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

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FOREWORD: This report presents the results of the bioassay of estradiol mustard conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of estradiol mustard 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]. Mr. J. Belzer[1] and Mr. I. Brown[1] were responsible for the care of the laboratory animals and the administration of the test chemical. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation.

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] and Ms. P. L. Yong[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed by Drs. P. Lim[7], E. Tong[7], and J. Jee[7], and the results of the analyses were reviewed by Dr. C. W. Jameson[5].

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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[8], and Dr. Jerrold M. Ward.

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SUMMARY

A bioassay of the experimental anticancer drug estradiol mustard for possible carcinogenicity was conducted by administering the chemical by gavage to Sprague-Dawley rats and B6C3F1 mice.

Groups of 35 rats and 34-36 mice of each sex were administered estradiol mustard at one of the following doses, either 0.62 or 1.25 mg/kg body weight for rats and either 15 or 30 mg/kg body weight for mice. The vehicle used for the test chemical consisted of 0.05% polysorbate 80 in phosphate-buffered saline. The rats and mice were dosed three times per week for 52 weeks, then observed for an additional 30-34 weeks. Controls consisted of groups of 10 rats and 15 mice of each sex that were not administered the chemical (untreated controls) and also of groups of 10 rats of each sex, 14 male mice, and 16 female mice administered the vehicle alone (vehicle controls). Pooled controls were also used. All surviving rats were killed at 84-86 weeks and all surviving mice at 82-86 weeks.

Mean body weights of male rats and male and female mice administered estradiol mustard were lower throughout the greater part of the study than those of corresponding vehicle or untreated controls; mean body weights of dosed female rats were unaffected. Administration of the test chemical had no significant effect on the survival of either male or female rats. A large number of dosed mice died prior to the end of the study. The numbers of dosed male mice which were at risk as long as 52 weeks were sufficient, however, for development of tumors appearing up to that time. Time-adjusted analysis and life-table analyses were applied to data obtained with the mice.

In rats, no tumors were observed in a statistically significant incidence in the animals administered estradiol mustard.

In mice, lymphoma or lymphocytic leukemia occurred at significant incidences in low-dose ($P = 0.018$) and high-dose ($P < 0.001$)

groups of males compared with those in the pooled vehicle controls (controls 0/28, low-dose 6/32, high-dose 17/29) and at significant incidences in low-dose (P = 0.020) and high-dose (P = 0.002) groups of females compared with those in the corresponding vehicle controls (controls 0/14, low-dose 9/30, high-dose 11/23). In addition, the incidences of lymphoma were statistically significant for dose-related trend for both the males (P < 0.001) and the females (P = 0.003). Since lymphoma was observed in male mice as early as 25 weeks, life-table analyses of the incidence in each sex were performed. The results indicated a dose association (P = 0.001) between the administration of estradiol mustard and the time of observation of lymphoma in either sex of mice.

In mice, alveolar/bronchiolar adenoma or carcinoma occurred at a significant incidence (P = 0.004) in the low-dose group of males compared with the pooled vehicle controls (controls 2/28, low-dose 12/30, high-dose 5/24) and at a significant incidence (P = 0.022) in the low-dose group of females compared with the pooled vehicle controls (controls 1/28, low-dose 7/27, high-dose 1/18). Sarcoma of the myocardium similarly occurred at a significant incidence (P = 0.015) in the low-dose group of males compared with the pooled vehicle controls (controls 0/28, low-dose 6/30, high-dose 2/24) and at a significant incidence (P = 0.002) in the low-dose group of females compared with the pooled vehicle controls (controls 0/28, low-dose 8/27, high-dose 1/12). The survival of both high-dose males and high-dose females was slightly lower than that of the respective low-dose groups and may account for the higher numbers of pulmonary tumors and myocardial sarcomas among low-dose mice of both sexes. The association of myocardial sarcoma with administration of the chemical in both dosed groups of each sex is strengthened by the fact that these tumors of the myocardium have not occurred in the more than 500 male and 500 female historical-control mice of this strain at the laboratory.

Squamous-cell carcinoma of the stomach occurred in the dosed male mice (high-dose 2/29) and in the dosed female mice (low-dose 2/26, high-dose 2/14) but was absent in all controls. Although the incidences in this bioassay were too low to be statistically significant, the fact that no squamous-cell carcinomas of the stomach have occurred in the more than 500 male and 500 female historical-control mice of this strain at this laboratory

indicates that these gastric tumors were related to the administration of the estradiol mustard.

It is concluded that under the conditions of this bioassay, estradiol mustard administered in a buffered saline vehicle was not carcinogenic in Sprague-Dawley rats. Estradiol mustard was carcinogenic in both male and female B6C3F1 mice, inducing lymphoma, sarcoma of the myocardium, alveolar adenoma or carcinoma, and squamous-cell carcinoma of the stomach.

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I. INTRODUCTION

Estradiol mustard (CAS 22966-79-6; NCI C01570) is an experimental anticancer agent that has been proposed for use in patients with cancer of the ovary, breast, and prostate (Vollmer et al., 1973a). It is the ester of the estrogen, estradiol, and a nitrogen mustard alkylating agent, chlorphenacyl.

The manipulation of hormones in the body has been used for over 75 years to treat cancer in hormone-responsive tissues. Surgical removal of endocrine organs, as in ovariectomy, has been associated with the regression of certain breast tumors, and the administration of estrogens has also produced some tumor regressions (Vollmer, 1973b). The effects are believed to reflect the role of hormones in regulating growth and development of certain tissues (NCI, 1976). Tissues that respond to estrogen contain cellular components known as estrogen receptors. These receptors bind estrogen and transport it from the cytoplasm to the nucleus where it initiates the synthesis of proteins that will be used to express hormonal effects (Wittliff et al., 1976). The rationale behind the synthesis of estrogen-nitrogen mustard compounds was that the alkylating moiety, when coupled with an estrogen, could be transported directly to the nucleus of cells such as those in the mammary glands, ovaries, uterus, prostate, and testes, which have estrogen receptors (Wall et al., 1969). Cytotoxic effects

of the nitrogen mustards would then be expressed only in these organs.

Estradiol mustard was selected for testing for carcinogenic activity because of the possibility that this drug may be used on a repeated basis in humans.

II. MATERIALS AND METHODS

A. Chemical

Estradiol mustard (estradiol,bis((p-(bis(2-chloroethyl)amino) phenyl)acetate)) was obtained in two batches for the chronic study from the Upjohn Company, North Haven, Connecticut. The purity of the batch from Lot No. 80-4 was determined to be 97 $\pm$ 1% by ultraviolet spectroscopy and thin-layer chromatography analyses at the Stanford Research Institute. Elemental analyses (C, H, Cl, N) were correct for $C_{42}H_{50}Cl_4N_2O_4$, the molecular formula for estradiol mustard. The identity was confirmed by nuclear magnetic resonance, infrared, and ultraviolet spectra, which were in agreement with the structure and matched the spectra for a reference sample of estradiol mustard.

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

B. Dosage Preparation

The test solutions of the estradiol mustard were prepared fresh for each chemical administration. The chemical was suspended in a phosphate-buffered saline-polysorbate 80 vehicle by mixing in a Potter-Elvehjem tissue grinder. The buffered saline vehicle

(pH 6.9) contained 0.85% NaCl, 0.40% NaH_2PO_4, 0.65% Na_2HPO_4, and 0.05% polysorbate 80.

No concentration or stability analyses of the chemical in the buffered-saline vehicle were performed.

C. Animals

For the chronic study, Sprague-Dawley rats and B6C3F1 mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, under a contract with the Division of Cancer Treatment, National Cancer Institute. Male rats were received at 29 days of age, female rats at 36 days of age, and male and female mice at 37 days of age. On arrival at the laboratory, all animals were quarantined (rats for 6 days, mice for 12 days). Those animals with no visible 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 incoming 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 meal (Allied Mills, Inc., Chicago, Ill.) and water were supplied daily and were available ad libitum.

Rats were housed five per cage and mice seven per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The rat cages were provided with Iso-Dri® hardwood chip bedding (Carworth, Edison, N.J.), and cage tops were covered with disposable filter bonnets beginning at week 16; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective dosed animals. Animals administered estradiol mustard were maintained in the same rooms as animals of the same species being administered the following chemicals:

RATS

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate (phenesterin) (CAS 3546-10-9)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

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)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of estradiol mustard, on the basis of which two concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. In the subchronic studies, estradiol mustard was administered by gavage in a vehicle containing polysorbate 80 in saline at doses of 0.25, 0.62, 1.25, 2.5, or 5.0 mg/kg body weight to male Sprague-Dawley rats and at doses of 0.4, 1.0, 2.0,

4.0, 8.0, 16.0, or 32.0 mg/kg body weight to male Swiss mice. Animals were dosed three times per week for 45 days and observed for an additional 45 days before termination of the study. Five animals of each species were used at each dose, and 10 animals of each species were used as untreated or vehicle controls.

