National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 57
1978

BIOASSAY OF
□-TGdR
FOR POSSIBLE CARCINOGENICITY

CAS No. 789-61-7

NCI-CG-TR-57

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

BETA-2′-DEOXY-6-THIOGUANOSINE MONOHYDRATE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 78-1363

BIOASSAY OF
BETA-2′-DEOXY-6-THIOGUANOSINE MONOHYDRATE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

FOREWORD: This report presents the results of the bioassay of beta-2′-deoxy-6-thioguanosine monohydrate 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 environmental 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: The bioassay of beta-2′-deoxy-6-thioguanosine monohydrate 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 care of the animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and R. B. Thompson[1], and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled at 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 obtained through Mr. C. Hewitt[7] and were analyzed by Drs. R. H. Iwamoto[8] and W. J. Haggerty[9]; the results of the analyses were reviewed by Dr. S. S. Olin[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 R. W. Fogleman, 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 and Ms. P. J. Graboske.

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:

Dr. Kenneth C. Chu
Dr. Cipriano Cueto, Jr.
Dr. J. Fielding Douglas
Dr. Dawn G. Goodman
Dr. Richard A. Griesemer
Dr. Harry A. Milman
Dr. Thomas W. Orme
Dr. Robert A. Squire[10]
Dr. Jerrold M. Ward

---

[1]Southern Research Institute, 2000 Ninth Avenue South, Birmingham, Alabama.

[2]Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[3]Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammond House Road, Valhalla, New York.

[4]EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

[5]Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

[6]Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[7]Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[8]Stanford Research Institute, Menlo Park, California.

[9]Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri.

[10]Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of beta-2′-deoxy-6-thioguanosine monohydrate (β-TGdR) 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 β-TGdR in a buffered saline and polysorbate 80 vehicle at one of two doses, either 3.5 or 7 mg/kg body weight, three times per week for 52 weeks, then observed for an additional 26 weeks. Controls consisted of groups of 10 rats of each sex, which were either administered the vehicle alone (matched vehicle controls) or were untreated (matched untreated controls). Pooled controls consisted of the matched vehicle controls of each sex from the current bioassay, combined with 20 corresponding vehicle controls of each sex from similar bioassays of two other test chemicals. All surviving rats were killed at 78 or 79 weeks.

Groups of 35 mice of each sex were administered the chemical in a buffered saline and polysorbate 80 vehicle at one of two doses, either 2 or 4 mg/kg, three times per week for 52 weeks, then observed for periods of up to 27 weeks, depending on length of survival. Because of severe toxicity at the high dose, resulting in loss of all mice by week 12 (males) or week 25 (females), additional groups of 35 mice of each sex were administered 1 mg/kg on the same schedule. Controls consisted of groups of 15 mice of each sex, which were either administered the vehicle or were untreated. Pooled controls consisted of groups of 15 vehicle-control animals of each sex from studies using the doses of 2 or 4 mg/kg, combined with corresponding groups of 15 vehicle-control animals of each sex from the study using the dose of 1 mg/kg.

β-TGdR was toxic to rats at the doses used in this study. Mean body weights of the high- and low-dose rats of both sexes were lower than those of the corresponding vehicle controls throughout the study. There was also severe early mortality in the high-dose groups of both sexes and positive dose-related trends in mortality over the period of the bioassay. However, 66% of the

low-dose males and 77% of the low-dose females survived until termination of the study.

In mice, β-TGdR was toxic at the doses originally selected. Mean body weights were not consistently affected; however, at the high dose only three males and seven females lived past week 7, and all were dead by week 25. In the mid-dose group, only 14% of the males and 6% of the females survived until termination of the study at week 79; in the low-dose group, the survival rate was 31% for the males and 29% for the females.

Because of the high mortality, time-adjusted statistical analyses were performed for both rats and mice.

In rats, the incidence of carcinomas of the ear canal (combined carcinomas and squamous-cell carcinomas) was statistically significant in both sexes. In males, the results of the test for dose-related trend were significant using either matched vehicle ($P = 0.046$) or pooled vehicle ($P = 0.014$) controls, but direct comparisons of dosed male rats with matched vehicle or pooled vehicle controls did not show significant differences (matched vehicle controls 0/10, pooled vehicle controls 0/28, low-dose 1/31, high-dose 2/7). In females, the results of the test for dose-related trend were significant using either matched vehicle ($P = 0.002$) or pooled vehicle ($P < 0.001$) controls, and the incidence in the high-dose group was significantly higher than that in either the matched vehicle ($P = 0.023$) or pooled vehicle ($P < 0.001$) controls (matched vehicle controls 0/9, pooled vehicle controls 0/28, low-dose 2/32, high-dose 6/13). There were no such ear canal tumors among 165 historical vehicle controls of either sex or among 220 female untreated controls at the laboratory, and only two such tumors occurred among 215 male untreated controls.

In mice, no tumors appeared in statistically significant incidences in the dosed groups compared with the matched vehicle controls, and there was no significant evidence of dose-related trend for any tumors. The incidences of the combination of lymphoma and leukemia were significantly higher in the matched

vehicle controls of each sex than in the corresponding matched untreated controls (males: matched untreated controls 1/30, matched vehicle controls 19/29; females: matched untreated controls 2/30, matched vehicle controls 21/29). This high incidence in the matched vehicle controls may have been due to a systematic procedural problem associated with injection of the drug.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and vehicle-control groups of mice, as well as the possible procedural problem that may have affected the incidences of tumors in these groups, does not allow a determination to be made of the carcinogenic potential of β-TGdR in this species. β-TGdR in the vehicle of 0.05% polysorbate 80 was, however, carcinogenic in rats, producing carcinomas of the ear canal in the females and possibly also in the males.

TABLE OF CONTENTS

APPENDIXES

TABLES

Page

FIGURES

I. INTRODUCTION

Beta-2'-deoxy-6-thioguanosine monohydrate (β-TGdR) (CAS 789-61-7; NCI C01581) is an experimental anticancer drug and a derivative of the anticancer drug 6-thioguanine (6-TG). Both drugs are sulphur analogs of the purine base guanine, a similarity that allows them to be incorporated into DNA. In tests using extracts of murine and human tissues, β-TGdR was phosphorylated to nucleotide mono-, di-, and triphosphates; in further tests using extracts of human tissues, the nucleotide triphosphate that was formed was found to be incorporated into DNA (Peery and Lepage, 1969). Because of the carcinostatic effects of β-TGdR in certain mouse tumor systems that were resistant to 6-TG (Lepage et al., 1964), it was hoped that this compound could be used to bypass the resistance to 6-TG that develops in man. At this time, however, there are not sufficient data from clinical trials to determine whether β-TGdR has any real advantage over 6-TG (Loo et al., 1975). β-TGdR was selected for testing in the carcinogenesis bioassay program as part of an attempt to evaluate the carcinogenicity of drugs that may be used in humans for prolonged periods.

II. MATERIALS AND METHODS

A. Chemical

The β-TGdR used in this study was obtained through the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (NCI). Lot No. RN C19052F was manufactured by Riker Laboratories, Northbridge, California, and Lot No. AJ-31, by Aerojet General, Sacramento, California. Both lots were used during the chronic study.

The identity and purity of Lot No. RN C19052F were confirmed at Stanford Research Institute by melting point, elemental analyses (C, H, N) for $C_{10}H_{13}N_5O_3S \cdot H_2O$, and infrared and ultraviolet spectra. Karl-Fischer analysis for water (6.0%) was correct for one molecule of water of hydration. Paper chromatography showed no detectable thioguanine or 2-deoxyribose.

The identity and purity of Lot No. AJ-31 were confirmed at Midwest Research Institute by melting point (differential thermal analysis); elemental analyses (C, H, N, O, S); and infrared, ultraviolet, and nuclear magnetic resonance spectra. Karl-Fischer analysis for water (6.7 $\pm$ 0.4%) was slightly high (6.0% theoretical). The absence of thioguanine, alpha anomer, and 2-deoxyribose was confirmed by thin-layer and paper chromatography.

In conclusion, no impurities were found in either lot. Both lots appeared to be > 98% pure β-2′-deoxy-6-thioguanosine monohydrate.

The different lots of bulk chemical were stored in their original containers at 5°C.

B. Dosage Preparation

Test solutions of β-TGdR were prepared by adding the drug to a vehicle of 0.05% polysorbate 80 in saline (for prechronic studies) or buffered saline (for chronic studies) and mixing the final suspension in a Potter-Elvehjem tissue grinder with a Teflon pestle for 20 seconds. The concentrations of β-TGdR that were prepared were 0.01, 0.02, 0.04, 0.14, and 0.28%. The solutions were stored in glass bottles at 5°C for up to 1 week.

C. Animals

Female Sprague-Dawley rats and male Swiss mice were used in the subchronic studies. Sprague-Dawley rats and B6C3F1 mice of both sexes were used in the chronic studies. The Sprague-Dawley rats and B6C3F1 mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, through contracts of the Division of Cancer Treatment, NCI. The Swiss mice were obtained from Charles River Laboratories and from Purina Laboratories, Staatsburg, New York. Rats used in the chronic

studies were 30 days of age when received at the laboratory, and mice were 41 days of age. Groups of mice started later in the study were 32 days of age when received. On arrival at the laboratory, all animals were quarantined (rats for 8 days, mice in original study for 7 days, mice in later studies for 17 days). Following this period, animals with no clinical signs of disease were assigned to control or dosed 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%. The room 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 light for 9 hours per day. Wayne® Lab Blox animal feed (Allied Mills, Inc., Chicago, Ill.) and water were supplied daily and were available ad libitum.

Rats and mice were housed five per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). Rat cages were provided with Iso-Dri® hardwood chip bedding (Carworth, Edison, N.J.), and the cage tops were covered with

disposable filter bonnets throughout the study. Mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.), and the cage tops were covered with disposable filter bonnets for mid- and high-dose mice beginning at week 63 and for low-dose mice beginning at week 51. Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; 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 β-TGdR were maintained in the same rooms as animals of the same species administered 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)
5-azacytidine (CAS 320-67-2)
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)
(±)-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 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)
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)
(±)-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 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)
adriamycin (CAS 23214-92-8)

E. Subchronic Studies

In the subchronic studies, β-TGdR in 0.05% polysorbate 80 in saline was administered by intraperitoneal injection to female Sprague-Dawley rats and male Swiss mice to estimate the maximum tolerated doses (MTD) of β-TGdR, on the basis of which "high" (estimated MTD) and "low" (1/2 estimated MTD) doses were determined for administration in the chronic studies. Rats received doses of 3, 7, 15, 30, or 60 mg/kg body weight. Doses of 5, 12, 25, 50, or 100 mg/kg were given to mice initially, and doses of 0.38, 0.75, 1.5, or 3 mg/kg were given in a second study. The animals were injected three times per week for 45 days, then observed for an additional 45 days. Five animals of each species were tested at each dose, 10 animals of each species were maintained as vehicle controls, and 10 as untreated controls.

In rats, death occurred in none of the animals administered 3 or

7 mg/kg, in 3/5 administered 15 mg/kg, in 3/5 administered 30 mg/kg, and in 4/5 administered 60 mg/kg. Mean body weights of the rats at doses of 3 or 7 mg/kg were essentially unaffected; mean body weights of animals at 15, 30, or 60 mg/kg were markedly depressed at the end of the period of administration, but were comparable to those of the controls by the termination of the study. No gross abnormalities were seen in any of the animals at necropsy. The low and high doses for the chronic studies using rats were set at 3.5 and 7 mg/kg.

In mice, death occurred in all animals administered doses of 5 to 100 mg/kg and in 1/5 administered 3 mg/kg; there were no deaths at doses below 3 mg/kg. No gross abnormalities were found at necropsy. Because mean body weights of the mice at 3 mg/kg or lower were comparable to those of the controls, the high dose was set midway between the lowest dose causing excessive death and the highest dose at which no effects on body weight were observed. Thus, the low and high doses for the chronic studies using mice were set at 2 and 4 mg/kg.

