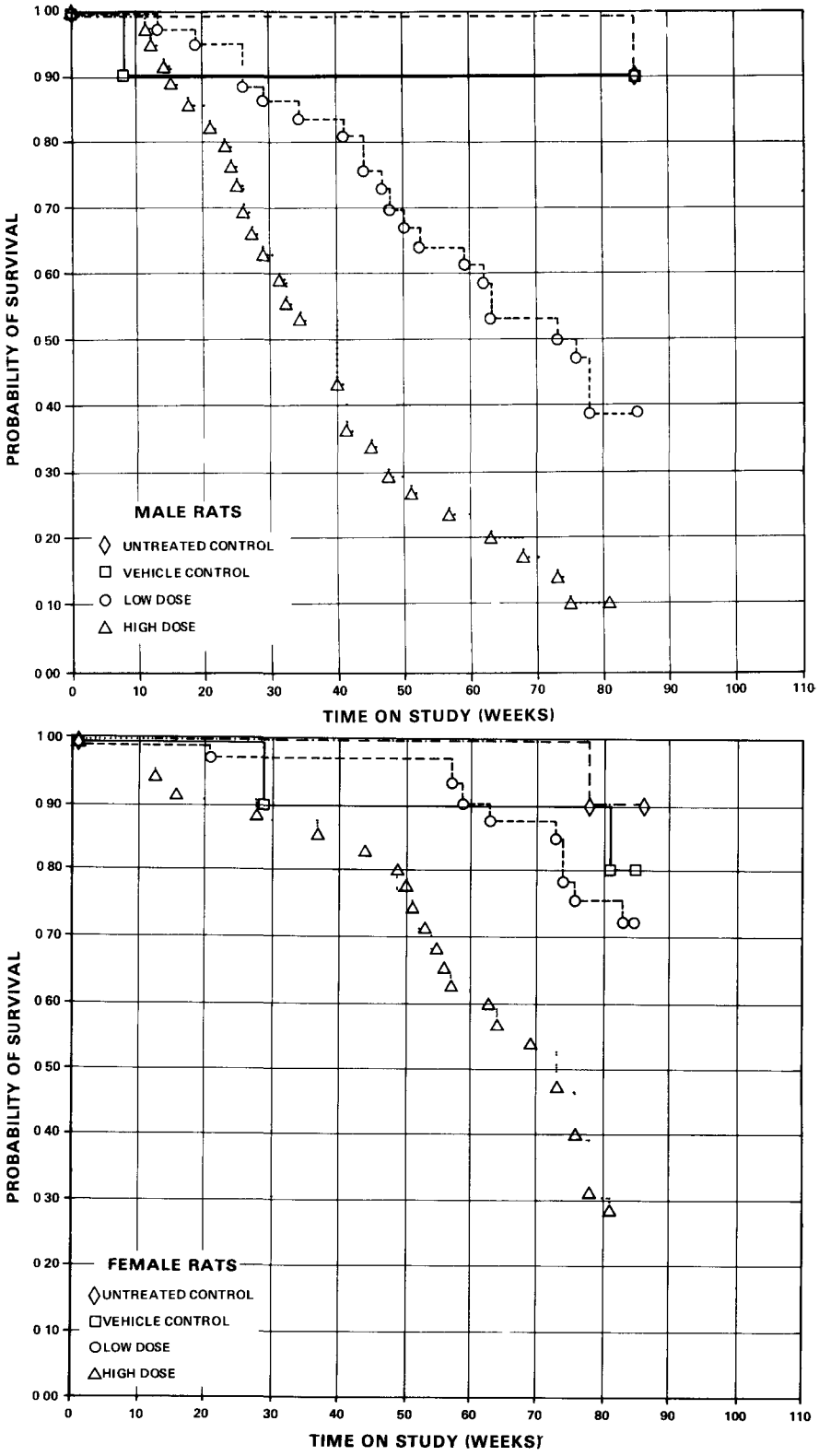


Figure 2. Survival Curves For Rats Treated With ICRF-159

least as long as week 52. In female rats, 10/10 (100%) of the untreated controls, 9/10 (90%) of the vehicle controls, 32/34 (94%) of the low-dose group, and 26/35 (74%) of the high-dose group were alive at week 52. Sufficient numbers of females were at risk for development of tumors.

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.

There was an increased incidence of uterine neoplasms in dosed female rats.

	FEMALE RATS			
Site/Neoplasm	Untreated Control	Vehicle Control	Low Dose	High Dose
Uterus				
Number of animals with tissues examined microscopically	(10)	(10)	(33)	(32)
Adenocarcinoma, NOS*	0	0	10	11
Endometrial stromal polyp	0	1	2	0
Cervix				
Number of animals with tissues examined microscopically	(10)	(10)	(33)	(32)
Squamous-cell carcinoma	0	0	0	1

*Not otherwise specified

The uterine tumors were primarily adenocarcinomas arising from the endometrial glandular epithelium. These tumors were

characterized by neoplastic epithelial cells having large vesicular nuclei, prominent eosinophilic nucleoli, and moderate amounts of eosinophilic cytoplasm. The tumor cells formed glands, ducts, and acini that were separated by a fibrovascular stroma. The neoplastic glandular tissue originated in the endometrium and projected into the uterine lumen and also infiltrated the overlying muscle layers of the uterine wall. The neoplastic cells had frequently penetrated the serosal surface of the uterus and transplanted to multiple sites throughout the abdominal cavity. Metastases were found at multiple sites in the abdominal cavity of the females, but no tumors of the abdominal cavity were observed in the dosed or control males. The increased incidence in the females appears to be the result of administration of the test chemical.

Endometrial glandular hyperplasias occurred in 1/32 (3%) high-dose females and 1/33 (3%) low-dose females. Inflammatory lesions of the uterus occurred in both dosed and control rats. The incidence of suppurative uterine lesions were: untreated-control females 5/10 (50%), vehicle-control females 1/10 (10%), low-dose females 20/33 (61%) and high-dose females 11/32 (34%).

With the exception of uterine tumors, neoplasms listed in Appendix A occurred with approximately equal frequency in control

and dosed rats or occurred in insignificant numbers. All neoplasms have been encountered previously as spontaneous lesions in Sprague-Dawley rats.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals of the dosed and control groups (Appendix C). These nonneoplastic lesions are commonly seen in aged Fischer 344 rats.

The small number of animals bearing tumors in the high-dose group of male rats (4/30 [13%]) compared with the number in the low-dose group (11/33 [33%]) may have been due to the decreased life span in the high-dose males. The increased mortality in the high-dose males may have been associated with inflammatory lesions in the lungs, liver, and serous cavities (pleural and peritoneal).

Based on the histologic examination, the administration of ICRF-159 by intraperitoneal injection to Sprague-Dawley rats at doses of 48 or 96 mg/kg was associated with uterine adenocarcinomas.

D. Statistical Analyses of Results (Rats)

Tables E1-E3 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least

two animals in one group and with an incidence of at least 5% in one or more than one group.