In rats, there were no deaths at any of the doses tested. After 45 days of chemical administration, mean body weight gains were depressed 13-18% at doses of 0.62, 1.25, and 2.5 mg/kg and 25% at a dose of 5.0 mg/kg. The low and high doses for the chronic studies using rats were set at 0.62 and 1.25 mg/kg.

In mice, the only death due to toxicity of the chemical occurred in an animal at 0.4 mg/kg during week 3 of the study. Mean body weight gains in groups of animals at doses of 8.0 mg/kg or lower were similar to those of controls; mean body weight gains in groups of animals at doses of 16 or 32 mg/kg were slightly lower than those of controls. The low and high doses for the chronic studies using mice were set at 15 and 30 mg/kg.

Doses for both rats and mice were purposely set low because of the wide variation in the acute toxicity data previously reported for this chemical (Vollmer et al., 1973).

F. Designs of Chronic Studies

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

Since the numbers of animals in the untreated-control and vehicle-control groups were small, pooled-vehicle control groups also were used for statistical evaluation. For the rats, the 10 matched vehicle-control animals of each sex from the current bioassay of estradiol mustard were combined with groups of 10 vehicle-control animals of each sex from the bioassay of phenesterin (CAS 3546-10-9) to give pooled groups of 20 vehicle-control animals of each sex. For the mice, the 14 male and 16 female matched vehicle-control animals from the current bioassay of estradiol mustard were combined with groups of 15 vehicle-control animals of each sex from the bioassay of phenesterin to give pooled groups of 29-31 vehicle-control animals of each sex. The vehicle-control groups of rats and mice for the different test chemicals were of the same strains and from the same supplier. The various control groups were started at times no more than 3 months apart, all at Southern Research Institute, and the diagnoses were made by the same pathologists. The vehicle used for the controls in the bioassay of phenesterin was the same as that used for the controls in the bioassay of estradiol mustard.

Table 1. Design of Chronic Studies of Estradiol Mustard in Rats

Sex and Test Group	Initial No. of Animals[a]	Estradiol Mustard Dose[b] (mg/kg)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Untreated-Control	10	0		86
Vehicle-Control	10	0[c]	52	34
Low-Dose	35	0.62	52	33
High-Dose	35	1.25	52	33
Female				
Untreated-Control	10	0		86
Vehicle-Control	10	0[c]	52	34
Low-Dose	35	0.62	52	33-34
High-Dose	35	1.25	52	32-33

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

[b]Estradiol mustard was administered by gavage in a vehicle consisting of 0.05% polysorbate 80 in phosphate-buffered saline at a volume of 0.25 ml/100 g body weight three times per week; doses were based on individual body weights.

[c]Vehicle-control groups received only polysorbate 80 in phosphate-buffered saline at the same volume as dosed rats.

Table 2. Design of Chronic Studies of Estradiol Mustard in Mice

Sex and Test Group	Initial No. of Animals[a]	Estradiol Mustard Dose[b] (mg/kg)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Untreated-Control	15	0		86
Vehicle-Control	14	0[c]	52	32
Low-Dose	34	15	52	32
High-Dose	35	30	52	30-31
Female				
Untreated-Control	15	0		85-86
Vehicle-Control	16	0[c]	52	32
Low-Dose	36	15	52	32
High-Dose	35	30	52	24[d]

[a]Mice were 49 days of age when placed on study.

[b]Estradiol mustard was administered by gavage in a vehicle consisting of 0.05% polysorbate 80 in phosphate-buffered saline at a volume of 1 ml/100 g body weight three times per week; doses were based on the mean weights of the animals in each cage.

[c]Vehicle-control groups received only polysorbate 80 in phosphate-buffered saline at the same volume as dosed mice.

[d]All high-dose females died or were killed by week 76.

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 and mice were weighed individually each week for 8 weeks and every 2 weeks thereafter. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal whenever possible. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals 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 indi-

cates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

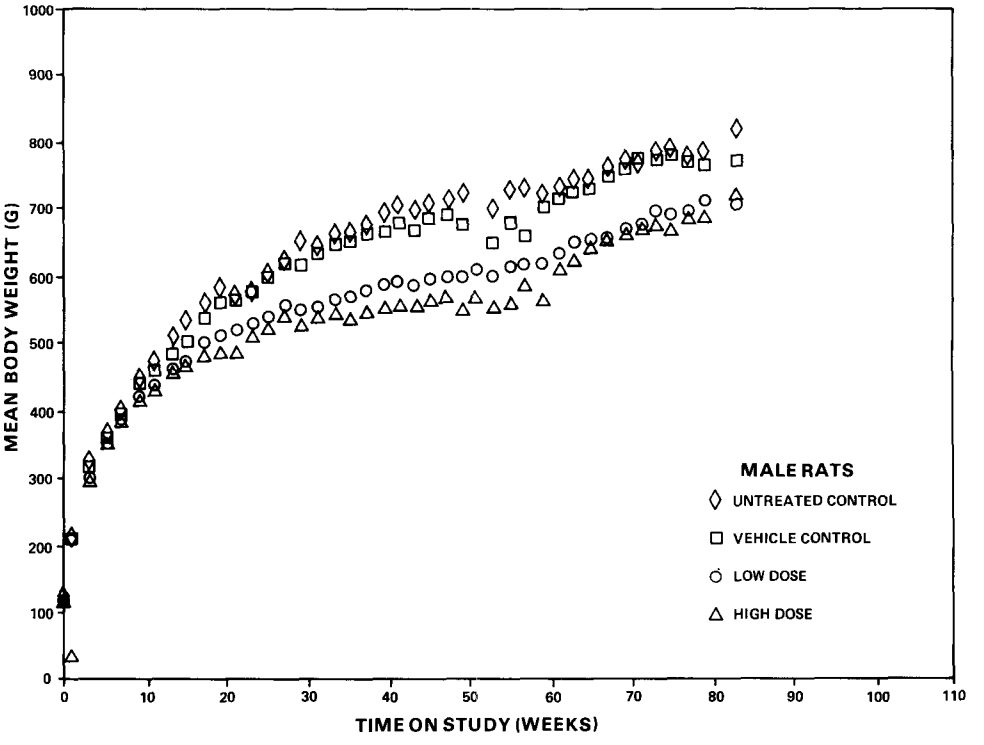
A. Body Weights and Clinical Signs (Rats)

Mean body weights of the dosed male rats were lower than those of the corresponding controls, and the depressions in weight were dose related (figure 1). Mean body weights of the dosed female rats were approximately the same as those of either the untreated or vehicle controls. 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 wide variation. No other clinical signs attributable to administration of estradiol mustard were reported.

B. Survival (Rats)

Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered estradiol mustard by gavage at the doses of this bioassay, together with those of the matched vehicle and untreated controls, are shown in figure 2.

In each sex, the results of the Tarone test for positive dose-related trend in mortality are not significant. In male rats, all of the 35 high-dose animals, 34/35 (97%) of the low-dose group, and all of the 10 animals in either the vehicle- or untreated-control group were alive at week 52. In females, 34/35