F. Designs of Chronic Studies

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

Since the number of rats in the matched vehicle-control group was small, a pooled vehicle-control group also was used for

Table 1. Design of Chronic Studies of β-TGdR in Rats

Sex and Test Group	Initial No. of Animals[a]	β-TGdR Dose[b] mg/kg/day	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched Untreated-Control	10	0		79
Matched Vehicle-Control	10	0[c]	52	27
Low-Dose	35	3.5	52	26
High-Dose	35	7	52	26
Female				
Matched Untreated-Control	10	0		79
Matched Vehicle-Control	10	0[c]	52	27
Low-Dose	35	3.5	52	26
High-Dose	35	7	52	26

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

[b]The drug was administered intraperitoneally in a vehicle of phosphate-buffered saline and polysorbate 80 at a volume of 0.25 ml/100 g body weight, three times per week during the period of administration of the drug. Doses were based on individual weights. The same needle for injection was used for each group of animals within a cage.

[c]Vehicle-control groups received only phosphate-buffered saline and polysorbate 80 at a volume 0.25 ml/100 g body weight. The same bottle of vehicle solution was used for all vehicle-control animals on test at any given time.

Table 2. Design of Chronic Studies of β-TGdR in Mice

Sex and Test Group	Initial No. of Animals[a]	β-TGdR Dose[b] mg/kg/day	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Low-Dose Matched Untreated-Control	15	0		78
Low-Dose Matched Vehicle-Control	15	0[c]	52	26
Low-Dose	35	1	52	25
Mid- and High-Dose Matched Untreated-Control	15	0		79
Mid- and High-Dose Matched Vehicle-Control	15	0[c]	52	17[d]
Mid-Dose[e]	35	2	52	27
High-Dose	35	4	12[f]	
Female				
Low-Dose Matched Untreated-Control	15	0		78
Low-Dose Matched Vehicle-Control	15	0[c]	52	26
Low-Dose	35	1	52	25
Mid- and High-Dose Matched Untreated-Control	15	0		79
Mid- and High-Dose Matched Vehicle-Control	15	0[c]	52	17[d]
Mid-Dose[e]	35	2	52	27
High-Dose	35	4	25[f]	

[a]All animals were 48-49 days of age when placed on study.

Table 2. Design of Chronic Studies of β-TGdR in Mice

(continued)

[b]The drug was administered intraperitoneally in a vehicle of phosphate-buffered saline and polysorbate 80 at a volume of 1 ml/100 g body weight, three times per week during the period of administration of the drug. Doses were based on the mean weight of the animals in each cage. The same needle for injection was used for each group of animals within a cage.

[c]Matched vehicle-control groups received only phosphate-buffered saline and polysorbate 80 at a volume of 1 ml/100 g body weight. The same bottle of vehicle solution was used for all vehicle-control animals on test at any given time.

[d]Observation of animals terminated at the time indicated, due to the death of all animals.

[e]The mid-dose group was originally the low-dose group; however, because of toxicity, a new low-dose group was started after 12 weeks of the study.

[f]Administration of β-TGdR to the animals terminated at the time indicated, due to the death of all animals.

statistical comparisons. The 10 matched vehicle-control rats of each sex from the current studies on β-TGdR were combined with 10 rerun vehicle-control rats of each sex from studies on thio-TEPA and 10 rerun vehicle-control rats of each sex from studies on 3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride (IPD), to give pooled vehicle-control groups of 30 rats of each sex. The vehicle-control rats that were used in the pooled vehicle-control groups were of the same strain and from the same supplier, and they were examined by the same pathologists; further, the different vehicle-control groups of which the pooled groups were composed were placed on study at starting times differing by no more than 3 months.

For statistical tests using mice, the 15 untreated-control mice of each sex used for the low-dose groups in the current bioassay were combined with the 15 untreated-control mice of each sex used for the mid- and high-dose groups in the current bioassay, to give final groups of 30 untreated-control mice of each sex. Groups of 30 vehicle-control mice of each sex were made up similarly.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied, except for those dying prior to day 100, due, presumably, to toxicity of the

test chemical. Rats were individually weighed every 2 weeks for 62 weeks and once per month thereafter; mice were individually weighed every 2 weeks for 62 weeks in the original study, for 51 weeks in the rerun study, and once per month 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 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 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 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 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 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

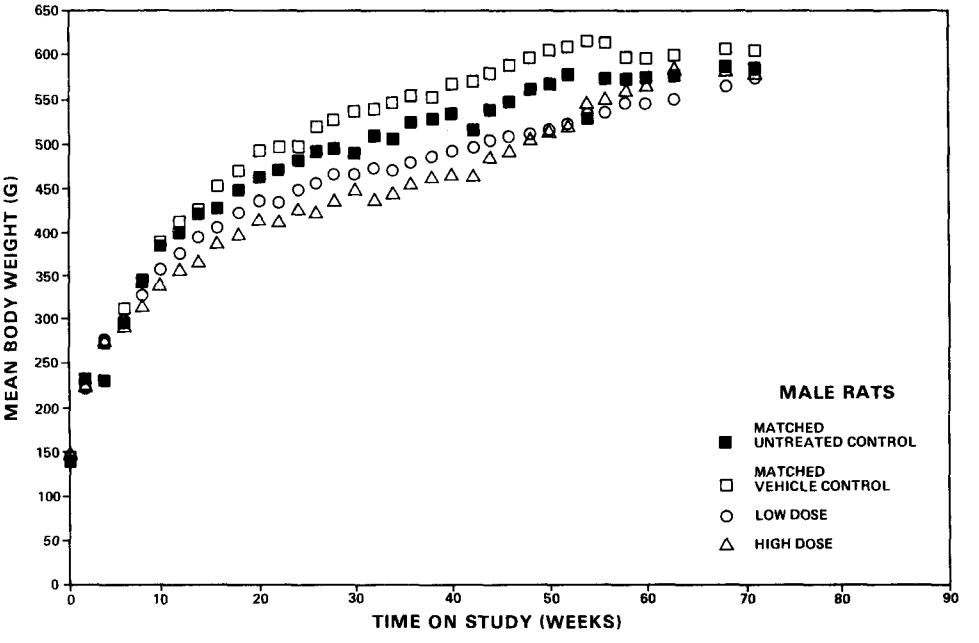
A. Body Weights and Clinical Signs (Rats)

Mean body weights for the low- and high-dose rats of each sex were lower than those for the corresponding matched vehicle controls throughout the study (figure 1). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Clinical signs, other than those of body weights, observed sporadically in a few animals included alopecia, cutaneous erythema and sores, tilting of the head, and exophthalmos.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered β-TGDR at the doses of this bioassay, together with those of the controls, are shown in figure 2.

The result of the Tarone test for positive dose-related trend in mortality is significant in either sex ($P < 0.001$), but an indicated departure from linear trend is observed ($P = 0.008$ in males, $P = 0.003$ in females), due to the steep increase in mortality in the high-dose rats. In male rats, 6/35 (17%) of the high-dose group, 23/35 (66%) of the low-dose group, 9/10 (90%) of



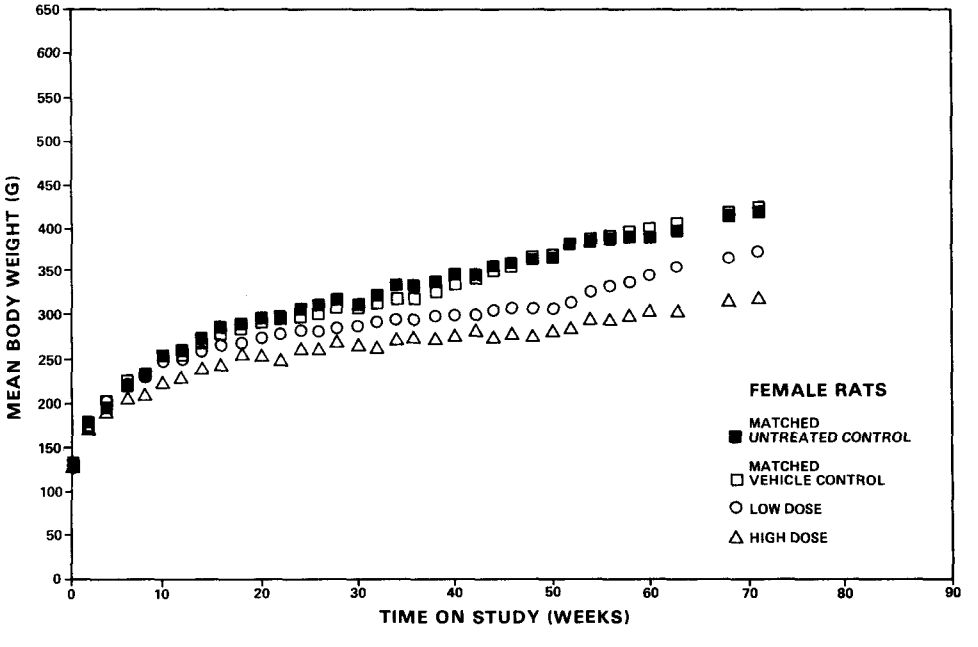


Figure 1. Growth Curves For Rats Treated With β-TGdR

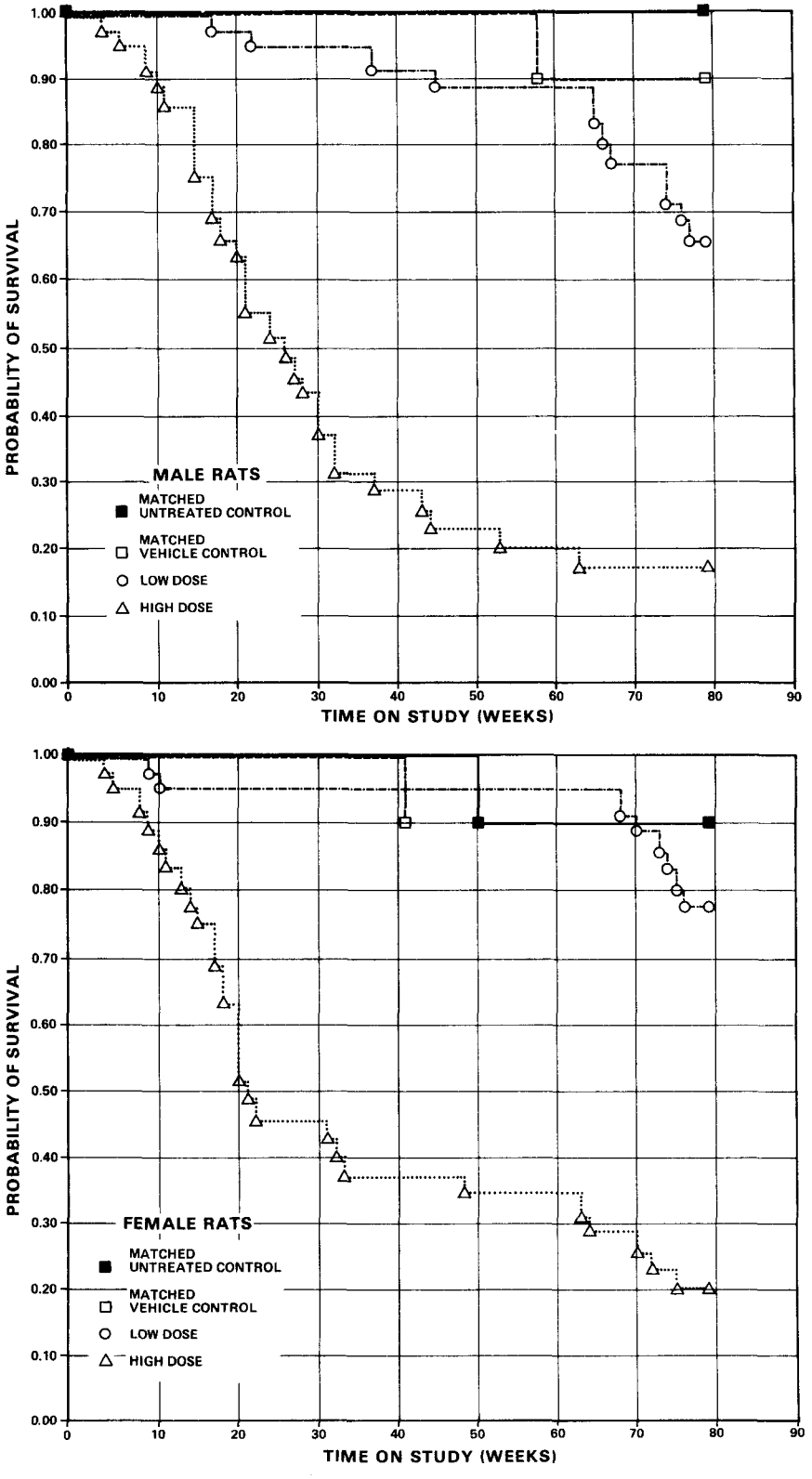


Figure 2. Survival Curves For Rats Treated With β-TGdR

the matched vehicle controls, and all 10 of the matched untreated controls lived to the end of the study. In female rats, 7/35 (20%) of the high-dose group, 27/35 (77%) of the low-dose group, 9/10 (90%) of the matched vehicle controls, and 9/10 (90%) of the matched untreated controls lived to the end of the study. In spite of the early death in the high-dose groups, a sufficient number of animals of each sex survived to exhibit a significant increase in the incidence of tumors in the dosed groups.