The untreated controls are not included in the tables and analyses, because the test conditions of the vehicle controls more closely resemble those of the dosed animals. Due to the high mortality of the high-dose male rats, time-adjusted analyses eliminating animals that died before 1 year on study are performed; however, the first leukemia was observed at week 44, and time-adjusted analyses on the incidence of this particular tumor are based on animals that lived at least as long as week 44 on study. These time-adjusted analyses are shown in table E3 in Appendix E, and the statistical narrative below on male rats is based on the time-adjusted data only.

In male rats, neither the results of the Cochran-Armitage test for positive dose-related trend in incidences of tumors nor the results of the Fisher exact test for direct comparison of incidences of tumors in the dosed groups with those in the controls are significant. In females, the result of the Cochran-Armitage test for dose-related trend in the incidence of adenocarcinomas of the uterus is significant ($P < 0.001$) when the pooled-control group is used, and the results of the Fisher exact test show that the incidences of the tumor are significantly higher ($P < 0.001$) in each of the dosed groups than that in the

pooled controls. The Fisher exact comparison of the incidences of each of the dosed groups with that of the vehicle-control group indicates probability levels that are above the 0.025 level required by the Bonferroni inequality criterion when multiple comparison is considered. The statistical conclusion suggests that the incidence of adenocarcinomas of the uterus in female rats is associated with administration of the test chemical. The historical vehicle controls of 165 female Sprague-Dawley rats compiled to date at this laboratory show no such tumor occurrence.

A significant dose-related trend in the negative direction is observed in the incidence of fibroadenomas of the mammary gland in female rats when the pooled-control group is used. This significant negative trend may be explained by the shortened survival of the dosed animals, thus suppressing the possibility of late tumor development in these groups.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the high-dose mice were lower than those of both the untreated and vehicle controls; body weights of the low-dose mice were lower than those of the untreated controls, but were comparable to those of the vehicle controls (figure 3). Fluctuation in the growth curves may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation.

There were no other clinical signs recorded which were indicative of drug-related toxicity in the mice.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered ICRF-159 by intraperitoneal injection at the doses of this bioassay, together with those of the untreated and vehicle controls, are shown in figure 4.

In male mice, the result of the Tarone test for positive dose-related trend in mortality over the bioassay is significant ($P < 0.001$). Twenty-three out of 35 (66%) of the high-dose group, 26/35 (74%) of the low-dose group, 11/15 (73%) of the vehicle