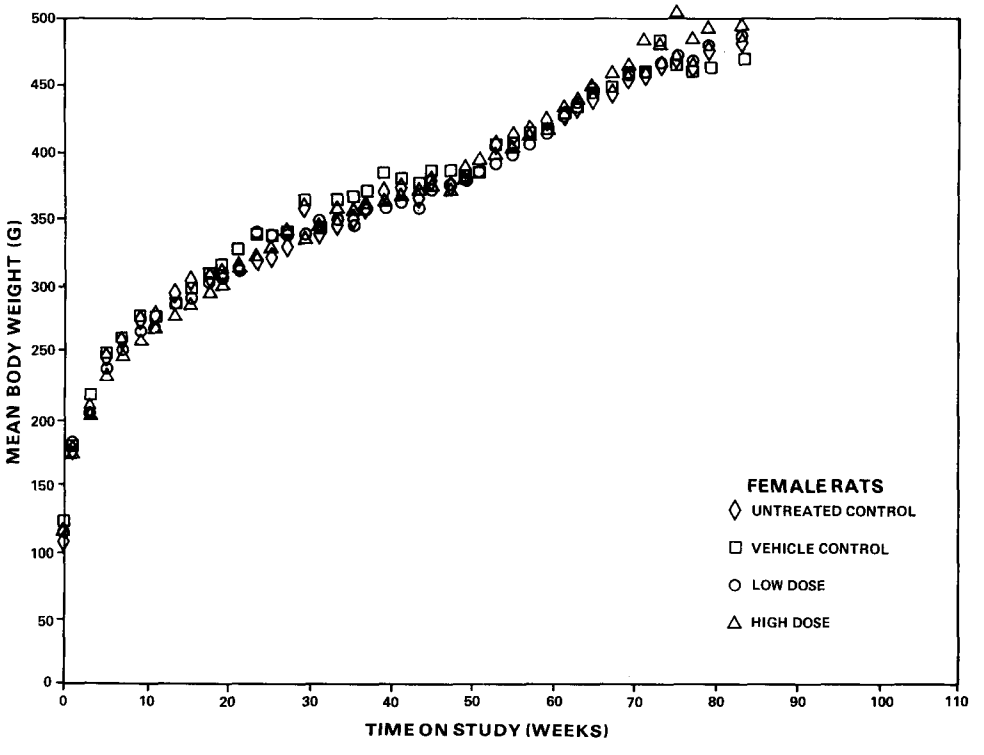


Figure 1. Growth Curves For Rats Administered Estradiol Mustard by Gavage

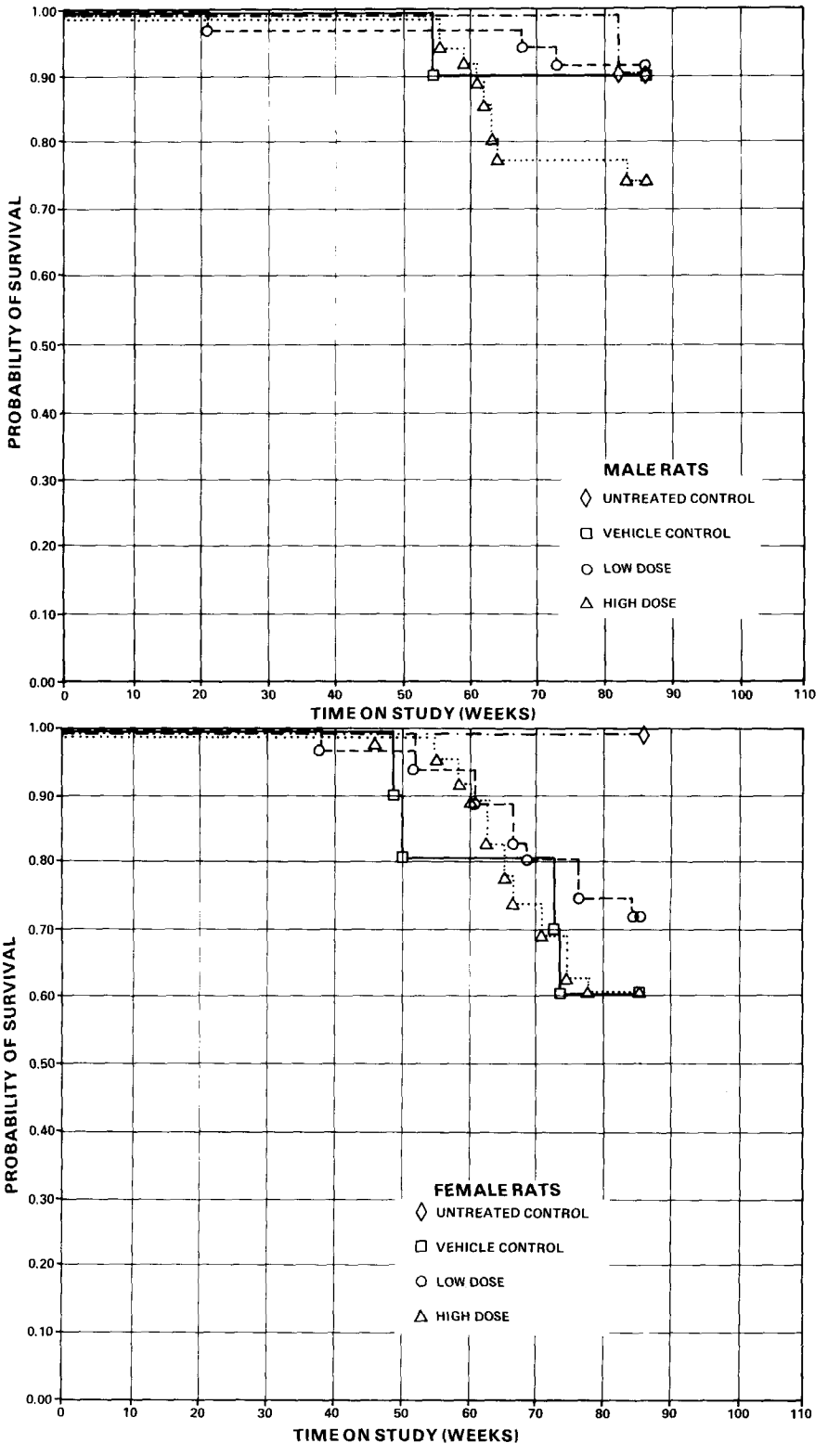


Figure 2. Survival Curves For Rats Administered Estradiol Mustard by Gavage

(97%) of the high-dose group, 34/35 (97%) of the low-dose group, 8/10 (80%) of the vehicle controls, and all of the 10 untreated controls were alive at week 52. A sufficient number of dosed rats of each sex was at risk for the development of late-appearing tumors.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, tables C1 and C2.

Mammary gland tumors occurred in 3/35 (9%) low-dose and 5/33 (15%) high-dose male rats. None occurred in males of the untreated (0/10) and vehicle (0/9) controls. In the females, tumors of the mammary glands occurred in 5/10 (50%) untreated-control, 4/8 (50%) vehicle-control, 20/34 (59%) low-dose, and 21/33 (63%) high-dose rats. The majority of the tumors in the dosed groups were benign. Mammary tumors of the same type have been observed previously as spontaneous lesions in Sprague-Dawley rats (Prejean et al., 1973; Young and Hallowes, 1973), and the incidences observed in this study do not support the conclusion of a compound-related effect.

A variety of other neoplasms occurred both in the control groups (untreated and vehicle) and in the dosed groups. Some of these

neoplasms occurred only, or with a greater frequency, in rats of dosed groups as compared with controls. These lesions, however, are not uncommon in this strain of rat independent of the administration of any chemical.

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). These nonneoplastic lesions are commonly seen in aged Sprague-Dawley rats.

Based on the histopathologic examination, estradiol mustard administered by oral gavage to Sprague-Dawley rats may have had an effect on the mammary gland under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group. Vehicle-control groups and pooled vehicle-control groups are used in the statistical analyses. The untreated-control groups are not included in the statistical analyses, since the test conditions of the vehicle controls are more similar to those of the dosed animals.

In male rats, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of fibroadenoma of the mammary gland is significant (P = 0.043) when the pooled vehicle controls are used, but the results of the Fisher exact test are not significant. The results of the tests made on the incidence of the combination of fibroadenoma or adenocarcinoma are not significant. The results of the bioassay program at this laboratory to date indicate a spontaneous tumor rate of fibroadenoma or adenocarcinoma in the mammary gland in male rats of 6/215 (2.8%).

In female rats, the results of the Cochran-Armitage test on the incidence of adenocarcinoma of the mammary gland, the incidence of fibroadenoma of the mammary gland, and the incidence of the combination of adenoma, adenocarcinoma, or fibroadenoma of the mammary gland are not significant. The results of the Fisher exact test show levels of probability above 0.05 when the incidences in either the low- or high-dose groups are compared with those in the controls. The historical data for female rats concerning the spontaneous occurrence of adenocarcinoma at this laboratory to date show an incidence of 15/220 (6.8%).

In male rats, significant results in the negative direction are indicated in the incidences of pituitary tumors, where the

incidence in the controls exceeds that observed in the dosed groups.