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 tumors occurred both in the untreated controls and the vehicle controls (phosphate-buffered saline and polysorbate 80) and in the dosed groups. Some types of neoplasms occurred only in rats of dosed groups, or with greater frequency in dosed groups when compared with controls. Most of these lesions are not uncommon in this strain of rat independent of any treatment. However, carcinomas or squamous-cell carcinomas of the ear canal occurred in 1/35 low-dose males, 2/30 high-dose males, 2/32 low-dose females, and 6/27 high-dose females. None were seen in the controls.

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 C). For the most part, these nonneoplastic lesions are commonly seen in aged rats.

The small number of tumors observed in the high-dose groups may have been influenced by the decreased life span in these animals. The lack of tumors in these animals is an indication of toxicity and not of a lack of carcinogenic activity. The shortened life spans of males and females in the high-dose groups, which were frequently associated with bone-marrow atrophy and bronchopneumonia, reduced the likelihood of detecting any possible carcinogenic activity of the test chemical in these groups.

In the judgment of the pathologists, β-TGdR in phosphate-buffered saline and polysorbate 80 induced squamous-cell carcinomas of the ear canal in female Sprague-Dawley rats.

D. Statistical Analyses of Results (Rats)

Tables E1-E4 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals and with an incidence of at least 5% in one or more than one group. Two sets of controls are used in the analyses:

the matched vehicle controls and the pooled vehicle controls. Untreated controls are not included in the tables and analyses, because the test conditions of the matched vehicle controls more closely resemble those of the dosed animals; however, the incidences in the untreated and vehicle controls are not statistically different.

In male rats, none of the incidences of tumors at any site are significant in the positive direction. The analyses of the incidences of squamous-cell carcinomas of the ear canal in female rats show a significant trend by the Cochran-Armitage test when the pooled vehicle controls are used (P = 0.010). The Fisher exact test shows that the incidence in the high-dose group is significantly greater than that in the pooled vehicle controls (P = 0.019). The statistical conclusion suggests the possibility of dose association. When the combined incidence of carcinomas and squamous-cell carcinomas of the ear canal is considered, increased significance is observed over that of squamous-cell carcinomas alone.

Due to the severe early mortality in the high-dose rats of each sex, time-adjusted analyses are performed, eliminating animals that died before week 52 on study; however, when a tumor is found at the body site of interest before week 52, comparisons are based exclusively on animals that survived at least as long as

the animal in which the first tumor was found. These time-adjusted analyses are shown in tables E3 and E4.

In male rats, there is a significant trend in the time-adjusted incidence of the combined incidence of carcinomas and squamous-cell carcinomas of the ear canal. These time-adjusted incidences become 0/28, 0/10, 1/31 (3%), and 2/7 (29%) in the pooled vehicle-control, matched vehicle-control, low-dose, and high-dose groups, respectively. The Cochran-Armitage test result is significant when either the pooled vehicle controls ($P = 0.014$) or the matched vehicle controls ($P = 0.046$) are used. The Fisher exact comparison of incidences between the high-dose and pooled vehicle-control groups shows a probability level of 0.035, which is above the 0.025 level required by the Bonferroni inequality criterion when multiple comparison is considered. The time-adjusted incidence of lymphoma in male rats shows a significant trend ($P = 0.025$) by the Cochran-Armitage test when the pooled vehicle-control group is used; however, the Fisher exact test results are not significant.

In female rats, the only significant time-adjusted incidences of tumors in the positive direction are those of the ear canal. The time-adjusted incidences of squamous-cell carcinomas of the ear canal in female rats become 0/30, 0/10, 2/31 (6%), and 5/12 (42%) in the pooled vehicle-control, matched vehicle-control, low-dose,

and high-dose groups, respectively. The results of the Cochran-Armitage test are significant ($P < 0.005$) when either control group is used, but an indicated departure from linear trend is observed ($P = 0.043$) when the pooled-control group is used, because of the steep increase in incidence in the high-dose group. The Fisher exact test shows that the incidence in the high-dose group is significantly higher ($P = 0.001$) than that in the pooled vehicle-control group. The Fisher exact comparison of incidences between the high-dose and matched vehicle-control groups indicates a probability level of 0.030, which is above the 0.025 level required by the Bonferroni inequality criterion when multiple comparison is considered. When the combined time-adjusted incidence of carcinomas and squamous-cell carcinomas of the ear canal is considered, increased significance is observed over that of the squamous-cell carcinomas alone.

The spontaneous rate of tumors compiled to date at this laboratory in the historical vehicle controls shows no incidence of tumors of the ear canal in the 165 Sprague-Dawley rats of each sex; however, 2/215 (1%) untreated male rats were observed to have such tumors. No tumors of this type were seen in the 220 untreated female controls at this laboratory.

The time-adjusted analysis of female rats indicates a significant ($P = 0.022$) negative result in the Fisher exact test when the

incidence of chromophobe adenomas of the pituitary gland in the high-dose group of female rats is compared with that in the matched vehicle controls. Except for this comparison, all of the Fisher exact test results of comparisons of the incidences of this tumor are above or equal to the 0.025 level required when the multiple comparison criterion is considered. The additional deaths which occurred in each of the dosed groups of female rats compared with the controls may account for this negative result. No other Fisher exact test result was significant in the negative direction. The analysis of this tumor in male rats indicates no significant results of the Fisher exact test when time-adjusted data are used.

Due to the early deaths in the high-dose groups, several of the results for the Cochran-Armitage test are in the direction of a negative trend, as indicated in the tables.

In the non-adjusted tables, significant results in the negative direction were obtained for the incidence of tumors of the pituitary gland in both sexes, and of fibroadenomas of the mammary gland in female rats.

In summary, the statistical conclusion suggests that the incidence of neoplasms of the ear canal in dosed rats is associated with the administration of β-TGdR.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

In spite of the toxicity of β-TGdR and the many early deaths in the high-dose groups, mean body weights were not consistently affected by administration of β-TGdR (figures 3 and 4). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Progressive weight loss occurred in a few animals. No other clinical signs that could be related to administration of the drug were recorded.

To control respiratory disease, the high- and mid-dose groups, together with corresponding controls, received oxytetracycline in the drinking water at 0.6 mg/ml for 5 days during week 42 and at 0.3 mg/ml for the following 5 days. The low-dose group, with controls, received oxytetracyline in the same pattern beginning at week 31. Also, propylene glycol was vaporized for about 2 months in the rooms housing the mice, beginning at week 42 for the high- and mid-dose groups and at week 30 for the low-dose groups.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of