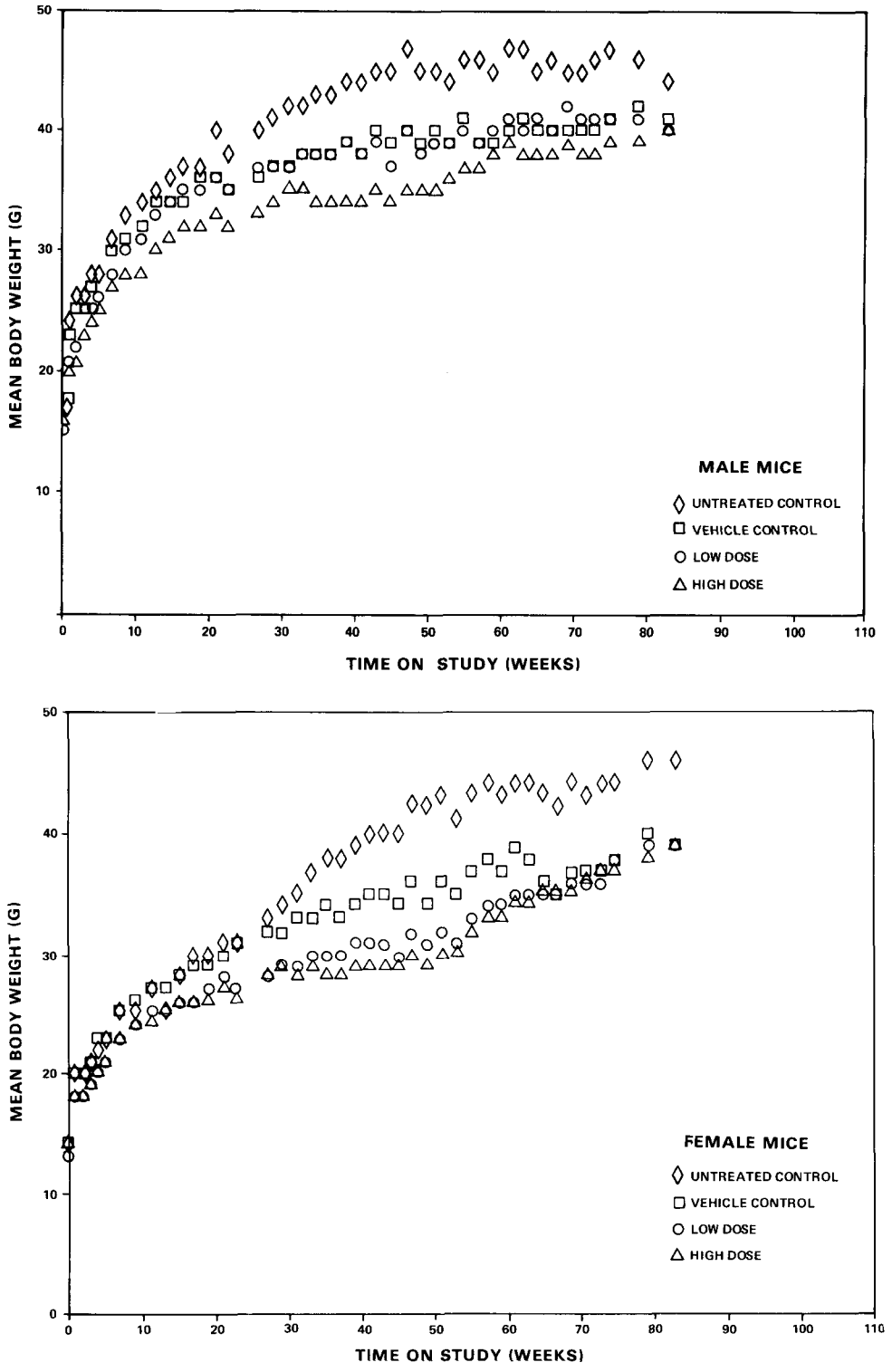


Figure 3. Growth Curves For Mice Treated With ICRF-159

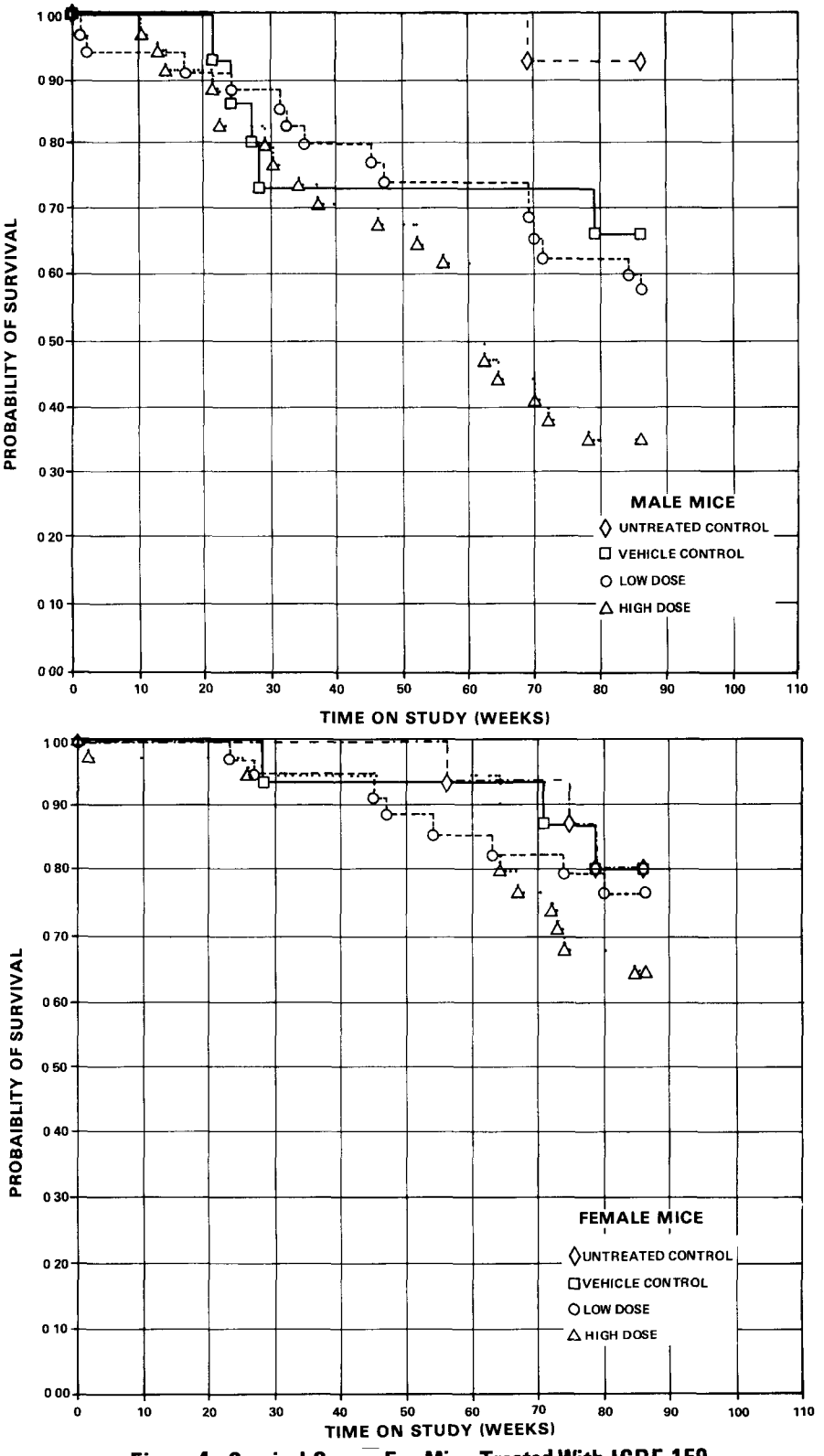


Figure 4. Survival Curves For Mice Treated With ICRF-159

controls, and all (15) of the untreated controls were alive at week 52. In females, the result of the Tarone test is not significant. Twenty-one of 35 (60%) high-dose animals, 26/35 (74%) low-dose animals, and 12/15 (80%) of the vehicle or untreated controls were alive at week 86. Sufficient numbers of male and female mice were at risk for the development of tumors.

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 tumors occurred in both the control and dosed groups.

With the exception of neoplastic lesions of the lymphoreticular system (tabulated below), the neoplasms listed in Appendix B appeared with approximately equal frequency in dosed and control mice or appeared in low numbers.

	MICE			
	MALE		FEMALE	
	Low Dose	High Dose	Low Dose	High Dose
Multiple Organs				
Number of Animals Necropsied	(34)	(30)	(31)	(34)
Malignant lymphoma, histiocytic type	1	1	1	3
Lymphocytic leukemia	1	1	4	3

	MICE			
	MALE		FEMALE	
	Low Dose	High Dose	Low Dose	High Dose
Spleen				
Number of animals with tissues examined microscopically	(34)	(29)	(31)	(34)
Malignant lymphoma, histiocytic type	0	0	0	2
Thymus				
Number of animals with tissues examined microscopically	(34)	(29)	(31)	(34)
Malignant lymphoma, lymphocytic type	0	0	0	1

There was a slight increase in the incidence of neoplastic lesions of the lymphoreticular system. These lesions were confined to the dosed mice.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups (Appendix D). For the most part, the nonneoplastic lesions are commonly seen in aged mice and were not associated with increased mortalities or decreased life spans.

Based on this histologic examination, the administration of ICRF-159 by intraperitoneal injection to B6C3F1 mice at doses of 40 or 80 mg/kg was associated with an increase in lymphoreticular neoplasms in the dosed females.

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 occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group. The untreated controls are not included in the tables and analyses, because the test conditions of the vehicle controls more closely resemble those of the dosed animals.

In male mice, neither the results of the Cochran-Armitage test for positive dose-related trend in incidences of tumors nor the results of the Fisher exact test for direct comparison of incidences of tumors in dosed groups with those in the controls are significant. However, a significant trend in the negative direction is observed in the incidences of tumors of the liver, where the incidences in the control groups exceed those in the dosed groups. These significant negative results may be due to the shortened survival of the dosed animals, which suppressed late tumor development in these animals.

In females, the results of the Cochran-Armitage test on the incidence of histiocytic lymphomas are significant when either the pooled-control group ($P = 0.006$) or the vehicle-control group ($P = 0.041$) is used. The results of the Fisher exact test show

that the incidence in the high-dose group is significantly higher ($P = 0.012$) than that in the pooled controls. When all of the tumors of the hematopoietic system are combined for analyses, the statistical results show increased significance. Data for the laboratory historical vehicle controls indicate an incidence of 1/111 (0.9%) of female mice with any type of hematopoietic tumor (lymphomas or leukemias). The statistical analyses indicate the possibility that hematopoietic tumors are associated with the administration of ICRF-159.

V. DISCUSSION

Under the conditions of this bioassay, ICRF-159 administered by intraperitoneal injection was toxic to both Sprague-Dawley rats and B6C3F1 mice. Mean body weights of both species were depressed by administration of the chemical, and tests for dose-related trend in survival were positive for male and female rats and for male mice. The high mortality among the high-dose male rats may have been associated with inflammatory lesions in the lungs, the liver, and the pleural and peritoneal cavities. Sufficient numbers of female rats and of both male and female mice were at risk beyond 52 weeks for development of tumors. For the male rats, time-adjusted analysis of the incidence of tumors was used.

In female rats, the incidence of uterine adenocarcinomas was significantly higher in the low- and high-dose groups ($P < 0.001$) than in the pooled controls (controls 0/38, low-dose 10/33, high-dose 11/32); the incidence was also dose related ($P < 0.001$). Metastases of the adenocarcinomas were found in the abdominal cavity of the females. In male rats, no tumors occurred in the dosed groups in a significantly increased incidence. In the high-dose males, tumors were observed in only four animals. This small number may have been due to the increased mortality among these animals.