In each of the 95% confidence intervals of relative risk shown in the tables, except that for the incidence of pituitary tumors in the high-dose males, one is included; this indicates the absence of significant positive results. It should also be noted that each of these intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by estradiol mustard, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

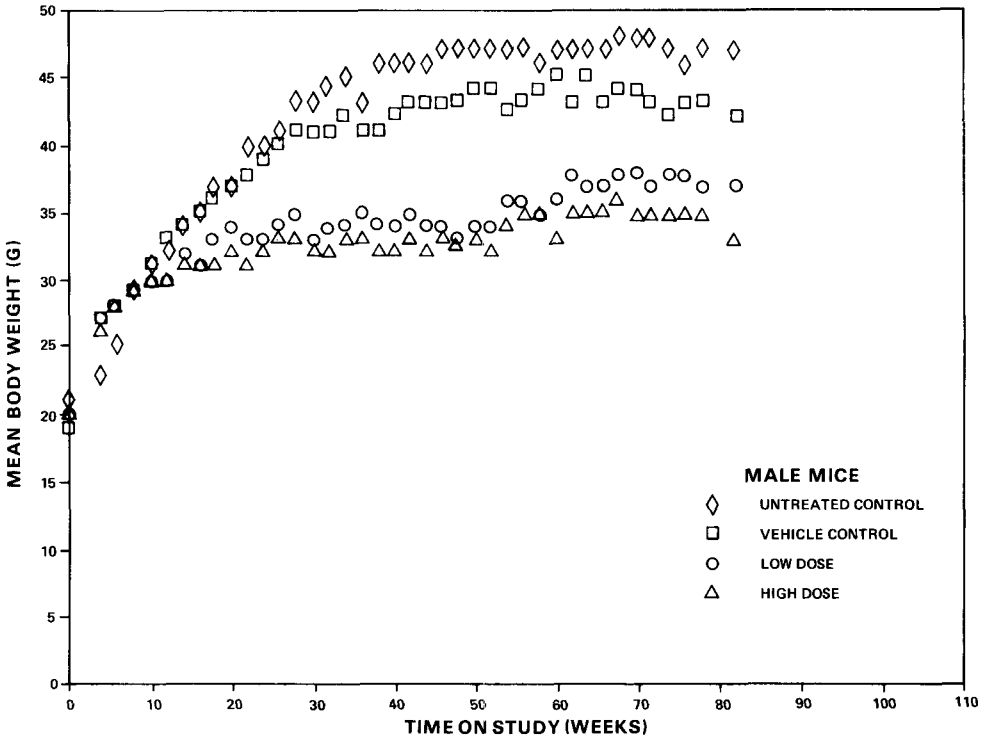
A. Body Weights and Clinical Signs (Mice)

Mean body weights of both the low- and high-dose male and female mice were markedly lower than those of either the untreated- or vehicle-control mice throughout most of the bioassay (figure 3). 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 wide variation. No other clinical signs attributable to administration of estradiol mustard were reported.

B. Survival (Mice)

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

In each sex, the results of the Tarone test for positive dose-related trend in mortality are significant ($P < 0.001$). In the male mice, 13/14 (93%) of the vehicle controls, all of the 15 untreated controls, 30/34 (88%) of the low-dose group, and 24/35 (69%) of the high-dose group were alive at week 52. Thus, sufficient numbers of dosed male mice were at risk for 52 weeks for development of tumors appearing up to that time. In females,



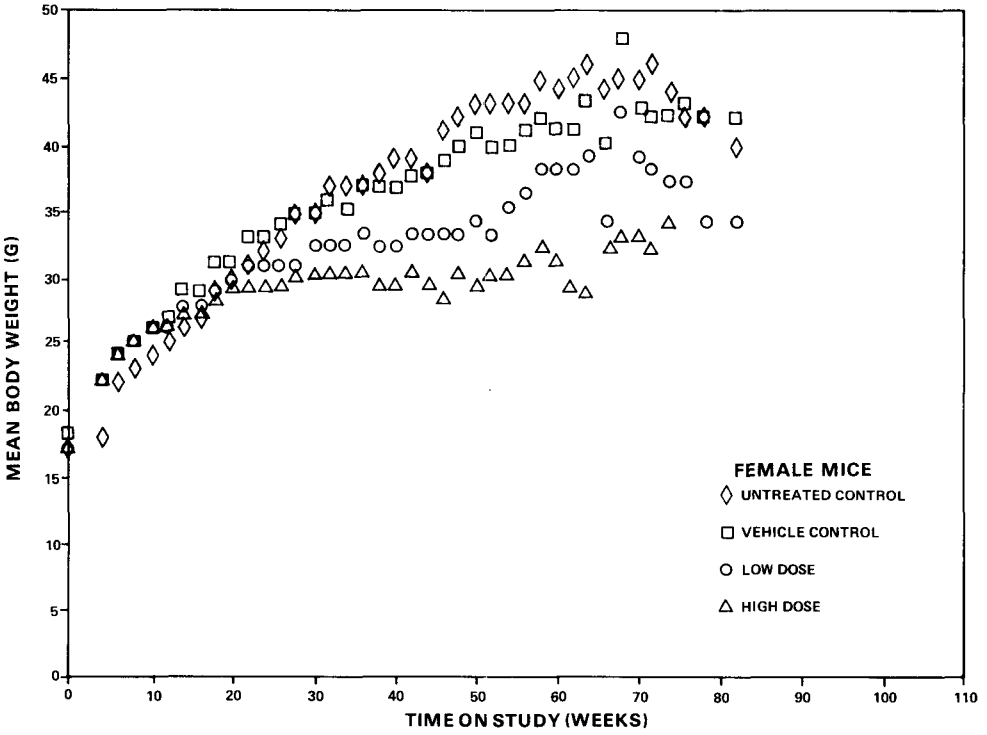


Figure 3. Growth Curves For Mice Administered Estradiol Mustard by Gavage

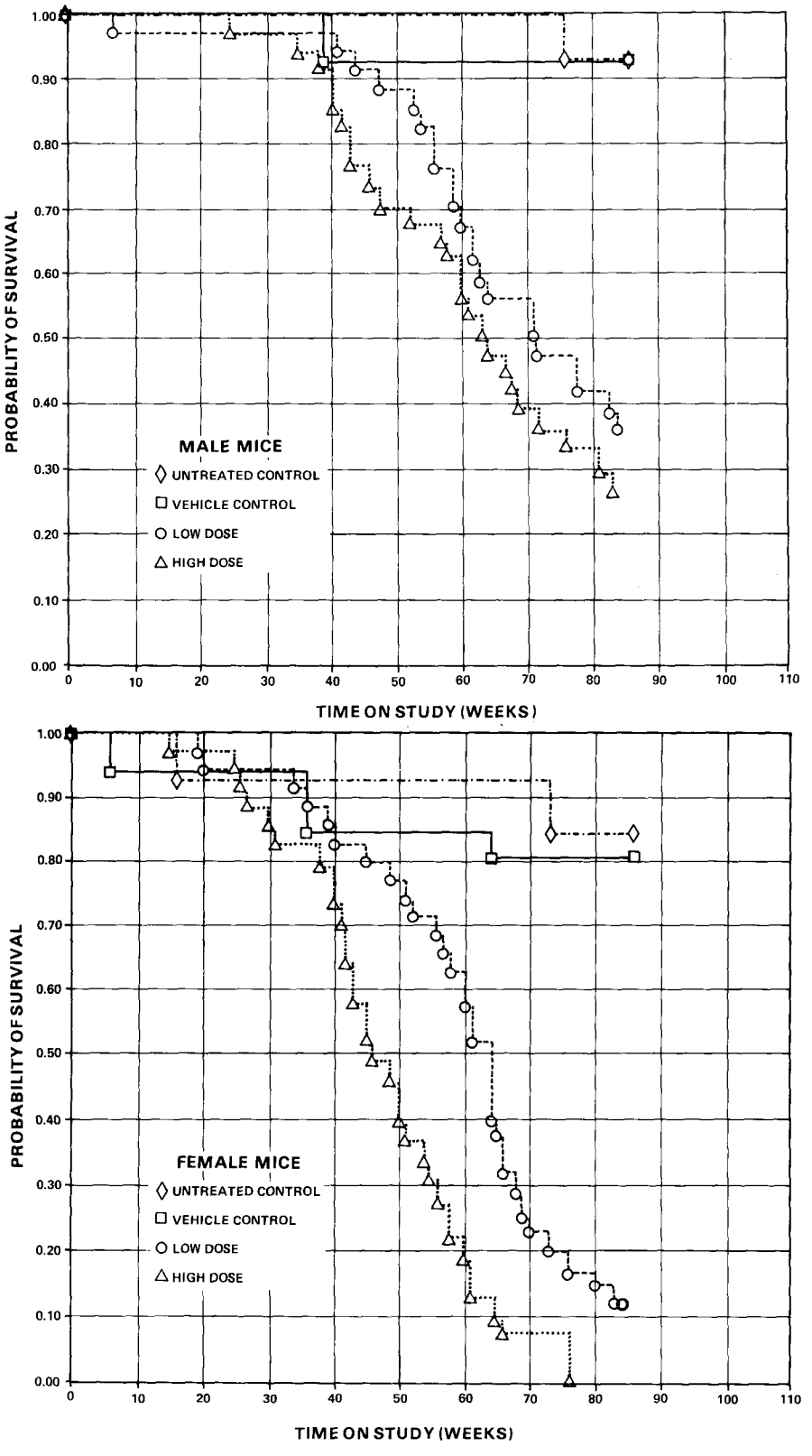


Figure 4. Survival Curves For Mice Administered Estradiol Mustard by Gavage

13/16 (81%) of the vehicle controls, 12/15 (80%) of the untreated controls, 26/36 (72%) of the low-dose group, and 12/35 (34%) of the high-dose group survived at least as long as 52 weeks on study.