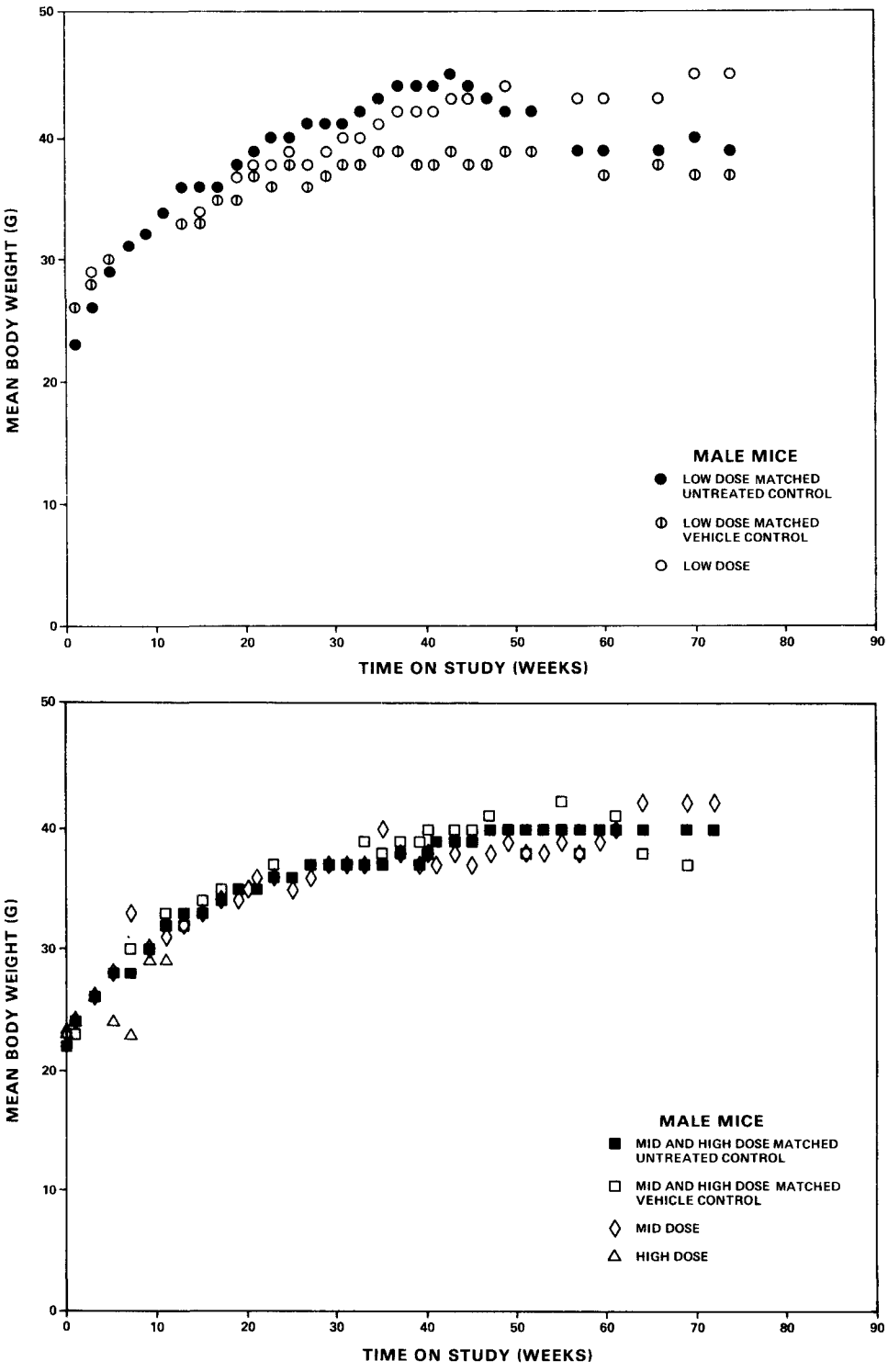


Figure 3. Growth Curves For Male Mice Treated With β-TGdR

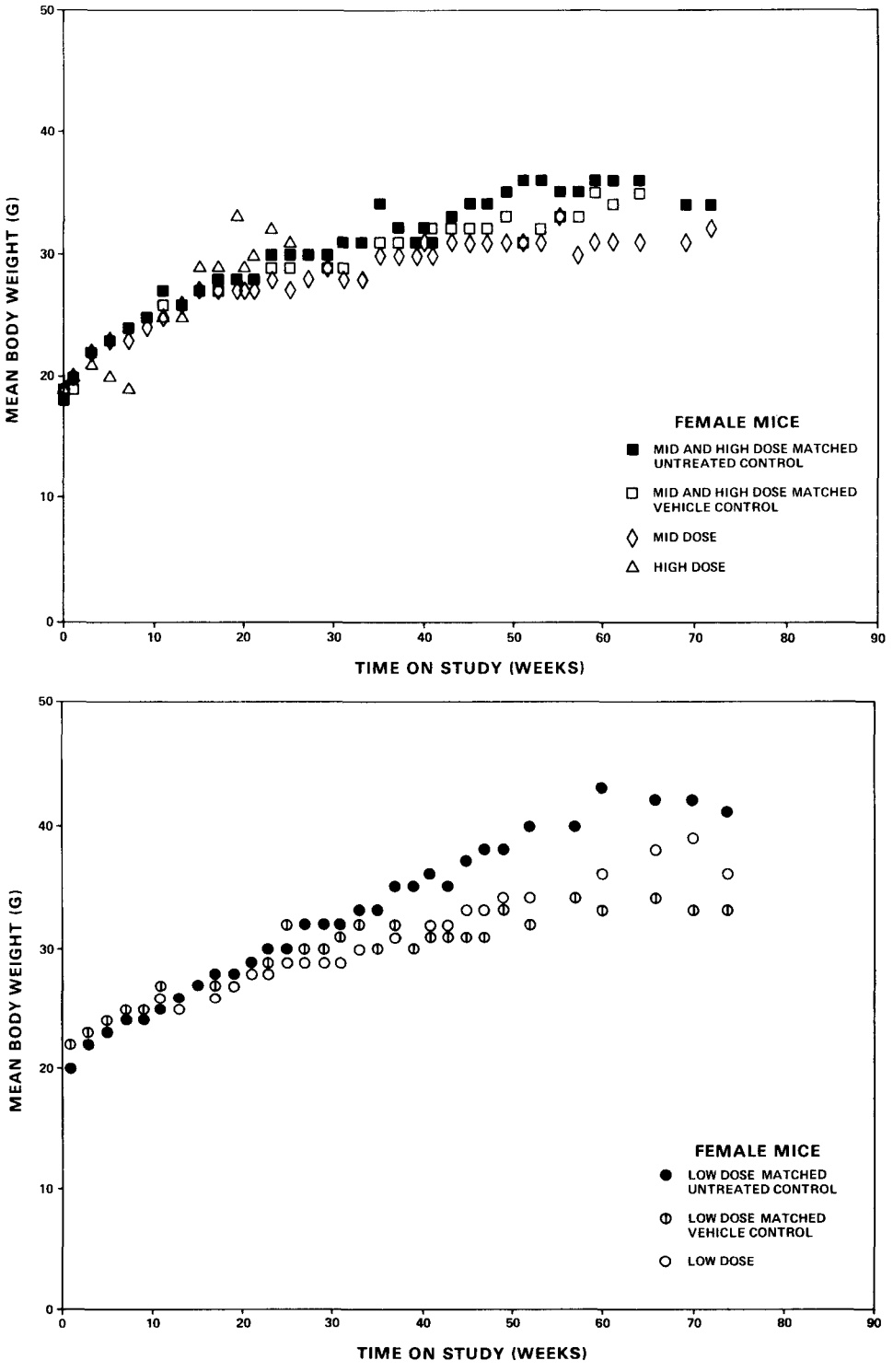


Figure 4. Growth Curves For Female Mice Treated With β-TGdR

survival for male and female mice administered β-TGdR by intraperitoneal injection at the doses of this bioassay, together with those of the controls, are shown in figures 5 and 6.

The results of the Tarone tests for positive dose-related trend in mortality are significant ($P < 0.001$) in either sex. In male mice, 0/35 (0%) of the high-dose group, 5/35 (14%) of the mid-dose group, 11/35 (31%) of the low-dose group, 4/15 (27%) of the low-dose matched vehicle-control group, 0/15 (0%) of the mid- and high-dose matched vehicle-control group, 9/15 (60%) of the low-dose matched untreated-control group, and 10/15 (67%) of the mid- and high-dose matched untreated-control group lived to the end of the study. In the females, 0/35 (0%) of the high-dose group, 2/35 (6%) of the mid-dose group, 10/35 (29%) of the low-dose group, 3/15 (20%) of the low-dose matched vehicle controls, 0/15 (0%) of the mid- and high-dose matched vehicle controls, 12/15 (80%) of the low-dose untreated controls, and 13/15 (87%) of the mid- and high-dose untreated controls survived to the end of the study. The severe early mortality in the dosed male and female mice may have prevented the observation of late-appearing tumors.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1-B4; findings on nonneoplastic lesions are summarized in Appendix D, tables D1-D4.

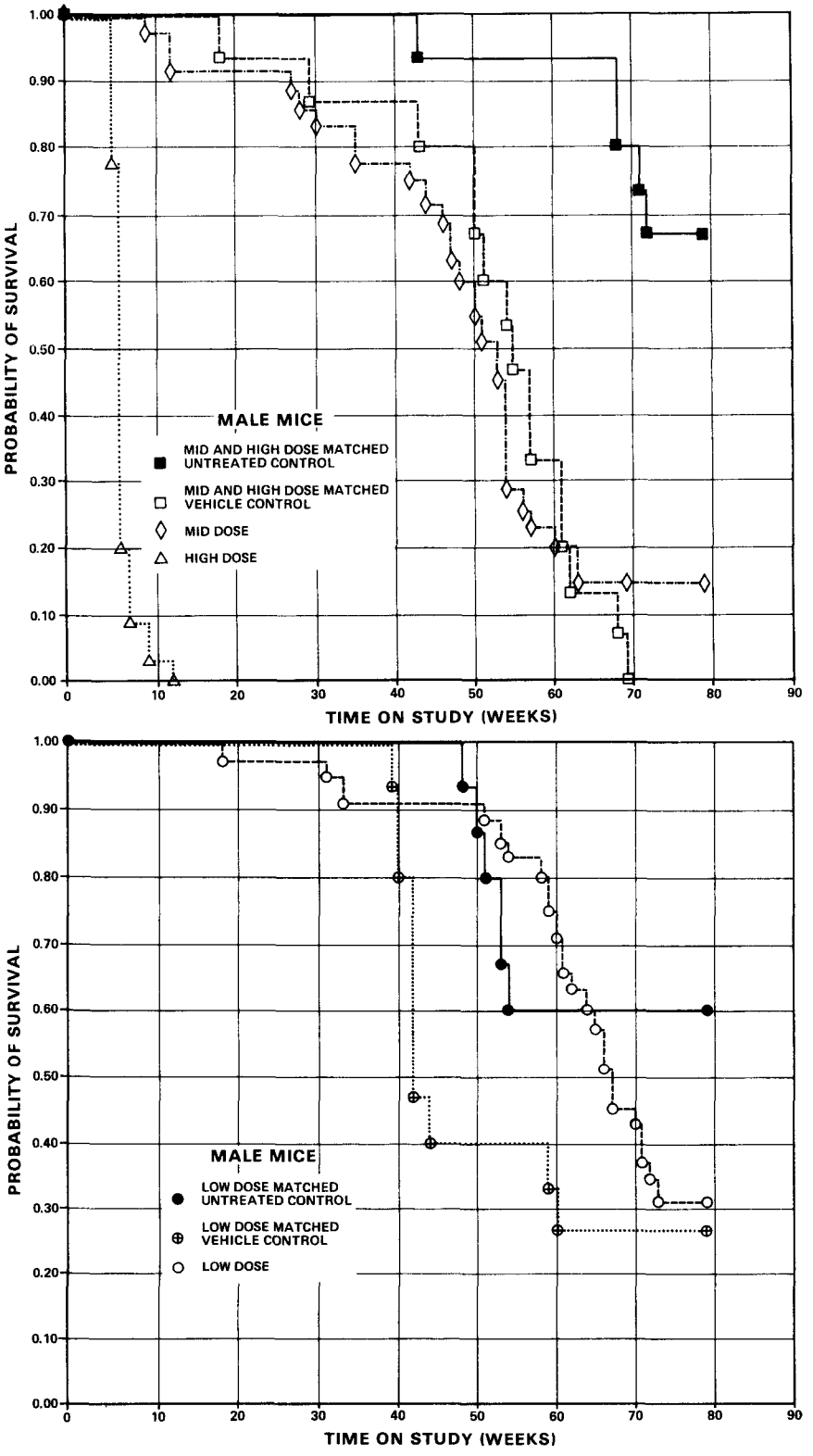


Figure 5. Survival Curves For Male Mice Treated With β-TGdR

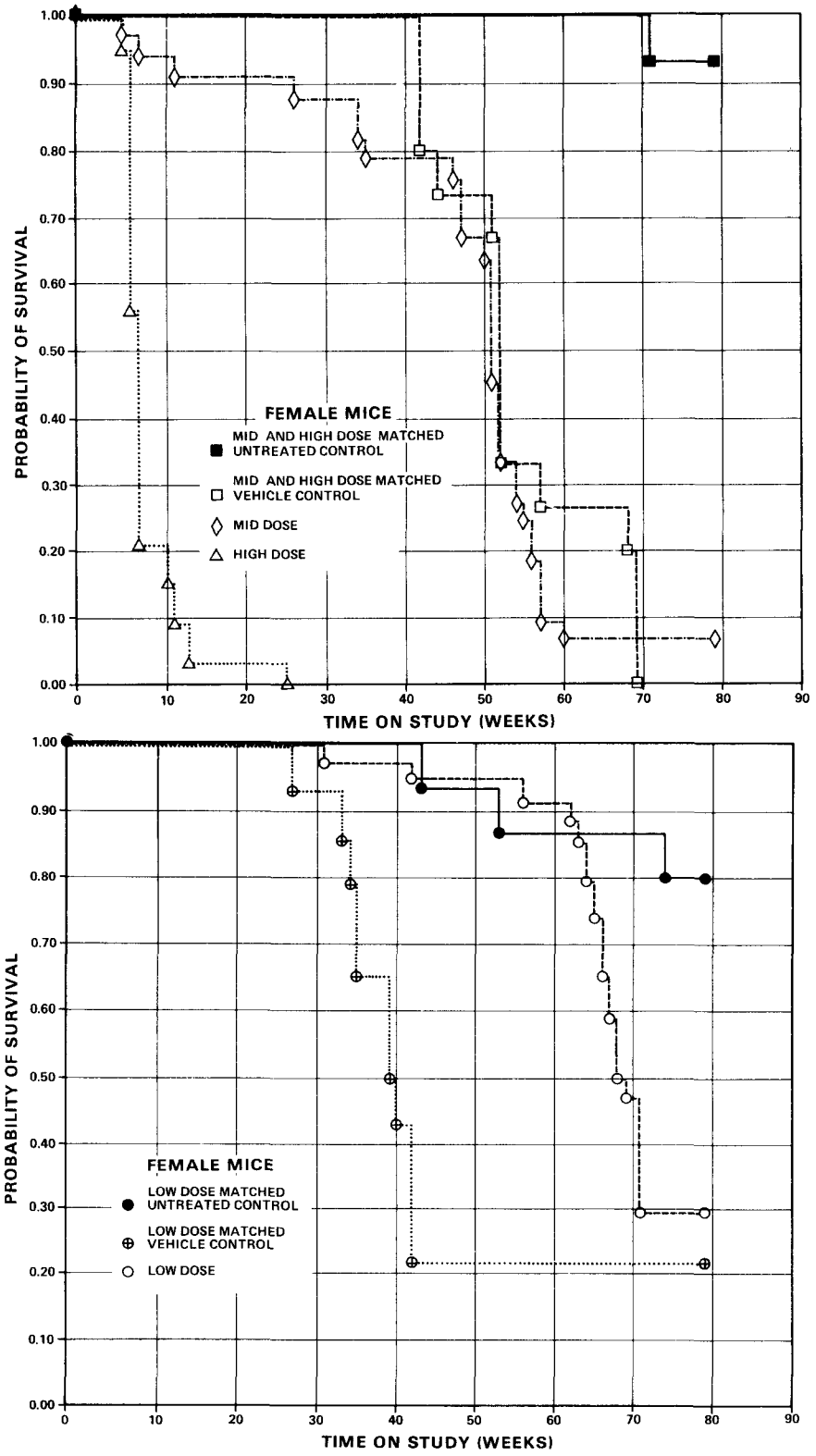


Figure 6. Survival Curves For Female Mice Treated With β-TGdR

There was a high incidence of lymphoreticular neoplasms, primarily malignant lymphomas, in the matched vehicle controls (phosphate-buffered saline and polysorbate 80) and the low- and mid-dose groups. The incidences of animals bearing these neoplasms were as follows:

	Matched Untreated Controls	Matched Vehicle Controls	Low Dose	Mid Dose
MALES				
Number of animals necropsied	30	29	35	32
Multiple organs (lymphoreticular)				
Malignant lymphoma, NOS*	0	2	1	1
Malignant lymphoma, undifferentiated type	0	0	2	6
Malignant lymphoma, lymphocytic type	1	4	7	9
Malignant lymphoma, histiocytic type	0	12	4	0
Malignant lymphoma, mixed type	0	1	3	0
Lymphocytic leukemia	0	0	0	2
Total number of animals with neoplasms	1	19	17	18
Percent of animals with neoplasms	3	66	49	56

*Not otherwise specified

	Matched Untreated Controls	Matched Vehicle Controls	Low Dose	Mid Dose
FEMALES				
Number of animals necropsied	30	29	33	29
Multiple Organs (lymphoreticular)				
Malignant lymphoma, NOS	1	4	2	2
Malignant lymphoma, undifferentiated type	0	4	1	17
Malignant lymphoma, lymphocytic type	0	0	2	2
Malignant lymphoma, histiocytic type	1	11	14	0
Lymphocytic leukemia	0	1	0	0
Total number of animals with neoplasms	2	20	19	21
Percent of animals with neoplasms	7	69	58	72

Data for the high-dose groups are not presented, since all but one of the animals in these groups died early, and they were not examined.

Lymphocytic leukemia was diagnosed in one vehicle-control female and two dosed males. This diagnosis was used because of the presence of numerous neoplastic lymphoid cells in the vascular sinusoids of the livers and spleens and peripheral blood smears. Since there was extensive lymphoid cell infiltration in many organs and tissues, these cases probably represented malignant lymphomas with accompanying leukemia.

Malignant lymphomas appeared with approximately equal frequency in the vehicle-control and dosed groups of both sexes. Thus, the neoplasms do not appear to be chemical related.

The lack of tumors in the high-dose male and female groups of mice could be related to marked decrease in life span, which is an indication of the toxicity of β-TGdR and does not necessarily indicate a lack of carcinogenic activity.

The other neoplasms listed in Appendix B appeared with approximately equal frequency in dosed and control mice, or appeared in insignificant numbers.

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

In the judgment of the pathologists, this study failed to provide conclusive evidence of carcinogenic activity of β-TGdR in B6C3F1 mice.

D. Statistical Analyses of Results (Mice)

Tables F1-F4 in Appendix F contain the statistical analyses of

the incidences of those primary tumors that occurred in at least two animals and with an incidence of at least 5% in one or more than one group. The high-dose groups are not included in the tables, since all but one of the animals in these groups died early and they were not examined. The matched 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.

The combined incidences of lymphoma and leukemia in the matched vehicle controls are significantly higher than those in the matched untreated controls in both sexes. In male mice, they are 1/30 in the matched untreated controls and 19/29 in the matched vehicle controls; in females they are 2/30 and 21/29 in the same order. These differences may indicate a difficulty in the execution of this bioassay.

Due to the severe early mortality in the dosed mice and in the matched vehicle controls, time-adjusted analyses are performed, eliminating animals that died before week 52 on study; however, when a tumor is found at the body site of interest before week 52, comparisons are based exclusively on animals that survived at least as long as the animal in which the first tumor was found. These time-adjusted analyses are shown in tables F3 and F4.

The results of the Cochran-Armitage test for positive dose-related trend in incidences and those of the Fisher exact test for direct comparison of control and dosed groups are not significant in either sex, before or after time adjustment.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the absence of significant results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by this compound, which could not be detected under the conditions of this test.

V. DISCUSSION

β-TGdR administered intraperitoneally was toxic to rats at the doses used in this study. Mean body weights of the high- and low-dose rats of both sexes were lower than those of the corresponding vehicle controls throughout the study. There was also severe early mortality in the high-dose groups of both sexes and positive dose-related trends in mortality over the period of the bioassay. However, 66% of the low-dose males and 77% of the low-dose females survived until termination of the study.

In mice, β-TGdR was toxic at the doses originally selected. Mean body weights were not consistently affected; however, at the high dose, only three males and seven females lived past week 7, and all were dead by week 25. In the mid-dose group, only 14% of the males and 6% of the females survived until termination of the study at week 79; in the low-dose group, the survival rate was 31% for the males and 29% for the females.

Because of the high mortality, time-adjusted statistical analyses were performed for both rats and mice.

In rats, the incidence of carcinomas of the ear canal (combined carcinoma and squamous-cell carcinoma) was statistically significant in both sexes. In males, the results of the test for dose-related trend were significant using either matched vehicle

(P = 0.046) or pooled vehicle (P = 0.014) controls, but direct comparisons of dosed male rats with matched vehicle or pooled vehicle controls did not show significant differences (matched vehicle controls 0/10, pooled vehicle controls 0/28, low-dose 1/31, high-dose 2/7). In females, the results of the test for dose-related trend were significant using either matched vehicle (P = 0.002) or pooled vehicle (P < 0.001) controls, and the incidence in the high-dose group was significantly higher than that in either the matched vehicle (P = 0.023) or pooled vehicle (P < 0.001) controls (matched vehicle controls 0/9, pooled vehicle controls 0/28, low-dose 2/32, high-dose 6/13). There were no such tumors of the ear canal among 165 historical vehicle controls of either sex or 220 untreated female controls at the laboratory, and only two such tumors among 215 untreated male controls.

In mice, no tumors appeared in statistically significant incidences in the dosed groups compared with the vehicle controls, and there was no significant evidence of dose-related trend for any tumors. The incidences of the combination of lymphoma and leukemia were significantly higher in the matched vehicle controls of either sex than in the corresponding untreated controls (males: matched untreated controls 1/30, matched vehicle controls 19/29; females: matched untreated

controls 2/30, matched vehicle controls 21/29). This high incidence in the matched vehicle controls may have been due to a systematic procedural problem associated with injection of the drug, which made possible the transplantation of tumor cells. The same needle for injection was used for each group of five animals within a cage; furthermore, the same bottle of vehicle was used for all vehicle-control animals.

The vehicle used for administering the β-TGdR to all groups in this bioassay contained polysorbate 80, which in itself has been implicated as a carcinogen, but only in the production of local sarcomas following subcutaneous injection (Grasso et al., 1971). However, in these bioassays no local sarcomas were observed in the vehicle-control animals administered polysorbate 80 by intraperitoneal injection.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and vehicle-control groups of mice, as well as the possible procedural problem that may have affected the incidences of tumors in these groups, does not allow a determination to be made of the carcinogenic potential of β-TGdR in this species. β-TGdR in the vehicle of 0.05% polysorbate 80 was, however, carcinogenic in rats, producing carcinomas of the ear canal in the females and possibly also in the males.

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.

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. 39:148-169, 1971.

Grasso, P., Gangolli, S. D., Golberg, L., and Hooson, J., Physicochemical and other factors determining local sarcoma production by food additives. J. Fd. Cosmet. Toxicol. 9:463-478, 1971.

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

Lepage, G. A., Junga, I. G., and Bowman, B., Biochemical and carcinostatic effects of 2′-deoxythioguanosine. Cancer Res. 24:835-840, 1964.

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.

Loo, T. L., Ho, D. H. W., Bodey, G. P., and Freireich, E. J., Pharmacological and clinical studies of some nucleoside analogs. Ann. N. Y. Acad. Sci. 255:252-260, 1975.

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

Peery, A. and Lepage, G. A., Nucleotide formation from α- and β-2′-deoxythioguanosine in extracts of murine and human tissues. Cancer Res. 29:617-623, 1969.

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.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH β-TGdR

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH β-TGdR

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	35	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	35	30
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(35)	(30)
PAPILLOMA, NOS		1 (10%)		
SQUAMOUS CELL CARCINOMA				1 (3%)
TRICHOEPITHELIOMA			1 (3%)	
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(10)	(35)	(30)
MALIG.LYMPHOMA, UNDIFFER-TYPE				2 (7%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)	
#MANDIBULAR L. NODE	(10)	(10)	(17)	(7)
SQUAMOUS CELL CARCINOMA, METASTA			1 (6%)	
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	(9)	(9)	(25)	(20)
CHROMOPHOBE ADENOMA	2 (22%)	4 (44%)	3 (12%)	
#ADRENAL	(10)	(10)	(34)	(29)
PHEOCHROMOCYTOMA		1 (10%)		
#PANCREATIC ISLETS	(10)	(10)	(33)	(27)
ISLET-CELL ADENOMA		1 (10%)	1 (3%)	
REPRODUCTIVE SYSTEM				
#TESTIS	(10)	(10)	(34)	(28)
INTERSTITIAL-CELL TUMOR			1 (3%)	
NERVOUS SYSTEM				
#BRAIN	(10)	(10)	(33)	(27)
ASTROCYTOMA	1 (10%)			1 (4%)
SPECIAL SENSE ORGANS				
*EAR CANAL	(10)	(10)	(35)	(30)
CARCINOMA,NOS				1 (3%)
SQUAMOUS CELL CARCINOMA			1 (3%)	1 (3%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PELVIS	(10)	(10)	(35)	(30)
ADENOCARCINOMA, NOS		1 (10%)		
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(10)	(10)	(35)	(30)
SQUAMOUS CELL CARCINOMA			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
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@			6	13
MORIBUND SACRIFICE		1	6	16
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	10	9	23	6
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	7	9	5
TOTAL PRIMARY TUMORS	3	8	9	6
TOTAL ANIMALS WITH BENIGN TUMORS	2	6	6	
TOTAL BENIGN TUMORS	2	7	6	
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	1	3	5
TOTAL MALIGNANT TUMORS	1	1	3	6
TOTAL ANIMALS WITH SECONDARY TUMORS#			1	
TOTAL SECONDARY TUMORS			1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH β-TGDR