In female mice, the incidence of all hematopoietic neoplasms (histiocytic lymphomas, lymphocytic lymphomas, or lymphocytic leukemias), taken together, was higher in the low-dose group (P = 0.038) and in the high-dose group (P = 0.002) than in the pooled controls (controls 1/45, low-dose 5/31, high-dose 9/34); and the incidence was significantly dose related (P = 0.002). In addition, the incidence of these tumors in the high-dose group was higher (P = 0.026) than that in the vehicle controls (0/15), and the incidence was dose related (P = 0.021) using the vehicle controls. In male mice, lymphocytic neoplasms occurred only in two low-dose and two high-dose animals.

Chronic toxicity studies with ICRF-159 in rats (Hellman, 1972) showed gross depletion of lymphoid elements in the spleen and thymus but only slight depletion in the lymph nodes. Short-term toxicity studies in dogs produced leucopenia and thrombocytopenia, together with cytotoxic changes in gastrointestinal, bone marrow, and male reproductive tissues (Wasserman et al., 1973).

It is concluded that under the conditions of this bioassay, ICRF-159 was carcinogenic for female Sprague-Dawley rats, producing uterine adenocarcinomas, and was also carcinogenic for female B6C3F1 mice, producing lymphomas.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	36	35
ANIMALS NECROPSIED	10	10	33	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	33	30
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(33)	(30)
SQUAMOUS CELL CARCINOMA			1 (3%)	
*SUBCUT TISSUE	(10)	(10)	(33)	(30)
SQUAMOUS CELL PAPILLOMA				1 (3%)
SARCOMA, NOS				1 (3%)
FIBROMA			2 (6%)	2 (7%)
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(10)	(33)	(30)
GRANULOCYTIC LEUKEMIA	1 (10%)		2 (6%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(32)	(28)
HEPATOCELLULAR CARCINOMA			1 (3%)	
#SMALL INTESTINE	(10)	(10)	(33)	(30)
MUCINOUS ADENOCARCINOMA		1 (10%)		
#JEJUNUM	(10)	(10)	(33)	(30)
ADENOCARCINOMA, NOS			1 (3%)	

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
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(9)	(8)	(30)	(22)
CHROMOPHOBE ADENOMA	1 (11%)		2 (7%)	1 (5%)
CHROMOPHOBE CARCINOMA	1 (11%)		1 (3%)	
#THYROID	(10)	(9)	(28)	(25)
FOLLICULAR-CELL CARCINOMA				1 (4%)
C-CELL ADENOMA			1 (4%)	
#PANCREATIC ISLETS	(10)	(10)	(33)	(30)
ISLET-CELL CARCINOMA	1 (10%)			
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(10)	(10)	(33)	(30)
FIBROMA	1 (10%)			
*MESENTERY	(10)	(10)	(33)	(30)
LIPOMA			1 (3%)	

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
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	36	35
NATURAL DEATH@	1		13	18
MORIBUND SACRIFICE		1	9	10
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				4
TERMINAL SACRIFICE	9	9	14	3
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	1	11	4
TOTAL PRIMARY TUMORS	5	1	12	6
TOTAL ANIMALS WITH BENIGN TUMORS	2		6	4
TOTAL BENIGN TUMORS	2		6	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	1	6	2
TOTAL MALIGNANT TUMORS	3	1	6	2
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 ICRF-159

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	34	35
ANIMALS NECROPSIED	10	10	33	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	33	32
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(33)	(32)
SQUAMOUS CELL PAPILLOMA			1 (3%)	
SQUAMOUS CELL CARCINOMA				1 (3%)
*SUBCUT TISSUE	(10)	(10)	(33)	(32)
SQUAMOUS CELL CARCINOMA				1 (3%)
SARCOMA, NOS				1 (3%)
FIBROSARCOMA				1 (3%)
RESPIRATORY SYSTEM				
*LUNG	(10)	(10)	(33)	(32)
SQUAMOUS CELL CARCINOMA				1 (3%)
ADENOCARCINOMA, NOS, METASTATIC			3 (9%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (3%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(10)	(33)	(32)
LYMPHOCYTIC LEUKEMIA				1 (3%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
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
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(9)	(31)	(30)
CHROMOPHOBE ADENOMA	2 (20%)	1 (11%)	7 (23%)	2 (7%)
#ADRENAL	(10)	(10)	(33)	(32)
PHEOCHROMOCYTOMA			1 (3%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(33)	(32)
ADENOCARCINOMA, NOS		1 (10%)	2 (6%)	3 (9%)
CYSTADENOCARCINOMA, NOS				1 (3%)
FIBROSARCOMA			1 (3%)	
FIBROADENOMA	3 (30%)	2 (20%)	5 (15%)	4 (13%)
#UTERUS	(10)	(10)	(33)	(32)
ADENOCARCINOMA, NOS			10 (30%)	11 (34%)
ENDOMETRIAL STROMAL POLYP		1 (10%)	2 (6%)	
#CERVIX UTERI	(10)	(10)	(33)	(32)
SQUAMOUS CELL CARCINOMA				1 (3%)
#OVARY	(10)	(10)	(33)	(32)
ADENOCARCINOMA, NOS				1 (3%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(10)	(33)	(32)
ADENOCARCINOMA, NOS, METASTATIC			9 (27%)	1 (3%)

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
SARCOMA, NOS			1 (3%)	2 (6%)
MESOTHELIOMA, MALIGNANT			1 (3%)	
*PERITONEUM	(10)	(10)	(33)	(32)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
*PELVIS	(10)	(10)	(33)	(32)
ADENOCARCINOMA, NOS, METASTATIC				1 (3%)
*MESENTERY	(10)	(10)	(33)	(32)
ADENOCARCINOMA, NOS, METASTATIC				2 (6%)
SARCOMA, NOS			1 (3%)	
ALL OTHER SYSTEMS				
THORAX				
SARCOMA, NOS				1
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	34	35
NATURAL DEATH@		2	5	9
MORIBUND SACRIFICE	1		4	16
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED			1	
TERMINAL SACRIFICE	9	8	24	10
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	4	24	22
TOTAL PRIMARY TUMORS	5	5	33	32
TOTAL ANIMALS WITH BENIGN TUMORS	4	4	16	6
TOTAL BENIGN TUMORS	5	4	17	6
TOTAL ANIMALS WITH MALIGNANT TUMORS		1	16	19
TOTAL MALIGNANT TUMORS		1	16	26
TOTAL ANIMALS WITH SECONDARY TUMORS#			10	3
TOTAL SECONDARY TUMORS			13	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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	15	14	34	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	34	29
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(34)	(29)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (20%)			
ALVEOLAR/BRONCHIOLAR CARCINOMA				1 (3%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(14)	(34)	(30)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	1 (3%)
LYMPHOCYTIC LEUKEMIA			1 (3%)	1 (3%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(33)	(29)
HEPATOCELLULAR ADENOMA	1 (7%)	1 (8%)		
HEPATOCELLULAR CARCINOMA	1 (7%)	1 (8%)		
URINARY SYSTEM				
NONE				
ENDOCRINE 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
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@		5	12	13
MORIBUND SACRIFICE	1		3	9
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				1
TERMINAL SACRIFICE	14	10	20	12
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*	3	2	2	3
TOTAL PRIMARY TUMORS	5	2	2	3
TOTAL ANIMALS WITH BENIGN TUMORS	3	1		
TOTAL BENIGN TUMORS	4	1		
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	1	2	3
TOTAL MALIGNANT TUMORS	1	1	2	3
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 GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS MISSING				1
ANIMALS NECROPSIED	14	15	31	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	31	34
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(15)	(31)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	3 (9%)
LYMPHOCYTIC LEUKEMIA			4 (13%)	3 (9%)
#SPLEEN	(13)	(15)	(31)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				2 (6%)
#THYMUS	(12)	(15)	(31)	(34)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				1 (3%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NONE				