C. Pathology (Mice)

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

A variety of neoplasms occurred both in control groups (untreated and vehicle) and in dosed groups. Some of the tumors appeared to be compound related.

In the males, alveolar-cell (alveolar-bronchiolar) tumors were observed in 2/15 (13%) untreated-control, 2/14 (14%) vehicle-control, 12/32 (44%) low-dose, and 5/29 (17%) high-dose males. In the females, 7/31 (23%) low-dose and 1/24 (4%) high-dose mice had these lung tumors. No alveolar-cell tumors occurred in untreated- (0/14) or vehicle-control (0/16) female mice. These alveolar-cell tumors included alveolar-cell adenocarcinomas (alveolar/bronchiolar carcinomas) which were present in 6/32 (19%) low-dose and 1/29 (3%) high-dose males and in 4/31 (13%) low-dose females. Both the benign and malignant neoplasms were comprised of cuboidal to columnar cells aligned along the

alveolar septa. Often the cells projected into the alveolar spaces, resulting in the formation of numerous papillary structures. Adenomas were differentiated from carcinomas by size and the degree of cellular pleomorphism, nuclear atypism, and extension into adjacent tissues. The alveolar-cell carcinomas were all well differentiated with few mitotic figures being observed. These tumors did not metastasize.

The hematopoietic and lymphoreticular neoplasms were observed in 17/29 (58%) high-dose and 6/32 (18%) low-dose males and in 11/24 (45%) high-dose and 9/31 (29%) low-dose females. None occurred in any control groups except for one lymphoma in the untreated-control females. The malignant lymphomas were classified as lymphocytic and histiocytic. The lymphocytic type was comprised of cells having a small, darkly basophilic to large lightly basophilic, vesicular nucleus and a rim of eosinophilic cytoplasm. Malignant lymphomas composed of lymphoblastic (undifferentiated) cells were included in the lymphocytic type. The histiocytic type was comprised primarily of cells with a large open-faced nucleus, distinct eosinophilic nucleolus, and abundant eosinophilic cytoplasm. However, some histiocytic tumors contained many cells having a smaller, pleomorphic, often elliptical or indented nucleus. The malignant lymphomas, NOS (not otherwise specified), could not be further classified. A

malignant lymphoma, lymphocytic type, was observed in 1/14 (7%) untreated-control females.

The malignant lymphomas were observed to be either generalized, involving several organs, or solitary, involving only one organ. The lymphocytic type of lymphomas appeared first in the thorax, with the thymus and lungs most frequently involved. Other organs, such as the liver, spleen, kidneys, and lymph nodes, were involved, but less frequently. In two mice, only the thymus was involved. The histiocytic type involved primarily the spleen, liver, lungs, and mesenteric lymph nodes. In one mouse, only the spleen contained neoplastic cells.

Lymphocytic leukemia was differentiated from malignant lymphoma, lymphocytic type, by the diffuse infiltration of the neoplastic cells within the involved organs, especially the liver. In the case of lymphoma, the neoplastic cells were more solid in arrangement.

The myocardial sarcomas were observed in 6/32 (19%) low-dose and 2/29 (7%) high-dose males and in 8/31 (26%) low-dose and 1/24 (4%) high-dose females. The neoplastic cells were pleomorphic and varied from those with a small round, basophilic nucleus to those with a large, vesicular nucleus. The larger nuclei contained one or more distinct nucleoli. All cells had an

abundant eosinophilic cytoplasm. The origin of the neoplastic cells was not determined, but they appeared to arise from the perimysial connective tissue surrounding myocardial fibers. The marked proliferation of neoplastic cells resulted in displacement and distortion of normal parenchyma. In some of the mice, the neoplastic cells had invaded through the endocardium and existed as large tumor thrombi within the ventricles. The neoplastic cells had metastasized to the lungs in 3/32 (9%) low-dose males and in 5/31 (16%) low-dose females.

Carcinoma, NOS, or squamous-cell carcinomas arising from the stomach were observed in 1/32 low-dose and 2/29 (7%) high-dose males as well as in 2/31 (6%) low-dose and 2/24 (8%) high-dose females. The carcinomas were characterized by large polyhedral cells that extended deep into the muscular wall of the stomach. The neoplastic cells had a large open-faced nucleus and prominent nucleolus. Large keratin pearls were numerous throughout the neoplasms. The primary tumor had invaded through the stomach wall and transplanted to other abdominal organs in 1/31 (3%) low-dose and 1/24 (4%) high-dose females. In the high-dose female, the tumor also had metastasized to the lungs. In addition, a carcinoma, NOS, of the stomach occurred in a low-dose male.

In addition to the neoplastic lesions, a number of degenerative,

proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups (Appendix D). These nonneoplastic lesions are commonly seen in aged B6C3F1 mice.

Administration of estradiol mustard by oral gavage to B6C3F1 mice for 19 months resulted in an increased incidence of hematopoietic tumors and myocardial sarcomas. It was also associated with a slight increase in the incidence of primary lung neoplasms and of squamous-cell carcinomas of the stomach. Because of the rare occurrence of squamous-cell carcinomas as spontaneous tumors, the few such tumors observed in this study must be considered as related to the administration of estradiol mustard. Based on the histopathologic examination, estradiol mustard administered by oral gavage to B6C3F1 mice appeared to be carcinogenic under the conditions of this bioassay.

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 in one group and with an incidence of at least 5% in one or more than one group. Vehicle-control and pooled vehicle-control groups are used in the statistical analyses. The untreated-control groups are not included in the statistical

analyses, since the test conditions of the vehicle controls are more similar to those of the dosed animals.

Due to the high mortality in the high-dose female mice, time-adjusted analyses are performed in female mice, eliminating animals that died before week 52 on study, unless a tumor was found at the anatomic site of interest before week 52. In such instances, comparisons are based exclusively on the female mice that survived at least as long as the animal in which the first tumor was found. In male mice, time-adjusted analyses are also performed on the incidences of tumors of the lung, liver, and myocardium. Thus, the statistical narrative regarding these incidences of tumors is based on time-adjusted data; however, the non-adjusted statistical data are also included in tables F1 and F2 of Appendix F.

In male mice, the results of the Cochran-Armitage test on the combined incidence of lymphoma or lymphocytic leukemia are significant ($P < 0.001$), and the results of the Fisher exact test show that the incidence in the high-dose group is significantly higher ($P < 0.001$) than the incidence in either group of controls. The results of the Fisher exact test comparing the incidence in the low-dose group with that in the pooled controls is significant ($P = 0.018$). Since the earliest occurrence of lymphoma was observed in male mice at week 25, the time-

adjustment process would not materially affect the results of this analysis. Life tables affecting the time of observation of lymphoma in both male and female mice are shown in figures 5 and 6. The results in the life-table analysis using vehicle-control, low-dose, and high-dose groups indicate a dose association ($P < 0.001$) between the administration of estradiol mustard and the time of observation of lymphoma in either sex of mice. In female mice, the results of the Cochran-Armitage test indicate a probability level of 0.003, and the results of the Fisher exact test show that the incidences in both the low- and high-dose groups are significantly higher than that in the vehicle-control group ($P = 0.020$ and $P = 0.002$, respectively). Results using the pooled-control group are more significant. The statistical conclusion suggests that the incidence of lymphoma in mice is dose associated. The significance of the combined incidence of lymphoma and lymphocytic leukemia in male mice is contributed by the lymphoma and not by the leukemia, which was reported only in one low-dose animal. No leukemia was observed in the female mice.