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	32	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	32	27
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(32)	(27)
SARCOMA, NOS	1 (10%)			
*SUBCUT TISSUE	(10)	(10)	(32)	(27)
SARCOMA, NOS			1 (3%)	
AMELOBLASTOMA			1 (3%)	
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(10)	(32)	(27)
MALIGNANT LYMPHOMA, NOS				1 (4%)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (10%)		1 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				1 (4%)
*THYMUS	(8)	(10)	(31)	(25)
THYMOMA			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)	(10)	(31)	(23)
CHROMOPHOBE ADENOMA	7 (70%)	8 (80%)	12 (39%)	2 (9%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(32)	(27)
CARCINOMA,NOS		1 (10%)		
ADENOCARCINOMA, NOS			3 (9%)	
FIBROADENOMA	4 (40%)	2 (20%)	5 (16%)	
#UTERUS	(9)	(10)	(32)	(26)
ENDOMETRIAL STROMAL POLYP		1 (10%)	1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(10)	(10)	(32)	(27)
CARCINOMA,NOS				1 (4%)
SQUAMOUS CELL CARCINOMA			2 (6%)	5 (19%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MEDIASTINUM	(10)	(10)	(32)	(27)
SARCOMA, NOS				1 (4%)
ALL OTHER SYSTEMS				
THORACIC CAVITY				
ADENOCARCINOMA, NOS				

\# 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	10	10	35	35
NATURAL DEATH@	1		3	13
MORIBUND SACRIFICE		1	5	15
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	9	9	27	7
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	9	17	10
TOTAL PRIMARY TUMORS	12	14	26	12
TOTAL ANIMALS WITH BENIGN TUMORS	8	9	15	2
TOTAL BENIGN TUMORS	11	11	20	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	3	6	10
TOTAL MALIGNANT TUMORS	1	3	6	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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH β-TGdR

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH β-TGdR (CONTROL GROUPS)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	15	15	15	15
ANIMALS NECROPSIED	15	15	14	15
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	14	15
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(14)	(15)	(14)	(15)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (14%)	1 (7%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (7%)			
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(15)	(14)	(15)
MALIGNANT LYMPHOMA, NOS				2 (13%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)		3 (21%)	1 (7%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			6 (43%)	6 (40%)
MALIGNANT LYMPHOMA, MIXED TYPE				1 (7%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(15)	(14)	(15)
HEPATOCELLULAR ADENOMA		1 (7%)	1 (7%)	1 (7%)
HEPATOCELLULAR CARCINOMA		1 (7%)		
*BILE DUCT	(15)	(15)	(14)	(15)
BILE DUCT CARCINOMA	1 (7%)			
URINARY SYSTEM				
#URINARY BLADDER	(15)	(15)	(14)	(15)
HEMANGIOMA		1 (7%)		

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

TABLE B1. MALE MICE (CONTROL GROUPS): NEOPLASMS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ENDOCRINE SYSTEM				
NONE				
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	15	15
NATURAL DEATH@	3	1	10	4
MORIBUND SACRIFICE	2	5	5	7
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	10	9		4
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE (CONTROL GROUPS): NEOPLASMS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	3	9	11
TOTAL PRIMARY TUMORS	5	4	10	11
TOTAL ANIMALS WITH BENIGN TUMORS	2	2	1	1
TOTAL BENIGN TUMORS	2	3	1	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	1	9	10
TOTAL MALIGNANT TUMORS	3	1	9	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 B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH β-TGdR (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	35	32	0
ANIMALS EXAMINED HISTOPATHOLOGICALLY	35	32	0
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(35)	(32)	
MALIGNANT LYMPHOMA, NOS	1 (3%)	1 (3%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE	2 (6%)	6 (19%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	7 (20%)	9 (28%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	4 (11%)		
MALIGNANT LYMPHOMA, MIXED TYPE	3 (9%)		
LYMPHOCYTIC LEUKEMIA		2 (6%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(34)	(31)	
HEPATOCELLULAR ADENOMA	1 (3%)	1 (3%)	
HEMANGIOSARCOMA	1 (3%)		
URINARY SYSTEM			
NONE			

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

TABLE B2. MALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(31)	(22)	
FOLLICULAR-CELL ADENOMA		1 (5%)	
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	35	35	35
NATURAL DEATH@	5	15	35
MORIBUND SACRIFICE	19	15	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	11	5	
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. MALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	19	
TOTAL PRIMARY TUMORS	19	20	
TOTAL ANIMALS WITH BENIGN TUMORS	1	1	
TOTAL BENIGN TUMORS	1	2	
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	18	
TOTAL MALIGNANT TUMORS	18	18	
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 B3.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH β-TGdR (CONTROL GROUPS)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	15	15	15	15
ANIMALS NECROPSIED	15	15	15	14
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	15	14
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(15)	(15)	(14)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (7%)		
OSTEOSARCOMA, METASTATIC		1 (7%)		
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(15)	(15)	(14)
MALIGNANT LYMPHOMA, NOS		1 (7%)	2 (13%)	2 (14%)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (7%)	3 (21%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (7%)	7 (47%)	4 (29%)
LYMPHOCYTIC LEUKEMIA				1 (7%)
#LIVER	(15)	(15)	(15)	(14)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (7%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(15)	(15)	(14)
HEPATOCELLULAR ADENOMA	1 (7%)			
URINARY SYSTEM				
NONE				

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

TABLE B3. FEMALE MICE (CONTROL GROUPS): NEOPLASMS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*VERTEBRA	(15)	(15)	(15)	(14)
OSTEOSARCOMA		1 (7%)		
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	15	15
NATURAL DEATH@	1	1	3	6
MORIBUND SACRIFICE		2	12	5
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED	1			1
TERMINAL SACRIFICE	13	12		3
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B3. FEMALE MICE (CONTROL GROUPS): NEOPLASMS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	4	11	10
TOTAL PRIMARY TUMORS	1	4	11	10
TOTAL ANIMALS WITH BENIGN TUMORS	1	1		
TOTAL BENIGN TUMORS	1	1		
TOTAL ANIMALS WITH MALIGNANT TUMORS		3	11	10
TOTAL MALIGNANT TUMORS		3	11	10
TOTAL ANIMALS WITH SECONDARY TUMORS#		1		
TOTAL SECONDARY TUMORS		1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE B4.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH β-TGdR (CONTROL GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	33	29	1
ANIMALS EXAMINED HISTOPATHOLOGICALLY	33	29	1
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(33)	(28)	(1)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(33)	(29)	(1)
MALIGNANT LYMPHOMA, NOS	2 (6%)	2 (7%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (3%)	17 (59%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (6%)	2 (7%)	1 (100%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	14 (42%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(30)	(25)	(1)
FOLLICULAR-CELL ADENOMA	1 (3%)		

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

TABLE B4. FEMALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
#OVARY	(32)	(29)	(1)
HEMANGIOSARCOMA	1 (3%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	35	35	35
NATURAL DEATH@	9	14	33
MORIBUND SACRIFICE	15	17	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	1	2	1
TERMINAL SACRIFICE	10	2	
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B4. FEMALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	21	1
TOTAL PRIMARY TUMORS	21	22	1
TOTAL ANIMALS WITH BENIGN TUMORS	1	1	
TOTAL BENIGN TUMORS	1	1	
TOTAL ANIMALS WITH MALIGNANT TUMORS	20	21	1
TOTAL MALIGNANT TUMORS	20	21	1
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 TREATED WITH β-TGdR

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH β-TGdR

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	35	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	35	30
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(35)	(30)
INFLAMMATION, FOCAL				1 (3%)
ULCER, CHRONIC			1 (3%)	
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(10)	(34)	(29)
LYMPHOCYTIC INFLAMMATORY INFILTR			2 (6%)	2 (7%)
INFLAMMATION, SUPPURATIVE				2 (7%)
#LUNG/BRONCHUS	(10)	(10)	(34)	(27)
BRONCHIECTASIS				1 (4%)
INFLAMMATION, SUPPURATIVE				1 (4%)
HYPERPLASIA, LYMPHOID	1 (10%)		3 (9%)	3 (11%)
#LUNG	(10)	(10)	(34)	(27)
BRONCHOPNEUMONIA SUPPURATIVE	1 (10%)			2 (7%)
PNEUMONIA, CHRONIC MURINE	1 (10%)	1 (10%)	10 (29%)	4 (15%)
INFLAMMATION, CHRONIC SUPPURATIV				1 (4%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(10)	(9)	(34)	(25)
ATROPHY, NOS				8 (32%)
CIRCULATORY SYSTEM				
#MYOCARDIUM	(10)	(10)	(34)	(29)
INFLAMMATION, INTERSTITIAL			1 (3%)	1 (3%)
*PULMONARY ARTERY	(10)	(10)	(35)	(30)
HYPERTROPHY, 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
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(33)	(28)
THROMBOSIS, NOS			1 (3%)	
INFLAMMATION, PYOGRANULOMATOUS				1 (4%)
NECROSIS, COAGULATIVE			2 (6%)	1 (4%)
#HEPATIC CAPSULE	(10)	(10)	(33)	(28)
HEMORRHAGE			1 (3%)	
#LIVER/CENTRILOBULAR	(10)	(10)	(33)	(28)
NECROSIS, FOCAL				1 (4%)
#PANCREAS	(10)	(10)	(33)	(27)
INFLAMMATION, CHRONIC				1 (4%)
INFLAMMATION, CHRONIC DIFFUSE	1 (10%)			
#STOMACH	(9)	(10)	(33)	(27)
ULCER, FOCAL		1 (10%)		
#JEJUNUM	(10)	(10)	(33)	(24)
ULCER, FOCAL		1 (10%)		
URINARY SYSTEM				
#KIDNEY	(10)	(10)	(34)	(28)
INFLAMMATION, CHRONIC			1 (3%)	
INFLAMMATION, CHRONIC FOCAL			1 (3%)	
INFLAMMATION, CHRONIC DIFFUSE	4 (40%)	3 (30%)	5 (15%)	1 (4%)
ENDOCRINE SYSTEM				
#ADRENAL CORTEX	(10)	(10)	(34)	(29)
CYTOLOGIC DEGENERATION				1 (3%)
REPRODUCTIVE SYSTEM				
NONE				
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
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(10)	(10)	(35)	(30)
INFLAMMATION, CHRONIC FOCAL		1 (10%)		
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(10)	(10)	(35)	(30)
HYPERPLASIA, LYMPHOID			1 (3%)	
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1		6	7
NO NECROPSY PERFORMED				5

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH β-TGdR

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	32	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	32	27
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(10)	(10)	(32)	(27)
HYPERPLASIA, LYMPHOID			1 (3%)	2 (7%)
#LUNG	(10)	(10)	(32)	(27)
BRONCHOPNEUMONIA SUPPURATIVE				1 (4%)
PNEUMONIA, CHRONIC MURINE			1 (3%)	2 (7%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(8)	(10)	(32)	(26)
ATROPHY, NOS				7 (27%)
#SPLEEN	(10)	(10)	(32)	(27)
HEMATOPOIESIS			1 (3%)	
#MANDIBULAR L. NODE	(8)	(8)	(27)	(10)
INFLAMMATION, CHRONIC DIFFUSE				1 (10%)
HYPERPLASIA, LYMPHOID			1 (4%)	
CIRCULATORY SYSTEM				
#MYOCARDIUM	(10)	(10)	(32)	(27)
INFLAMMATION, CHRONIC				1 (4%)
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(32)	(27)
NECROSIS, COAGULATIVE				1 (4%)