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
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(14)	(15)	(31)	(34)
ADENOMA, NOS			1 (3%)	
ADENOCARCINOMA, NOS			1 (3%)	
#UTERUS	(13)	(15)	(31)	(34)
ADENOCARCINOMA, NOS			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(14)	(15)	(31)	(34)
CARCINOMA,NOS			1 (3%)	
*MESENTERY	(14)	(15)	(31)	(34)
LIPOMA				2 (6%)
ALL OTHER SYSTEMS				
NONE				

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
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@	3	2	7	4
MORIBUND SACRIFICE		1	1	8
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED			1	
TERMINAL SACRIFICE	12	12	26	22
ANIMAL MISSING				1
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*			8	11
TOTAL PRIMARY TUMORS			9	11
TOTAL ANIMALS WITH BENIGN TUMORS			1	2
TOTAL BENIGN TUMORS			1	2
TOTAL ANIMALS WITH MALIGNANT TUMORS			7	9
TOTAL MALIGNANT TUMORS			8	9
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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	36	35
ANIMALS NECROPSIED	10	10	33	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	33	30
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(33)	(30)
EPIDERMAL INCLUSION CYST		1 (10%)		
*SUBCUT TISSUE	(10)	(10)	(33)	(30)
ABSCESS, NOS		1 (10%)		
INFLAMMATION, ACUTE/CHRONIC			1 (3%)	
INFLAMMATION, CHRONIC		1 (10%)		
INFLAMMATION, CHRONIC FOCAL			1 (3%)	1 (3%)
INFLAMMATION WITH FIBROSIS				1 (3%)
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(10)	(32)	(30)
INFLAMMATION, CHRONIC			1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
#LUNG/BRONCHICLE	(10)	(10)	(33)	(30)
HYPERPLASIA, LYMPHOID			3 (9%)	2 (7%)
#LUNG	(10)	(10)	(33)	(30)
EDEMA, NOS				1 (3%)
INFLAMMATION, INTERSTITIAL			1 (3%)	3 (10%)
BRONCHOPNEUMONIA SUPPURATIVE			1 (3%)	3 (10%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (10%)		11 (33%)	4 (13%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(10)	(9)	(29)	(28)
ATROPHY, NOS	6 (60%)	4 (44%)	10 (34%)	11 (39%)
#SPLEEN	(10)	(10)	(33)	(30)
INFLAMMATION, 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
ATROPHY, NOS				1 (3%)
HEMATOPOIESIS				7 (23%)
#CERVICAL LYMPH NODE		(2)	(3)	(3)
HYPERPLASIA, LYMPHOID		1 (50%)		
#PANCREATIC L.NODE		(2)	(3)	(3)
INFLAMMATION, SUPPURATIVE		1 (50%)		
#MESENTERIC L. NODE		(2)	(3)	(3)
HYPERPLASIA, LYMPHOID				1 (33%)
CIRCULATORY SYSTEM				
#MYOCARDIUM	(10)	(10)	(33)	(30)
INFLAMMATICN, INTERSTITIAL				1 (3%)
#ENDOCARDIUM	(10)	(10)	(33)	(30)
INFLAMMATION, CHRONIC SUPPURATIV				1 (3%)
*CORONARY ARTERY	(10)	(10)	(33)	(30)
MINERALIZATION			1 (3%)	
INFLAMMATICN, CHRONIC				1 (3%)
*CELIAC ARTERY	(10)	(10)	(33)	(30)
NECROSIS, FIBRINOID			1 (3%)	
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(32)	(28)
INFLAMMATICN, SUPPURATIVE				1 (4%)
INFLAMMATICN, CHRONIC SUPPURATIV			1 (3%)	1 (4%)
INFLAMMATICN, CHRONIC NECROTIZIN				1 (4%)
PERIARTERITIS			1 (3%)	
DEGENERATION, NOS				1 (4%)
NECROSIS, FOCAL				1 (4%)
NECROSIS, COAGULATIVE				1 (4%)
#HEPATIC CAPSULE	(10)	(10)	(32)	(28)
HEMORRHAGE		1 (10%)	1 (3%)	1 (4%)
#LIVER/CENTRILOBULAR	(10)	(10)	(32)	(28)
NECROSIS, COAGULATIVE		1 (10%)		1 (4%)

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
#PANCREAS	(10)	(10)	(33)	(30)
INFLAMMATION, SUPPURATIVE				1 (3%)
PERIARTERITIS				2 (7%)
NECROSIS, FIBRINOID				1 (3%)
#STOMACH	(10)	(10)	(33)	(30)
CALCIFICATION, METASTATIC			1 (3%)	
#SMALL INTESTINE	(10)	(10)	(33)	(30)
PERIARTERITIS				1 (3%)
NECROSIS, FIBRINOID				1 (3%)
#JEJUNUM	(10)	(10)	(33)	(30)
HEMORRHAGE			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(10)	(10)	(33)	(30)
INFLAMMATION, CHRONIC	7 (70%)	6 (60%)	24 (73%)	17 (57%)
#URINARY BLADDER	(10)	(10)	(33)	(30)
CALCULUS, NOS				1 (3%)
ENDOCRINE SYSTEM				
#PARATHYROID	(7)	(7)	(9)	(17)
HYPERPLASIA, NOS		1 (14%)	2 (22%)	1 (6%)
REPRODUCTIVE SYSTEM				
#PROSTATE	(10)	(10)	(33)	(30)
INFLAMMATION, SUPPURATIVE	1 (10%)			1 (3%)
#TESTIS	(10)	(10)	(32)	(28)
INFLAMMATION, NECROTIZING				1 (4%)
INFLAMMATION, CHRONIC SUPPURATIV				1 (4%)
PERIARTERITIS				1 (4%)
*EPIDIDYMIS	(10)	(10)	(33)	(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				
*EYE/CORNEA	(10)	(10)	(33)	(30)
MINERALIZATION			1 (3%)	
INFLAMMATION, FIBRINOUS			1 (3%)	
*EYELID	(10)	(10)	(33)	(30)
HYPERKERATOSIS				1 (3%)
MUSCULOSKELETAL SYSTEM				
*KNEE JOINT	(10)	(10)	(33)	(30)
INFLAMMATION, CHRONIC SUPPURATIV				1 (3%)
BODY CAVITIES				
*PERITONEUM	(10)	(10)	(33)	(30)
INFLAMMATION, SUPPURATIVE		1 (10%)		
INFLAMMATION, NECROTIZING				1 (3%)
INFLAMMATION, CHRONIC				4 (13%)
*PLEURA	(10)	(10)	(33)	(30)
INFLAMMATION, CHRONIC				2 (7%)
*PERICARDIUM	(10)	(10)	(33)	(30)
INFLAMMATION, CHRONIC				1 (3%)
*EPICARDIUM	(10)	(10)	(33)	(30)
INFLAMMATION, CHRONIC				2 (7%)
*MESENTERY	(10)	(10)	(33)	(30)
MINERALIZATION	1 (10%)			
NECROSIS, FAT	1 (10%)			
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(10)	(10)	(33)	(30)
CALCIFICATION, METASTATIC			1 (3%)	
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	2		5	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
AUTOLYSIS/NO NECROPSY			3	5