In male mice, using the pooled-control group, the results of the Cochran-Armitage test on the incidence of alveolar/bronchiolar adenoma or carcinoma and on the incidence of sarcoma of the myocardium are not significant, but departure from linear trend

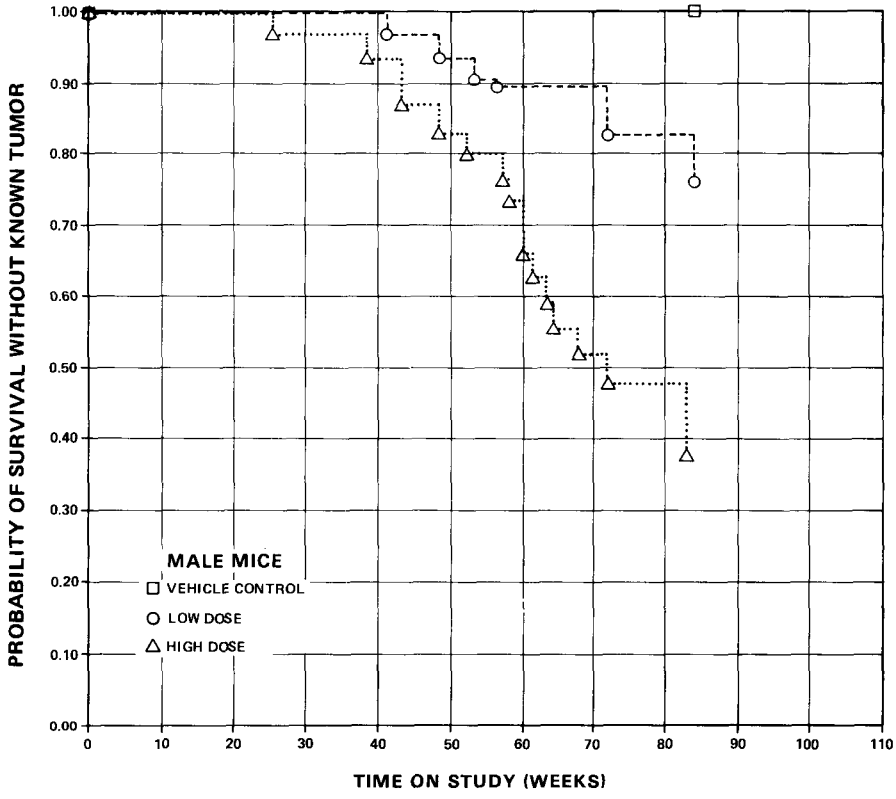


Figure 5. Life Table for Male Mice Administered Estradiol Mustard by Gavage: Combined Incidence of Lymphoma and Lymphocytic Leukemia

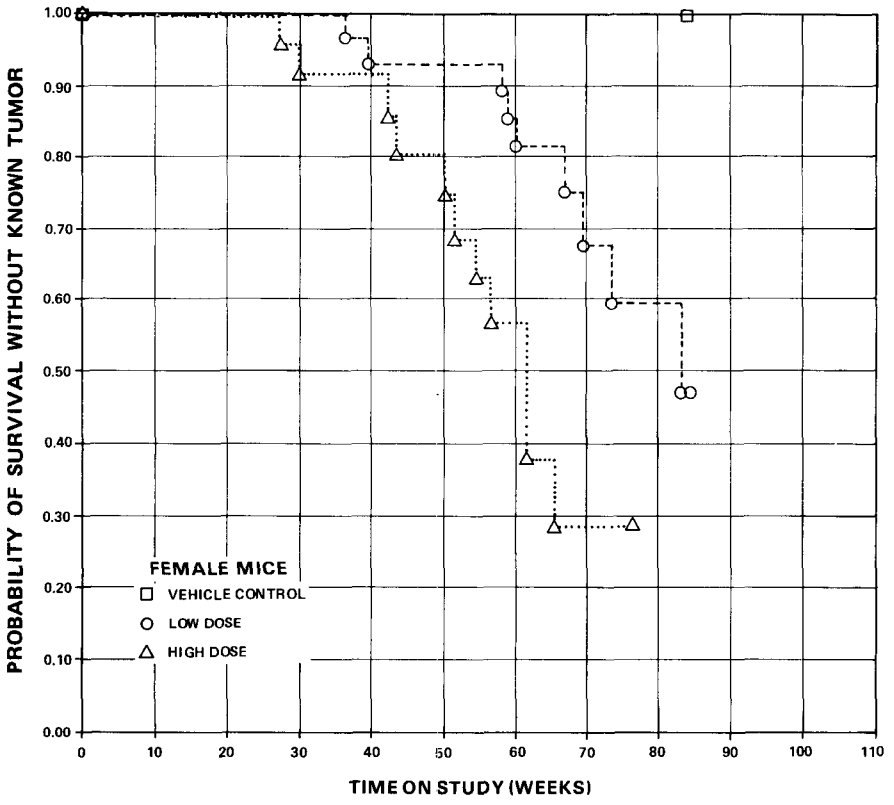


Figure 6. Life Table for Female Mice Administered Estradiol Mustard by Gavage: Lymphoma

is observed ($P \leq 0.020$), because the incidences in the low-dose groups are greater than those in the high-dose groups. The results of the Fisher exact test indicate that the incidences of these tumors in the low-dose groups are significantly higher than those in the pooled vehicle-control groups ($P \leq 0.015$), but no positive results are indicated in the high-dose groups.

Similarly, in female mice, using the pooled controls, the results of the Cochran-Armitage test on the incidence of alveolar/bronchiolar adenoma or carcinoma and on the incidence of sarcoma of the myocardium are not significant, but departure from linearity is observed ($P \leq 0.008$), because the incidences in the low-dose groups are greater than those in the high-dose groups. The results of the Fisher exact test show a P value equal to or less than 0.022 when the incidence of the combined lung tumors in the low-dose groups and the incidence of the myocardium tumors in the low-dose groups are compared with the incidences of their corresponding pooled vehicle controls, but the probability levels of comparisons made using the vehicle-control group are above the 0.025 level ($P = 0.048$ for the incidence in the lung, and $P = 0.029$ for the incidence in the myocardium) required by the Bonferroni inequality criterion when multiple comparison is considered. The results of the bioassay program at this laboratory to date indicate no sarcomas of the myocardium in more

than 500 male and 500 female mice. The statistical conclusion is that the occurrence of these sarcomas in male mice (20%) and in female mice (30%) in this study is associated with the administration of estradiol mustard. The increased mortality in both high-dose groups of mice after 40 weeks on study may have limited the development of tumors in those groups.

The results of the Cochran-Armitage test on the combined incidence of adenocarcinoma and carcinoma of the mammary gland in the female mice indicate a probability level of 0.035 when the vehicle-controls are used, but the results of the Fisher exact test are not significant.

V. DISCUSSION

Mean body weights of male rats and male and female mice administered estradiol mustard by gavage were lower throughout the greater part of the study than those of corresponding vehicle or untreated controls. The mean body weights of dosed female rats were unaffected when compared with controls, and administration of the chemical had no significant effect on the survival of either male or female rats. Thus, the female rats may have been able to tolerate a higher dose. However, the rats were dosed for only 52 weeks, a shorter period of time than is specified in the carcinogensis bioassay (Sontag et al., 1975). A large number of the dosed mice died prior to the end of the bioassay; however, sixty-nine percent or more of the male mice in either dosed group lived for at least 52 weeks on study. Thus, the number of dosed male mice at risk was sufficient for development of tumors appearing up to that time. Time-adjusted analysis and life-table analysis were applied to data obtained with the mice in most cases.

In rats, the incidence of fibroadenoma of the mammary gland in the males was dose related ($P = 0.043$), using pooled vehicle controls (controls 0/18, low-dose 2/35, high-dose 5/33), but the incidence in neither dosed group was significantly higher than that in the controls. Thus, the occurrence of mammary tumors in

the male rats cannot clearly be related to administration of the test chemical. The incidence of mammary tumors in the dosed females was not statistically significant.

In mice, lymphoma or lymphocytic leukemia occurred at significant incidences in low-dose ($P = 0.018$) and high-dose ($P < 0.001$) groups of males compared with those in the pooled vehicle controls (controls 0/28, low-dose 6/32, high-dose 17/29) and at significant incidences in low-dose ($P = 0.020$) and high-dose ($P = 0.002$) groups of females compared with those in the corresponding vehicle controls (controls 0/14, low-dose 9/30, high-dose 11/23). In addition, the incidences of lymphoma were statistically significant for dose-related trend for both the males ($P < 0.001$) and the females ($P = 0.003$). Since lymphoma was observed in male mice as early as 25 weeks, life-table analyses of the incidences in each sex were performed. The results indicated a dose association ($P < 0.001$) between the administration of estradiol mustard and the time of observation of lymphoma in either sex of mice.