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
#LIVER/CENTRILOBULAR	(10)	(10)	(32)	(27)
NECROSIS, FOCAL				1 (4%)
CYTOPLASMIC VACUOLIZATION				1 (4%)
*BILE DUCT	(10)	(10)	(32)	(27)
HYPERPLASIA, NOS			1 (3%)	
#PANCREAS	(10)	(10)	(32)	(27)
INFLAMMATION, CHRONIC			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(10)	(10)	(32)	(27)
INFLAMMATION, CHRONIC	1 (10%)	1 (10%)		
INFLAMMATION, CHRONIC DIFFUSE	1 (10%)	1 (10%)		
ENDOCRINE SYSTEM				
#ADRENAL	(10)	(10)	(32)	(26)
ANGIECTASIS	1 (10%)		4 (13%)	1 (4%)
#ADRENAL MEDULLA	(10)	(10)	(32)	(26)
HEMORRHAGE			1 (3%)	
REPRODUCTIVE SYSTEM				
#UTERUS/ENDOMETRIUM	(9)	(10)	(32)	(26)
INFLAMMATION, SUPPURATIVE				1 (4%)
#UTERUS/MYOMETRIUM	(9)	(10)	(32)	(26)
THROMBOSIS, NOS			1 (3%)	
#OVARY	(9)	(10)	(32)	(26)
INFLAMMATION, SUPPURATIVE			1 (3%)	5 (19%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
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
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MESENTERY	(10)	(10)	(32)	(27)
NECROSIS, FAT			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	2	1	10	4
NO NECROPSY PERFORMED			2	8
AUTOLYSIS/NO NECROPSY			1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN MICE TREATED WITH β-TGdR

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH β-TGdR (CONTROL GROUPS)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	15	15	15	15
ANIMALS NECROPSIED	15	15	14	15
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	14	15
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(15)	(15)	(14)	(14)
INFLAMMATION, SUPPURATIVE	5 (33%)	2 (13%)		
#LUNG/BRONCHIOLE	(14)	(15)	(14)	(15)
HYPERPLASIA, PLASMA CELL		3 (20%)		
HYPERPLASIA, LYMPHOID	1 (7%)			
#LUNG	(14)	(15)	(14)	(15)
BRONCHOPNEUMONIA SUPPURATIVE	6 (43%)	7 (47%)	1 (7%)	
HYPERPLASIA, PLASMA CELL			1 (7%)	
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
#MYOCARDIUM	(15)	(15)	(14)	(15)
INFLAMMATION, SUPPURATIVE		1 (7%)	1 (7%)	
INFLAMMATION, CHRONIC DIFFUSE	1 (7%)			
INFLAMMATION, CHRONIC SUPPURATIV			1 (7%)	
#ENDOCARDIUM	(15)	(15)	(14)	(15)
INFLAMMATION, CHRONIC			1 (7%)	
DIGESTIVE SYSTEM				
#LIVER	(15)	(15)	(14)	(15)
INFLAMMATION, SUPPURATIVE		1 (7%)		

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

TABLE D1. MALE MICE (CONTROL GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
INFLAMMATION, CHRONIC SUPPURATIV			1 (7%)	
NECROSIS, COAGULATIVE			2 (14%)	1 (7%)
HYPERPLASIA, NODULAR	2 (13%)			2 (13%)
URINARY SYSTEM				
#KIDNEY	(15)	(15)	(14)	(15)
PYELONEPHRITIS SUPPURATIVE			1 (7%)	1 (7%)
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#PROSTATE	(15)	(15)	(14)	(15)
INFLAMMATION, SUPPURATIVE			1 (7%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*MIDDLE EAR	(15)	(15)	(14)	(15)
INFLAMMATION, SUPPURATIVE	1 (7%)			
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(15)	(14)	(15)
INFLAMMATION, SUPPURATIVE				1 (7%)
ALL OTHER SYSTEMS				
NONE				

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

TABLE D1. MALE MICE (CONTROL GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	4	6	1	3
AUTO/NECROPSY/NO HISTO			1	
AUTOLYSIS/NO NECROPSY			1	

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH β-TGdR (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	35	32	0
ANIMALS EXAMINED HISTOPATHOLOGICALLY	35	32	0
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(35)	(32)	
INFLAMMATION, SUPPURATIVE	1 (3%)		
#LUNG/BRONCHIOLE	(35)	(31)	
HYPERPLASIA, PLASMA CELL	2 (6%)		
#LUNG	(35)	(31)	
BRONCHOPNEUMONIA SUPPURATIVE	8 (23%)		
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(34)	(31)	
INFLAMMATION, NECROTIZING		1 (3%)	
INFLAMMATION, CHRONIC NECROTIZIN		1 (3%)	
NECROSIS, COAGULATIVE	1 (3%)	1 (3%)	
HYPERPLASIA, NODULAR	2 (6%)		
#HEPATIC CAPSULE	(34)	(31)	
HEMORRHAGE		1 (3%)	

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

TABLE D2. MALE MICE (TREATED GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
#LIVER/CENTRILOBULAR	(34)	(31)	
NECROSIS, NOS		1 (3%)	
#PANCREAS	(33)	(32)	
INFLAMMATION, SUPPURATIVE		1 (3%)	
INFLAMMATION, CHRONIC		2 (6%)	
#STOMACH	(34)	(32)	
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
#ILEUM	(33)	(31)	
ULCER, NOS		1 (3%)	
INFLAMMATION, SUPPURATIVE		1 (3%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#PROSTATE	(35)	(32)	
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
*EPIDIDYMIS	(35)	(32)	
INFLAMMATION, CHRONIC SUPPURATIV		2 (6%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. MALE MICE (TREATED GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(35)	(32)	
INFLAMMATION, SUPPURATIVE		2 (6%)	
INFLAMMATION, CHRONIC SUPPURATIV		3 (9%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	8	6	
NO NECROPSY PERFORMED		3	35

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

TABLE D3.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH β-TGdR (CONTROL GROUPS)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	15	15	15	15
ANIMALS NECROPSIED	15	15	15	14
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	15	14
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(15)	(15)	(15)	(13)
INFLAMMATION, SUPPURATIVE			2 (13%)	
#LUNG/BRONCHIOLE	(15)	(15)	(15)	(14)
HYPERPLASIA, PLASMA CELL	2 (13%)	1 (7%)	2 (13%)	
HYPERPLASIA, LYMPHOID	1 (7%)			
#LUNG	(15)	(15)	(15)	(14)
BRONCHOPNEUMONIA SUPPURATIVE	5 (33%)		4 (27%)	1 (7%)
BRONCHOPNEUMONIA CHRONIC SUPPURA				1 (7%)
HYPERPLASIA, PLASMA CELL	1 (7%)			
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
#MYOCARDIUM	(15)	(15)	(15)	(14)
INFLAMMATION, SUPPURATIVE			1 (7%)	
INFLAMMATION, CHRONIC SUPPURATIV				1 (7%)
#ENDOCARDIUM	(15)	(15)	(15)	(14)
INFLAMMATION, CHRONIC			1 (7%)	1 (7%)
DIGESTIVE SYSTEM				
NONE				

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

TABLE D3. FEMALE MICE (CONTROL GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
URINARY SYSTEM				
#URINARY BLADDER	(15)	(15)	(15)	(14)
ULCER, NOS		1 (7%)		
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#UTERUS/ENDOMETRIUM	(15)	(15)	(15)	(14)
INFLAMMATION, SUPPURATIVE	1 (7%)			
#OVARY	(15)	(15)	(15)	(14)
INFLAMMATION, SUPPURATIVE				1 (7%)
NERVOUS SYSTEM				
#BRAIN	(15)	(15)	(15)	(13)
HEMORRHAGE				1 (8%)
SPECIAL SENSE ORGANS				
*EYE/CORNEA	(15)	(15)	(15)	(14)
INFLAMMATION, CHRONIC			1 (7%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(15)	(15)	(14)
INFLAMMATION, SUPPURATIVE			1 (7%)	
ALL OTHER SYSTEMS				
NONE				

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

TABLE D3. FEMALE MICE (CONTROL GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	9	11		2
ACCIDENTAL DEATH				1

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

TABLE D4.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH β-TGdR (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	33	29	1
ANIMALS EXAMINED HISTOPATHOLOGICALLY	33	29	1
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(33)	(29)	(1)
INFLAMMATION, SUPPURATIVE	5 (15%)	1 (3%)	
#LUNG/BRONCHIOLE	(33)	(28)	(1)
HYPERPLASIA, PLASMA CELL		1 (4%)	
#LUNG	(33)	(28)	(1)
BRONCHOPNEUMONIA SUPPURATIVE	6 (18%)	4 (14%)	
HEMATOPOIETIC SYSTEM			
#MESENTERIC L. NODE	(30)	(20)	
INFLAMMATION, NECROTIZING		1 (5%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			

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

TABLE D4. FEMALE MICE (TREATED GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*UTERUS/ENDOMETRIUM	(32)	(29)	(1)
INFLAMMATION, SUPPURATIVE	4 (13%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(33)	(29)	(1)
INFLAMMATION, SUPPURATIVE	1 (3%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(33)	(29)	(1)
INFLAMMATION, SUPPURATIVE		1 (3%)	
INFLAMMATION, CHRONIC FOCAL		1 (3%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	8	3	
ACCIDENTAL DEATH	1	2	1
NO NECROPSY PERFORMED		3	33
AUTOLYSIS/NO NECROPSY	1	1	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS TREATED WITH β-TGdR

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

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Hematopoietic System				
Lymphoma[b]	0/30 (0)	0/10 (0)	1/35 (3)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.047	0.301
Upper Limit			Infinite	Infinite
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.017	0.109
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	66	43
Pituitary: Chromophobe				
Adenoma[b]	7/26 (27)	4/9 (44)	3/25 (12)	0/20 (0)
P Values[c,d]	P = 0.008(N)	P = 0.003(N)	N.S.	P = 0.005*(N) P = 0.012**(N)
Relative Risk (Pooled Vehicle Control)[f]			0.446	0.000
Lower Limit			0.083	0.000
Upper Limit			1.710	0.637
Relative Risk (Matched Vehicle Control)[f]			0.270	0.000
Lower Limit			0.057	0.000
Upper Limit			1.344	0.449
Weeks to First Observed Tumor	79	79	77	--

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

(continued)

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Ear Canal: Carcinoma, NOS, or Squamous-cell Carcinoma[b]	0/30 (0)	0/10 (0)	1/35 (3)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.047	0.301
Upper Limit			Infinite	Infinite
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.017	0.109
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	76	63

[a]Dosed groups received 3.5 or 7 mg/kg by intraperitoneal injection.

[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 matched vehicle-control group (*) or with the pooled vehicle-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 Treated with β-TGdR[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 Treated with β-TGdR[a]

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Hematopoietic System:				
Lymphoma	1/30 (3)	1/10 (10)	0/32 (0)	3/27 (11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			0.000	3.333
Lower Limit			0.000	0.288
Upper Limit			17.314	167.925
Relative Risk (Matched Vehicle Control)[f]			0.000	1.111
Lower Limit			0.000	0.108
Upper Limit			5.791	56.038
Weeks to First Observed Tumor	41	41	--	31
Pituitary: Chromophobe				
Adenoma[b]	14/28 (50)	8/10 (80)	12/31 (39)	2/23 (9)
P Values[c,d]	P = 0.002(N)	P < 0.001(N)	P = 0.027*(N)	P < 0.001*(N) P = 0.002**(N)
Relative Risk (Pooled Vehicle Control)[f]			0.774	0.174
Lower Limit			0.407	0.022
Upper Limit			1.482	0.649
Relative Risk (Matched Vehicle Control)[f]			0.484	0.109
Lower Limit			0.342	0.012
Upper Limit			1.077	0.419
Weeks to First Observed Tumor	41	41	68	70

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

(continued)

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Mammary Gland:				
Adenocarcinoma, NOS[b]	1/30 (3)	0/10 (0)	3/32 (9)	0/27 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			2.813	0.000
Lower Limit			0.242	0.000
Upper Limit			142.727	20.405
Relative Risk (Matched Vehicle Control)[f]			Infinite	--
Lower Limit			0.209	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	80	--	74	--
Mammary Gland: Carcinoma or				
Adenocarcinoma, NOS[b]	2/30 (7)	1/10 (10)	3/32 (9)	0/27 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			1.406	0.000
Lower Limit			0.173	0.000
Upper Limit			15.880	3.673
Relative Risk (Matched Vehicle Control)[f]			0.938	0.000
Lower Limit			0.091	0.000
Upper Limit			47.629	6.825
Weeks to First Observed Tumor	79	79	74	--

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

(continued)

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	6/30 (20)	2/10 (20)	5/32 (16)	0/27 (0)
P Values[c,d]	P = 0.020(N)	P = 0.032(N)	N.S.	P = 0.016**(N)
Relative Risk (Pooled Vehicle Control)[f]			0.781	0.000
Lower Limit			0.211	0.000
Upper Limit			2.750	0.675
Relative Risk (Matched Vehicle Control)[f]			0.781	0.000
Lower Limit			0.165	0.000
Upper Limit			7.520	1.210
Weeks to First Observed Tumor	79	79	74	--
Ear Canal: Squamous-cell Carcinoma[b]	0/30 (0)	0/10 (0)	2/32 (6)	5/27 (19)
P Values[c,d]	P = 0.010	N.S.	N.S.	P = 0.019**
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.282	1.436
Upper Limit			Infinite	Infinite
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.102	0.524
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	70	63

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

(continued)

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Ear Canal: Carcinoma, NOS, or Squamous-cell Carcinoma[b]	0/30 (0)	0/10 (0)	2/32 (6)	6/27 (22)
P Values[c,d]	P = 0.004	N.S.	N.S.	P = 0.008**
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.282	1.824
Upper Limit			Infinite	Infinite
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.102	0.665
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	70	48

[a]Dosed groups received 3.5 or 7 mg/kg by intraperitoneal injection.