\# 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 ICRF-159

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	34	35
ANIMALS NECROPSIED	10	10	33	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	33	32
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(33)	(32)
ULCER, NOS				1 (3%)
*SUBCUT TISSUE	(10)	(10)	(33)	(32)
INFLAMMATION, CHRONIC				1 (3%)
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(10)	(33)	(32)
INFLAMMATION, SUPPURATIVE			2 (6%)	
INFLAMMATION, CHRONIC			2 (6%)	
#LUNG/BRONCHUS	(10)	(10)	(33)	(32)
INFLAMMATION, SUPPURATIVE			1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
#LUNG/BRONCHIOLE	(10)	(10)	(33)	(32)
HYPERPLASIA, LYMPHOID			2 (6%)	1 (3%)
#LUNG	(10)	(10)	(33)	(32)
INFLAMMATION, INTERSTITIAL			1 (3%)	1 (3%)
BRONCHOPNEUMONIA SUPPURATIVE		1 (10%)		1 (3%)
PNEUMONIA INTERSTITIAL CHRONIC		1 (10%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (10%)	6 (18%)	3 (9%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(10)	(10)	(30)	(31)
ATROPHY, NOS	5 (50%)	3 (30%)	9 (30%)	4 (13%)
#SPLEEN	(10)	(10)	(33)	(32)
HEMATOPOIESIS			5 (15%)	14 (44%)

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
#MESENTERIC L. NODE				(3)
INFLAMMATION, GRANULOMATOUS				1 (33%)
#THYMUS	(10)	(10)	(33)	(32)
HYPERPLASIA, LYMPHOID			1 (3%)	
CIRCULATORY SYSTEM				
#MYOCARDIUM	(10)	(10)	(32)	(32)
INFLAMMATION, FIBRINOUS				1 (3%)
CALCIFICATION, METASTATIC			1 (3%)	
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(33)	(32)
CONGESTION, PASSIVE				1 (3%)
NECROSIS, COAGULATIVE				1 (3%)
LIPOIDOSIS			1 (3%)	1 (3%)
#LIVER/CENTRILOBULAR	(10)	(10)	(33)	(32)
NECROSIS, COAGULATIVE			1 (3%)	
CYTOLOGIC DEGENERATION				1 (3%)
#PANCREAS	(10)	(10)	(33)	(31)
INFLAMMATION, INTERSTITIAL			1 (3%)	
INFLAMMATION, CHRONIC				1 (3%)
#GASTRIC MUSCULARIS	(10)	(10)	(33)	(32)
CALCIFICATION, METASTATIC			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(10)	(10)	(33)	(32)
HYDRONEPHROSIS			1 (3%)	
INFLAMMATION, CHRONIC		2 (20%)	12 (36%)	17 (53%)
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(9)	(31)	(30)
ABSCESS, CHRONIC				1 (3%)

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
#ADRENAL	(10)	(10)	(33)	(32)
ANGIECTASIS	5 (50%)	1 (10%)	5 (15%)	8 (25%)
#PARATHYROID	(6)	(6)	(20)	(11)
HYPERPLASIA, NOS				1 (9%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(33)	(32)
CYST, NOS	5 (50%)	3 (30%)	9 (27%)	4 (13%)
#UTERUS	(10)	(10)	(33)	(32)
INFLAMMATICN, CHRONIC SUPPURATIV				1 (3%)
#UTERUS/ENDOMETRIUM	(10)	(10)	(33)	(32)
INFLAMMATICN, SUPPURATIVE	3 (30%)	1 (10%)	8 (24%)	1 (3%)
INFLAMMATICN, CHRONIC SUPPURATIV	2 (20%)		12 (36%)	10 (31%)
INFLAMMATICN, CHRONIC NECROTIZIN				1 (3%)
HYPERPLASIA, NOS				1 (3%)
HYPERPLASIA, CYSTIC			1 (3%)	
#OVARY/OVIDUCT	(10)	(10)	(33)	(32)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
#OVARY	(10)	(10)	(33)	(32)
CYST, NOS				2 (6%)
INFLAMMATICN, SUPPURATIVE				1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	8 (25%)
METAPLASIA, SQUAMOUS				1 (3%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE	(10)	(10)	(33)	(32)
HEMORRHAGE				1 (3%)
MUSCULOSKELETAL SYSTEM				
*JOINT	(10)	(10)	(33)	(32)
INFLAMMATICN, CHRONIC				1 (3%)

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
*ABDOMINAL MUSCLE	(10)	(10)	(33)	(32)
INFLAMMATICN, CHRONIC NECROTIZIN				1 (3%)
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(10)	(33)	(32)
NECROSIS, FAT				1 (3%)
METAPLASIA, OSSEOUS				1 (3%)
*PERITONEUM	(10)	(10)	(33)	(32)
INFLAMMATION, SUPPURATIVE				1 (3%)
INFLAMMATICN, CHRONIC			2 (6%)	
INFLAMMATICN, CHRONIC NECROTIZIN				1 (3%)
*PLEURA	(10)	(10)	(33)	(32)
INFLAMMATICN, CHRONIC				1 (3%)
*PERICARDIUM	(10)	(10)	(33)	(32)
INFLAMMATICN, FIBRINOUS				1 (3%)
*EPICARDIUM	(10)	(10)	(33)	(32)
INFLAMMATICN, FIBRINOUS				1 (3%)
*MESENTERY	(10)	(10)	(33)	(32)
PERIARTERITIS			1 (3%)	
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
INFLAMMATICN, CHRONIC				1
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED		2	1	
AUTOLYSIS/NO NECROPSY			1	3