In mice, alveolar/bronchiolar adenoma or carcinoma occurred at a significant incidence ($P = 0.004$) in the low-dose group of males compared with the pooled vehicle controls (controls 2/28, low-dose 12/30, high-dose 5/24) and at a significant incidence ($P = 0.022$) in the low-dose group of females compared with the pooled vehicle controls (controls 1/28, low-dose 7/27, high-dose 1/18).

Sarcoma of the myocardium similarly occurred at a significant incidence (P = 0.015) in the low-dose group of males compared with the pooled vehicle controls (controls 0/28, low-dose 6/30, high-dose 2/24) and at a significant incidence (P = 0.002) in the low-dose group of females compared with the pooled vehicle controls (controls 0/28, low-dose 8/27, high-dose 1/12). Metastasis of the myocardial sarcoma to the lung occurred in low-dose males and low-dose females. The survival of both high-dose males and high-dose females was slightly lower than that of the respective low-dose groups and may account for the higher numbers of pulmonary tumors and myocardial sarcomas among low-dose mice of both sexes. The association of myocardial sarcoma with administration of the chemical in both dosed groups of each sex is strengthened by the fact that these tumors of the myocardium have not occurred in the more than 500 male and 500 female historical-control mice of this strain at the laboratory.

Squamous-cell carcinoma of the stomach occurred in the dosed male mice (high-dose 2/29) and in the dosed female mice (low-dose 2/26, high-dose 2/14), but was absent in all controls. A carcinoma, NOS, of the stomach also occurred in a low-dose male mouse. Although the incidences in this bioassay were too low to be statistically significant, the fact that no squamous-cell carcinomas of the stomach have occurred in the more than 500 male

and 500 female historical-control mice of this strain at this laboratory indicates that these gastric tumors were related to the administration of the estradiol mustard.

Two investigations of the carcinogenic potential of the chemotherapeutic agent estradiol mustard have been reported. Stoner et al. (1973), using a pulmonary tumor test system, found a high incidence of lung tumors in male and female A/He mice intraperitoneally injected three times per week for 8 weeks with doses totaling 1.6, 1.2, or 0.48 g/kg body weight, terminating the experiment 24 weeks after the first injection. Schmähl and Habs (1976) subcutaneously injected Sprague-Dawley rats once per week with 10 mg/kg or 20 mg/kg body weight for their life spans. No carcinogenic effects were observed, although the mean life span was considerably reduced (low-dose rats 513 $\pm$ 62 days, high-dose rats 492 $\pm$ 92 days, control rats 721 $\pm$ 123 days.

It is concluded that under the conditions of this bioassay, estradiol mustard administered in a buffered saline vehicle was not carcinogenic in Sprague-Dawley rats. Estradiol mustard was carcinogenic in both male and female B6C3F1 mice, causing lymphoma, sarcoma of the myocardium, alveolar adenoma or carcinoma, and squamous-cell carcinoma of the stomach.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	9	35	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	9	35	33
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(9)	(35)	(33)
SQUAMOUS CELL PAPILLOMA				1 (3%)
FIBROMA				1 (3%)
*SUBCUT TISSUE	(10)	(9)	(35)	(33)
SEBACEOUS ADENOMA				1 (3%)
SARCOMA, NOS			1 (3%)	
FIBROMA			1 (3%)	
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
NONE				
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	(8)	(8)	(35)	(31)
CHROMOPHOBE ADENOMA	2 (25%)	3 (38%)	4 (11%)	
CHROMOPHOBE CARCINOMA	2 (25%)	1 (13%)	1 (3%)	
#ADRENAL	(10)	(9)	(35)	(32)
PHEOCHROMOCYTOMA		1 (11%)		
#THYROID	(10)	(9)	(34)	(32)
FOLLICULAR-CELL ADENOMA			1 (3%)	
C-CELL CARCINOMA			2 (6%)	
#PANCREATIC ISLETS	(10)	(9)	(35)	(32)
ISLET-CELL ADENOMA				1 (3%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(9)	(35)	(33)
ADENOCARCINOMA, NOS			1 (3%)	
FIBROADENOMA			2 (6%)	5 (15%)
#TESTIS	(10)	(9)	(35)	(32)
INTERSTITIAL-CELL TUMOR	1 (10%)			
NERVOUS SYSTEM				
#BRAIN	(10)	(9)	(33)	(32)
GLIOMA, NOS				1 (3%)
ASTROCYTOMA				1 (3%)
SPECIAL SENSE ORGANS				
*EAR CANAL	(10)	(9)	(35)	(33)
SQUAMOUS CELL CARCINOMA			1 (3%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(9)	(35)	(33)
SARCOMA, NOS				1 (3%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@	1	1	2	6
MORIBUND SACRIFICE			1	3
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	9	9	32	26
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	4	10	10
TOTAL PRIMARY TUMORS	5	5	14	12
TOTAL ANIMALS WITH BENIGN TUMORS	3	3	7	8
TOTAL BENIGN TUMORS	3	4	8	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	1	6	3
TOTAL MALIGNANT TUMORS	2	1	6	3
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	8	34	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	8	34	33
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(10)	(8)	(34)	(33)
SARCOMA, NOS				1 (3%)
FIBROMA			1 (3%)	
RESPIRATORY SYSTEM				
#LUNG	(10)	(8)	(34)	(33)
SQUAMOUS CELL CARCINOMA, METASTA				1 (3%)
SARCOMA, NOS, METASTATIC				1 (3%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(8)	(34)	(33)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (3%)
LYMPHOCYTIC LEUKEMIA			1 (3%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(6)	(31)	(33)
CHROMOPHOBE ADENOMA	5 (50%)	4 (67%)	9 (29%)	9 (27%)

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
CHROMOPHOBE CARCINOMA			2 (6%)	2 (6%)
#THYROID	(10)	(7)	(32)	(33)
FOLLICULAR-CELL CARCINOMA				1 (3%)
C-CELL ADENOMA	1 (10%)			
C-CELL CARCINOMA				1 (3%)
#PANCREATIC ISLETS	(10)	(8)	(34)	(33)
ISLET-CELL CARCINOMA			1 (3%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(8)	(34)	(33)
ADENOMA, NOS			1 (3%)	
ADENOCARCINOMA, NOS			3 (9%)	5 (15%)
FIBROADENOMA	5 (50%)	4 (50%)	16 (47%)	16 (48%)
#UTERUS	(10)	(7)	(34)	(33)
SARCOMA, NOS			1 (3%)	
ENDOMETRIAL STROMAL POLYP	1 (10%)			
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(10)	(8)	(34)	(33)
SQUAMOUS CELL CARCINOMA				1 (3%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@		4	6	4
MORIBUND SACRIFICE			4	10
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	10	6	25	21
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	5	27	26
TOTAL PRIMARY TUMORS	12	8	35	37
TOTAL ANIMALS WITH BENIGN TUMORS	7	5	23	22
TOTAL BENIGN TUMORS	12	8	27	25
TOTAL ANIMALS WITH MALIGNANT TUMORS			7	10
TOTAL MALIGNANT TUMORS			8	12
TOTAL ANIMALS WITH SECONDARY TUMORS#				2
TOTAL SECONDARY TUMORS				2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	14	34	35
ANIMALS NECROPSIED	15	14	32	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	14	32	29
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(15)	(14)	(32)	(29)
SARCOMA, NOS			1 (3%)	
RESPIRATORY SYSTEM				
#LUNG	(15)	(14)	(32)	(29)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (13%)	2 (14%)	8 (25%)	4 (14%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			6 (19%)	1 (3%)
SARCOMA, NOS, METASTATIC			3 (9%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(14)	(32)	(29)
MALIGNANT LYMPHOMA, NOS			1 (3%)	7 (24%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			3 (9%)	8 (28%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	
LYMPHOCYTIC LEUKEMIA			1 (3%)	
#SPLEEN	(15)	(14)	(32)	(29)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (3%)
#THYMUS	(15)	(14)	(31)	(29)
MALIG.LYMPHOMA, UNDIFFER-TYPE				1 (3%)
CIRCULATORY SYSTEM				
#MYOCARDIUM	(15)	(14)	(32)	(29)
SARCOMA, NOS			6 (19%)	2 (7%)
HEMANGIOMA				1 (3%)
DIGESTIVE SYSTEM				
#LIVER	(15)	(14)	(32)	(29)
HEPATOCELLULAR ADENOMA	4 (27%)	2 (14%)	3 (9%)	1 (3%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA	1 (7%)		2 (6%)	
SARCOMA, NOS, METASTATIC			1 (3%)	
#STOMACH	(15)	(14)	(32)	(29)
CARCINOMA,NOS			1 (3%)	
SQUAMOUS CELL CARCINOMA				2 (7%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(14)	(32)	(29)
ADENOCARCINOMA, NOS			1 (3%)	
*PREPUTIAL GLAND	(15)	(14)	(32)	(29)
ADNEXAL CARCINOMA				1 (3%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	14	34	35
NATURAL DEATH@	1		19	21
MORIBUND SACRIFICE		1	3	4
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				1
TERMINAL SACRIFICE	14	13	12	9
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	4	24	23
TOTAL PRIMARY TUMORS	7	4	34	29
TOTAL ANIMALS WITH BENIGN TUMORS	5	4	10	6
TOTAL BENIGN TUMORS	6	4	11	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	1		18	20
TOTAL MALIGNANT TUMORS	1		23	23
TOTAL ANIMALS WITH SECONDARY TUMORS#			3	
TOTAL SECONDARY TUMORS			4	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	16	36	35
ANIMALS NECROPSIED	14	16	31	24
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	16	30	24
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(14)	(16)	(31)	(24)
SARCOMA, NOS			2 (6%)	1 (4%)
RESPIRATORY SYSTEM				
#LUNG	(14)	(16)	(31)	(24)
CARCINOMA, NOS, METASTATIC				1 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA			3 (10%)	1 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			4 (13%)	
SARCOMA, NOS, METASTATIC			5 (16%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(16)	(31)	(24)
MALIGNANT LYMPHOMA, NOS			3 (10%)	1 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)		2 (6%)	8 (33%
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			4 (13%)	1 (4%)
#THYMUS	(14)	(16)	(31)	(24)
MALIG.LYMPHOMA, UNDIFFER-TYPE				1 (4%)
CIRCULATORY SYSTEM				
#MYOCARDIUM	(14)	(16)	(31)	(24)
SARCOMA, NOS			8 (26%)	1 (4%)
DIGESTIVE SYSTEM				
#STOMACH	(14)	(16)	(31)	(24)
SQUAMOUS CELL CARCINOMA			2 (6%)	2 (8%)
URINARY SYSTEM				
NONE				