[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 matched vehicle-control group (*) or with the pooled vehicle-control group (**) when P < 0.05 for either control group; otherwise, not significant (N.S.) is indicated.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Treated with β-TGdR[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 E3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Treated with β-TGdR[a]

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Hematopoietic System:				
Lymphoma (43)[b]	0/29 (0)	0/10 (0)	1/32 (3)	2/10 (20)
P Values[c,d]	P = 0.025	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.049	0.885
Upper Limit			Infinite	Infinite
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.018	0.330
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	66	43
Pituitary: Chromophobe				
Adenoma (52)[b]	7/24 (29)	4/9 (44)	3/22 (14)	0/6 (0)
P Values[c,d]	N.S.	P = 0.027(N)	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			0.468	0.000
Lower Limit			0.088	0.000
Upper Limit			1.763	1.672
Relative Risk (Matched Vehicle Control)[f]			0.307	0.000
Lower Limit			0.065	0.000
Upper Limit			1.511	1.293
Weeks to First Observed Tumor	79	79	77	--

Table E3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Treated with β-TGdR[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Ear Canal: Carcinoma, NOS, or Squamous-cell Carcinoma (52)[b]	0/28 (0)	0/10 (0)	1/31 (3)	2/7 (29)
P Values[c,d]	P = 0.014	P = 0.046	N.S.	P = 0.035**
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.049	1.233
Upper Limit			Infinite	Infinite
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.019	0.475
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	76	63

[a]Dosed groups received 3.5 or 7 mg/kg by intraperitoneal injection.

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

[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 matched vehicle-control group (*) or with the pooled vehicle-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

Table E3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Rats Treated with β-TGdR[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 E4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Treated with β-TGdR[a]

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Hematopoietic System:				
Lymphoma (31)[b]	1/30 (3)	1/10 (10)	0/32 (0)	3/15 (20)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.026	P = 0.029		
Relative Risk (Pooled Vehicle Control)[f]			0.000	6.000
Lower Limit			0.000	0.526
Upper Limit			17.314	289.415
Relative Risk (Matched Vehicle Control)[f]			0.000	2.000
Lower Limit			0.000	0.198
Upper Limit			5.791	96.600
Weeks to First Observed Tumor	41	41	--	31
Pituitary: Chromophobe				
Adenoma (41)[b]	14/28 (50)	8/10 (80)	12/31 (39)	2/14 (14)
P Values[c,d]	P = 0.023(N)	P = 0.002(N)	P = 0.027*(N)	P = 0.002*(N) P = 0.025**(N)
Relative Risk (Pooled Vehicle Control)[f]			0.774	0.286
Lower Limit			0.407	0.036
Upper Limit			1.482	1.002
Relative Risk (Matched Vehicle Control)[f]			0.484	0.179
Lower Limit			0.342	0.046
Upper Limit			1.012	0.658
Weeks to First Observed Tumor	41	41	68	70

Table E4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Treated with β-TGdR[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Mammary Gland: Adenocarcinoma, NOS (52)[b]	1/29 (3)	0/10 (0)	3/31 (10)	0/12 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			2.806	0.000
Lower Limit			0.242	0.000
Upper Limit			142.233	42.376
Relative Risk (Matched Vehicle Control)[f]			Infinite	--
Lower Limit			0.216	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	80	--	74	--
Mammary Gland: Carcinoma or Adenocarcinoma, NOS (52)[b]	2/29 (7)	1/10 (10)	3/31 (10)	0/12 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			1.403	0.000
Lower Limit			0.174	0.000
Upper Limit			15.813	7.546
Relative Risk (Matched Vehicle Control)[f]			0.968	0.000
Lower Limit			0.094	0.000
Upper Limit			49.097	14.663
Weeks to First Observed Tumor	79	79	74	--

Table E4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Treated with β-TGdR[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Mammary Gland:				
Fibroadenoma (52)[b]	6/29 (21)	2/10 (20)	5/31 (16)	0/12 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			0.780	0.000
Lower Limit			0.211	0.000
Upper Limit			2.733	1.378
Relative Risk (Matched Vehicle Control)[f]			0.806	0.000
Lower Limit			0.170	0.000
Upper Limit			7.748	2.576
Weeks to First Observed Tumor	79	79	74	--
Ear Canal: Squamous-cell				
Carcinoma (52)[b]	0/30 (0)	0/10 (0)	2/31 (6)	5/12 (42)
P Values[c,d]	P < 0.001	P = 0.004	N.S.	P = 0.030* P = 0.001**
Departure from Linear Trend[e]	P = 0.043			
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.291	3.311
Upper Limit			Infinite	Infinite
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.105	1.201
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	70	63

Table E4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Treated with β-TGdR[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Matched Vehicle Control	Low Dose	High Dose
Ear Canal: Carcinoma, NOS, or Squamous-cell Carcinoma (48)[b]	0/28 (0)	0/9 (0)	2/32 (6)	6/13 (46)
P Values[c,d]	P < 0.001	P = 0.002	N.S.	P = 0.023* P < 0.001**
Departure from Linear Trend[e]	P = 0.024			
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.264	3.636
Upper Limit			Infinite	Infinite
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.093	1.286
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	70	48

[a]Dosed groups received 3.5 or 7 mg/kg by intraperitoneal injection.

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

Table E4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Treated with β-TGdR[a]

(continued)

[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 matched vehicle-control group (*) or with the pooled vehicle-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.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE TREATED WITH β-TGdR

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

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose
Hematopoietic System: Lymphoma[b]	19/29 (66)	17/35 (49)	16/32 (50)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.741	0.763
Lower Limit		0.473	0.482
Upper Limit		1.208	1.242
Weeks to First Observed Tumor	39	51	28
Hematopoietic System: Leukemia[b]	0/29 (0)	0/35 (0)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		--	Infinite
Lower Limit		--	0.272
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	54

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose
Hematopoietic System: Lymphoma or Leukemia[b]	19/29 (66)	17/35 (49)	18/32 (56)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.741	0.859
Lower Limit		0.473	0.558
Upper Limit		1.208	1.354
Weeks to First Observed Tumor	39	51	28

[a]Dosed groups received 1 or 2 mg/kg by intraperitoneal injection.

[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 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 specified control group.

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

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose
Hematopoietic System: Lymphoma[b]	20/29 (69)	19/33 (58)	21/29 (72)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.835	1.050
Lower Limit		0.560	0.728
Upper Limit		1.285	1.501
Weeks to First Observed Tumor	33	64	34
Hematopoietic System: Lymphoma or Leukemia[b]	21/29 (72)	19/33 (58)	21/29 (72)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.795	1.000
Lower Limit		0.547	0.708
Upper Limit		1.207	1.412
Weeks to First Observed Tumor	27	64	34

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

(continued)

[a]Dosed groups received 1 or 2 mg/kg by intraperitoneal injection.

[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 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 specified control group.

Table F3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Treated with β-TGdR[a]

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose
Hematopoietic System: Lymphoma[b]	19/29 (66)	17/35 (49)	16/31 (52)
P Values[d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.741	0.788
Lower Limit		0.473	0.498
Upper Limit		1.206	1.276
Hematopoietic System: Leukemia[c]	0/16 (0)	0/31 (0)	2/18 (11)
P Values[d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		--	Infinite
Lower Limit		--	0.279
Upper Limit		--	Infinite

Table F3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Treated with β-TGdR[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose
Hematopoietic System: Lymphoma or Leukemia[b]	19/29 (66)	17/35 (49)	18/31 (58)
P Values[d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.741	0.886
Lower Limit		0.473	0.578
Upper Limit		1.208	1.387

[a]Dosed groups received 1 or 2 mg/kg by intraperitoneal injection.

[b]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 28 weeks of the study.

[c]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 52 weeks of the study.

[d]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 when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[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 F4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Treated with β-TGdR[a]

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose
Hematopoietic System: Lymphoma[b]	20/28 (71)	19/32 (59)	21/28 (75)
P Values[e]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[g]		0.831	1.050
Lower Limit		0.568	0.740
Upper Limit		1.266	1.473
Hematopoietic System: Lymphoma or Leukemia[c]	21/29 (72)	19/33 (58)	21/28 (75)
P Values[e]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[g]		0.795	1.036
Lower Limit		0.547	0.736
Upper Limit		1.207	1.440

Table F4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Treated with β-TGdR[a]

(continued)

[a]Dosed groups received 1 or 2 mg/kg by intraperitoneal injection.

[b]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 33 weeks of the study.

[c]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 27 weeks of the study.

[d]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 52 weeks of the study.

[e]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 when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

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

Review of the Bioassay of Beta-Deoxythioguansine* (β-TGdR)
for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

January 18, 1978

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, biostatistics, biochemistry, 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 Beta-Deoxythioguansine (β-TGdR)was reviewed.

The primary reviewer noted the unusual occurrence of ear canal carcinomas in the β-TGdR treated rats. Despite the high mortality due to toxicity, he considered the results of the study to be valid. He said that the incidence of lymphomas and leukemias in the matched vehicle control mice may be related to the mineral oil vehicle. The primary reviewer opined that too much attention was given to statistical analyses to the detriment of biological considerations.

The secondary reviewer said he agreed that β-TGdR was carcinogenic in the treated rats. He questioned the primary reviewer's notion that Polysorbate 80 was related to the mouse lymphomas and leukemias, since it has not produced a similar effect in other studies in which it had been used.

A Program staff pathologist commented that the Polysorbate 80 may not act in the same way as mineral oil. He suggested that granulomas also would have been found if the reaction was similar to that of mineral oil. Another Program staff member said that it was clear that the rat ear canal tumors were related to β-TGdR treatment. He added that a question of the significance of the lymphomas in mice arose because of possible procedural irregularities.

A motion was made that Beta-Deoxythioguansine was carcinogenic in the rat and that the mouse study was uninterpretable because of its inadequacies. The motion was seconded and passed five to two. The opposing votes were cast by Mr. Garfinkel and Dr. Kensler.

Members Present Were:

Arnold Brown (Acting Chairman), Mayo Clinic
Lawrence Garfinkel, American Cancer Society
Joseph Highland, Environmental Defense Fund
Charles Kensler, Arthur D. Little Company
Verald K. Rowe, Dow Chemical, U.S.A.
Sheldon Samuels, Industrial Union Department, AFL-CIO
Louise Strong, University of Texas Health Sciences Center
Sidney Wolfe, Health Research Group

---

* 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 260-899/3042 1-3

DHEW Publication No. (NIH) 78-1363