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 ICRF-159

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	15	14	34	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	34	29
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(14)	(34)	(30)
INFLAMMATION, GRANULOMATOUS	1 (7%)			
*SUBCUT TISSUE	(15)	(14)	(34)	(30)
EPIDERMAL INCLUSION CYST	1 (7%)			
INFLAMMATION, CHRONIC FOCAL	1 (7%)			
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(34)	(29)
BRONCHOPNEUMONIA SUPPURATIVE			1 (3%)	
HYPERPLASIA, LYMPHOID			2 (6%)	1 (3%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(15)	(13)	(34)	(29)
INFLAMMATION, HEMORRHAGIC			1 (3%)	
HYPERPLASIA, HEMATOPOIETIC				1 (3%)
HYPERPLASIA, LYMPHOID			1 (3%)	1 (3%)
HEMATOPOIESIS	3 (20%)		2 (6%)	
#MESENTERIC L. NODE	(1)	(2)	(3)	(3)
CONGESTION, NOS	1 (100%)		2 (67%)	
INFLAMMATION, SUPPURATIVE	1 (100%)	2 (100%)		
HYPERPLASIA, LYMPHOID				1 (33%)
#AXILLARY LYMPH NODE	(1)	(2)	(3)	(3)
HYPERPLASIA, LYMPHOID				1 (33%)
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)	(33)	(29)
CYST, NOS			1 (3%)	
NECROSIS, COAGULATIVE		1 (8%)	1 (3%)	
HYPERPLASIA, NODULAR			7 (21%)	1 (3%)
#COLON	(15)	(13)	(33)	(29)
INFLAMMATION, NECROTIZING			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(15)	(13)	(34)	(29)
HYDRONEPHROSIS			2 (6%)	
PYELONEPHRITIS SUPPURATIVE			1 (3%)	
INFLAMMATION, CHRONIC		1 (8%)	1 (3%)	
HYPOPLASIA, NOS				1 (3%)
HYPERPLASIA, LYMPHOID			1 (3%)	1 (3%)
#URINARY BLADDER	(15)	(9)	(34)	(29)
CALCULUS, NOS				1 (3%)
INFLAMMATION, CHRONIC			1 (3%)	
ENDOCRINE SYSTEM				
#THYROID	(11)	(11)	(32)	(22)
HYPERPLASIA, FOLLICULAR-CELL			2 (6%)	
REPRODUCTIVE SYSTEM				
#PROSTATE	(15)	(13)	(33)	(29)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
#TESTIS	(15)	(13)	(32)	(29)
INFLAMMATION, SUPPURATIVE		1 (8%)		
#TUNICA ALBUGINEA	(15)	(13)	(32)	(29)
MINERALIZATION			3 (9%)	
NERVOUS SYSTEM				
#CEREBRUM	(14)	(13)	(33)	(28)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	

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
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(14)	(34)	(30)
HEMORRHAGE				1 (3%)
INFLAMMATION, SUPPURATIVE		1 (7%)		
*PELVIS	(15)	(14)	(34)	(30)
CYST, NOS			1 (3%)	
METAPLASIA, OSSEOUS			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	8	7	8	18
ACCIDENTAL DEATH				1
NECROPSY PERF/NO HISTO PERFORMED				1
AUTO/NECROPSY/HISTO PERF		1		
AUTO/NECROPSY/NO HISTO		1		
AUTOLYSIS/NO NECROPSY		1	1	4

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 ICRF-159

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS MISSING				1
ANIMALS NECROPSIED	14	15	31	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	31	34
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(15)	(31)	(34)
ULCER, CHRONIC		1 (7%)		
RESPIRATORY SYSTEM				
#LUNG/BRONCHIOLE	(13)	(15)	(31)	(34)
PLASMA-CELL INFILTRATE				1 (3%)
#LUNG	(13)	(15)	(31)	(34)
INFLAMMATION, INTERSTITIAL	1 (8%)	1 (7%)		1 (3%)
PNEUMONIA INTERSTITIAL CHRONIC			1 (3%)	
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (7%)		1 (3%)
HYPERPLASIA, PLASMA CELL		1 (7%)		
HYPERPLASIA, LYMPHOID				1 (3%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(13)	(15)	(29)	(33)
ATROPHY, NOS				1 (3%)
HYPERPLASIA, HEMATOPOIETIC				1 (3%)
#SPLEEN	(13)	(15)	(31)	(34)
INFLAMMATION, SUPPURATIVE		1 (7%)		
ATROPHY, NOS				1 (3%)
HYPERPLASIA, HEMATOPOIETIC				1 (3%)
HYPERPLASIA, LYMPHOID				2 (6%)
HEMATOPOIESIS	3 (23%)		3 (10%)	3 (9%)
#MESENTERIC L. NODE	(1)		(4)	(9)
LYMPHANGIECTASIS	1 (100%)			
HEMORRHAGE	1 (100%)			

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
INFLAMMATION, SUPPURATIVE			1 (25%)	
INFLAMMATION, ACUTE SUPPURATIVE				1 (11%)
HYPERPLASIA, LYMPHOID			1 (25%)	3 (33%)
#THYMUS	(12)	(15)	(31)	(34)
HYPERPLASIA, HEMATOPOIETIC				1 (3%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(13)	(15)	(31)	(34)
MINERALIZATION				1 (3%)
THROMBOSIS, NOS		1 (7%)		
CONGESTION, NOS	1 (8%)			
NECROSIS, COAGULATIVE				1 (3%)
HYPERPLASIA, NODULAR		1 (7%)		
ANGIECTASIS		1 (7%)		
#HEPATIC CAPSULE	(13)	(15)	(31)	(34)
FIBROSIS, FOCAL				1 (3%)
#PANCREAS	(13)	(15)	(31)	(34)
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)	
INFLAMMATION, PYOGRANULOMATOUS		1 (7%)		
#COLON	(13)	(15)	(31)	(33)
NEMATODIASIS		1 (7%)		
URINARY SYSTEM				
#KIDNEY	(13)	(15)	(31)	(34)
HYPERPLASIA, LYMPHOID				1 (3%)
#URINARY BLADDER	(13)	(15)	(31)	(34)
MINERALIZATION		1 (7%)		
ENDOCRINE SYSTEM				
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
REPRODUCTIVE SYSTEM				
#UTERUS/ENDOMETRIUM	(13)	(15)	(31)	(34)
INFLAMMATION, SUPPURATIVE			5 (16%)	3 (9%)
INFLAMMATION, NECROTIZING			1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV		3 (20%)		
HYPERPLASIA, CYSTIC	7 (54%)	11 (73%)	19 (61%)	18 (53%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(14)	(15)	(31)	(34)
INFLAMMATION, CHRONIC	1 (7%)			
INFLAMMATION, CHRONIC FOCAL				1 (3%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (3%)	
INFLAMMATION, PYOGRANULOMATOUS		1 (7%)		
*PLEURA	(14)	(15)	(31)	(34)
INFLAMMATION, FOCAL GRANULOMATOU			1 (3%)	
CYTOMEGALY			1 (3%)	
*MESENTERY	(14)	(15)	(31)	(34)
NECROSIS, FOCAL	1 (7%)			
ALL OTHER SYSTEMS				
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
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	3		3	8
ANIMAL MISSING/NO NECROPSY				1
NO NECROPSY PERFORMED			1	
AUTOLYSIS/NO NECROPSY	1		3	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of ICRF-159[a]

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Integumentary System: Fibroma[b]	1/40 (3)	0/10 (0)	2/33 (6)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			2.424	2.667
Lower Limit			0.132	0.145
Upper Limit			138.563	151.896
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.099	0.109
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	86	--	78	57
Hematopoietic System: Leukemia[b]	0/40 (0)	0/10 (0)	2/33 (6)	0/30 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.038			
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.361	--
Upper Limit			Infinite	--
Relative Risk (Vehicle Control)[f]			Infinite	--
Lower Limit			0.099	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	--	--	44	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of ICRF-159[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	3/37 (8)	0/8 (0)	3/30 (10)	1/22 (5)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.233	0.561
Lower Limit			0.177	0.011
Upper Limit			8.542	6.399
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.185	0.022
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	86	--	78	75

[a]Dosed groups received 48 or 96 mg/kg.

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

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

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of ICRF-159[a]

(continued)

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

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

[f]The 95% confidence interval of the relative risk between each dosed group and the specified control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of ICRF-159[a]

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Integumentary System:				
Squamous-cell Carcinoma[b]	0/38 (0)	0/10 (0)	0/33 (0)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			--	Infinite
Lower Limit			--	0.355
Upper Limit			--	Infinite
Relative Risk (Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.102
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	81
Pituitary: Chromophobe				
Adenoma[b]	9/37 (24)	1/9 (11)	7/31 (23)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.928	0.274
Lower Limit			0.331	0.031
Upper Limit			2.454	1.194
Relative Risk (Vehicle Control)[f]			2.032	0.600
Lower Limit			0.336	0.038
Upper Limit			88.007	34.226
Weeks to First Observed Tumor	84	85	84	73

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of ICRF-159[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Mammary Gland: Adenocarcinoma or Cystadenocarcinoma, NOS[b]	2/38 (5)	1/10 (10)	2/33 (6)	4/32 (13)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.152	2.375
Lower Limit			0.088	0.365
Upper Limit			15.075	24.769
Relative Risk (Vehicle Control)[f]			0.606	1.250
Lower Limit			0.037	0.152
Upper Limit			34.683	59.496
Weeks to First Observed Tumor	85	85	59	44
Mammary Gland: Fibroadenoma[b]	12/38 (32)	2/10 (20)	5/33 (15)	4/32 (13)
P Values[c,d]	P = 0.031(N)	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.480	0.396
Lower Limit			0.148	0.103
Upper Limit			1.292	1.158
Relative Risk (Vehicle Control)[f]			0.758	0.625
Lower Limit			0.160	0.114
Upper Limit			7.306	6.349
Weeks to First Observed Tumor	79	85	83	76

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of ICRF-159[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Uterus: Adenocarcinoma, NOS[b]	0/38 (0)	0/10 (0)	10/33 (30)	11/32 (34)
P Values[c,d]	P < 0.001	N.S.	P = 0.048* P < 0.001**	P = 0.030* P < 0.001**
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			3.477	4.008
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)f			Infinite	Infinite
Lower Limit			1.012	1.166
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	74	73
Uterus: Endometrial Stromal Polyp[b]	2/38 (5)	1/10 (10)	2/33 (6)	0/32 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)			1.152	0.000
Lower Limit			0.088	0.000
Upper Limit			15.075	3.957
Relative Risk (Vehicle Control)[f]			0.606	0.000
Lower Limit			0.037	0.000
Upper Limit			34.683	5.791
Weeks to First Observed Tumor	79	85	84	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of ICRF-159[a]

(continued)

[a]Dosed groups received 48 or 96 mg/kg.

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

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

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

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

[f]The 95% confidence interval of the relative risk between each dosed group and the specified control group.

Table E3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of ICRF-159[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Integumentary System: Fibroma (52)[b]	0/9 (0)	2/22 (9)	2/7 (29)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.136	0.434
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	78	57
Hematopoietic System: Leukemia (44)[b]	0/9 (0)	2/22 (9)	0/7 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		Infinite	--
Lower Limit		0.136	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	44	--

Table E3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Given Intraperitoneal Injections of ICRF-159[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma (52)[b]	0/8 (0)	3/21 (14)	1/6 (17)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.265	0.079
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	78	75

[a]Dosed groups received 48 or 96 mg/kg.

[b]Number of tumor-bearing animals/number of animals examined at site (percent), based on the number of animals that lived at least as long as the number of weeks shown in parenthesis after the description of morphology.

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

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF ICRF-159

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Given Intraperitoneal Injections of ICRF-159[a]

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Lymphocytic Leukemia[b]	1/42 (2)	0/14 (0)	2/34 (6)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			2.471	2.800
Lower Limit			0.134	0.152
Upper Limit			141.342	159.477
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.130	0.147
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	85	--	69	64
Liver: Hepatocellular Adenoma or Carcinoma[b]	3/42 (7)	2/13 (15)	0/33 (0)	0/29 (0)
P Values[c,d]	N.S.	P = 0.028(N)	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.045		
Relative Risk (Pooled Control)[f]			0.000	0.000
Lower Limit			0.000	0.000
Upper Limit			2.086	2.362
Relative Risk (Vehicle Control)[f]			0.000	0.000
Lower Limit			0.000	0.000
Upper Limit			1.303	1.475
Weeks to First Observed Tumor	86	86	--	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Given Intraperitoneal Injections of ICRF-159[a]

(continued)

[a]Dosed groups received 40 or 80 mg/kg.

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

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

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

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

[f]The 95% confidence interval of the relative risk between each dosed group and the specified control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of ICRF-159[a]

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System:				
Lymphoma, Histiocytic Type[b]	0/45 (0)	0/15 (0)	1/31 (3)	5/34 (15)
P Values[c,d]	P = 0.006	P = 0.041	N.S.	P = 0.012**
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.078	1.680
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.027	0.594
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	86	74
Hematopoietic System:				
Lymphocytic Leukemia or				
Lymphoma, Lymphocytic Type[b]	1/45 (2)	0/15 (0)	4/31 (13)	4/34 (12)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			5.806	5.294
Lower Limit			0.609	0.554
Upper Limit			275.610	252.309
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.479	0.436
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	86	--	27	64

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of ICRF-159[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System:				
All Neoplasms[b]	1/45 (2)	0/15 (0)	5/31 (16)	9/34 (26)
P Values[c,d]	P = 0.002	P = 0.021	P = 0.038**	P = 0.026* P = 0.002**
Relative Risk (Pooled Control)[f]			7.258	11.912
Lower Limit			0.866	1.778
Upper Limit			330.475	501.635
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.653	1.243
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	86	--	27	64
Mesentery: Lipoma[b]	0/45 (0)	0/15 (0)	0/31 (0)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			--	Infinite
Lower Limit			--	0.393
Upper Limit			--	Infinite
Relative Risk (Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.138
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	86

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of ICRF-159[a]

(continued)

[a]Dosed groups received 40 or 80 mg/kg.

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

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

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

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

[f]The 95% confidence interval of the relative risk between each dosed group and the specified control group.

Review of the Bioassay of ICRF-159* 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 ICRF-159 for carcinogenicity.

The primary reviewer agreed that the compound induced uterine adenocarcinomas in female rats and lymphomas in female mice. Although the intraperitoneal route of exposure was a limitation of the study, he said that the bioassay was adequate enough to support the conclusion regarding the carcinogenicity of ICRF-159. The primary reviewer pointed out a number of other tumors observed among the treated animals.

The secondary reviewer noted that the tumor incidence at some organ sites in treated animals was lower than in controls. He attributed this to the shorter lifespan of the treated animals. He added, however, that the life-shortening effect did not interfer with the conclusion regarding the carcinogenicity of ICRF-159.

It was moved that the report on the bioassay of ICRF-159 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
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Michael Shimkin, University of California at San Diego

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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.

☆ U. S. GOVERNMENT PRINTING OFFICE : 1978—260-899/3154

DHEW Publication No. (NIH) 78-1328