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(14)	(16)	(31)	(24)
CARCINOMA,NOS				1 (4%)
ADENOCARCINOMA, NOS				2 (8%)
*VAGINA	(14)	(16)	(31)	(24)
SQUAMOUS CELL CARCINOMA				1 (4%)
#UTERUS	(14)	(16)	(31)	(24)
ADENOCARCINOMA, NOS			1 (3%)	
ENDOMETRIAL STROMAL POLYP	1 (7%)			
#OVARY	(14)	(16)	(31)	(24)
CYSTADENOMA, NOS			1 (3%)	
HILAR-CELL TUMOR				1 (4%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(14)	(16)	(31)	(24)
ADENOMA, NOS				1 (4%)
*EAR CANAL	(14)	(16)	(31)	(24)
SQUAMOUS CELL CARCINOMA			1 (3%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(14)	(16)	(31)	(24)
SQUAMOUS CELL CARCINOMA, METASTA			1 (3%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SARCOMA, NOS			1 (3%)	1 (4%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(16)	(31)	(24)
SQUAMOUS CELL CARCINOMA, METASTA				1 (4%)
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	16	36	35
NATURAL DEATH@	1	1	24	19
MORIBUND SACRIFICE	1	2	7	14
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED	2	1	1	2
TERMINAL SACRIFICE	11	12	4	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2		25	20
TOTAL PRIMARY TUMORS	2		32	23
TOTAL ANIMALS WITH BENIGN TUMORS	1		4	3
TOTAL BENIGN TUMORS	1		4	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	1		25	19
TOTAL MALIGNANT TUMORS	1		28	20
TOTAL ANIMALS WITH SECONDARY TUMORS#			6	2
TOTAL SECONDARY TUMORS			6	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	9	35	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	9	35	33
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(9)	(35)	(33)
ULCER, CHRONIC				1 (3%)
INFLAMMATION, CHRONIC NECROTIZIN			1 (3%)	
HYPERKERATOSIS				1 (3%)
ACANTHOSIS				1 (3%)
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(9)	(35)	(33)
INFLAMMATION, SUPPURATIVE				1 (3%)
INFLAMMATION, CHRONIC	1 (10%)	3 (33%)	5 (14%)	5 (15%)
INFLAMMATION, CHRONIC SUPPURATIV			2 (6%)	
#LUNG/BRONCHIOLE	(10)	(9)	(35)	(33)
HYPERPLASIA, LYMPHOID		1 (11%)	1 (3%)	
#LUNG	(10)	(9)	(35)	(33)
EMPHYSEMA, NOS				1 (3%)
INFLAMMATION, INTERSTITIAL			1 (3%)	1 (3%)
PNEUMONIA, LIPID				1 (3%)
BRONCHOPNEUMONIA SUPPURATIVE	1 (10%)	1 (11%)		2 (6%)
BRONCHOPNEUMONIA CHRONIC SUPPURA		2 (22%)	5 (14%)	1 (3%)
METAPLASIA, OSSEOUS			1 (3%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(9)	(8)	(35)	(32)
ATROPHY, NOS	6 (67%)	6 (75%)	17 (49%)	19 (59%)
HYPERPLASIA, GRANULOCYTIC			1 (3%)	
#SPLEEN	(10)	(9)	(35)	(32)
INFLAMMATION, SUPPURATIVE			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
HYPERPLASIA, GRANULOCYTIC			1 (3%)	
HEMATOPOIESIS			1 (3%)	1 (3%)
#SPLENIC FOLLICLES	(10)	(9)	(35)	(32)
ATROPHY, NOS			1 (3%)	
#MANDIBULAR L. NODE		(1)	(1)	
HYPERPLASIA, LYMPHOID			1 (100%)	
#MESENTERIC L. NODE		(1)	(1)	
INFLAMMATION, CHRONIC SUPPURATIV		1 (100%)		
INFLAMMATION, CHRONIC NECROTIZIN			1 (100%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(10)	(9)	(35)	(32)
NECROSIS, COAGULATIVE				1 (3%)
HYPERPLASIA, GRANULOCYTIC			1 (3%)	
#LIVER/CENTRILOBULAR	(10)	(9)	(35)	(32)
LIPOIDOSIS				1 (3%)
#PANCREAS	(10)	(9)	(35)	(32)
PERIARTERITIS				1 (3%)
#PANCREATIC ACINUS	(10)	(9)	(35)	(32)
ATROPHY, NOS				2 (6%)
#STOMACH	(10)	(9)	(35)	(32)
NECROSIS, NOS				1 (3%)
#GASTRIC MUSCULARIS	(10)	(9)	(35)	(32)
MINERALIZATION				1 (3%)
#DUODENUM	(10)	(9)	(35)	(32)
CONGENITAL MALFORMATION, NOS		1 (11%)		
URINARY SYSTEM				
#KIDNEY	(10)	(9)	(35)	(32)
INFLAMMATION, CHRONIC	5 (50%)	8 (89%)	23 (66%)	22 (69%)

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
#URINARY BLADDER	(10)	(9)	(35)	(32)
ULCER, NOS				1 (3%)
ENDOCRINE SYSTEM				
#PITUITARY	(8)	(8)	(35)	(31)
CYST, NOS				1 (3%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(9)	(35)	(33)
CYST, NOS			1 (3%)	
#PROSTATE	(10)	(9)	(35)	(32)
INFLAMMATION, SUPPURATIVE				1 (3%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE/CORNEA	(10)	(9)	(35)	(33)
ULCER, CHRONIC				1 (3%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(9)	(35)	(33)
STEATITIS	1 (10%)			2 (6%)
*PLEURA	(10)	(9)	(35)	(33)
METAPLASIA, OSSEOUS			1 (3%)	
ALL OTHER SYSTEMS				
NONE				

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	2		4	2
AUTOLYSIS/NO NECROPSY		1		2

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	8	34	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	8	34	33
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(8)	(34)	(33)
INFLAMMATION, CHRONIC SUPPURATIV				2 (6%)
#LUNG/BRONCHIOLE	(10)	(8)	(34)	(33)
HYPERPLASIA, LYMPHOID			1 (3%)	
#LUNG	(10)	(8)	(34)	(33)
BRONCHOPNEUMONIA SUPPURATIVE		1 (13%)		4 (12%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (10%)			
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(9)	(8)	(32)	(32)
ATROPHY, NOS	6 (67%)	4 (50%)	16 (50%)	14 (44%)
#SPLEEN	(10)	(8)	(34)	(33)
HEMATOPOIESIS	1 (10%)	1 (13%)	3 (9%)	3 (9%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(10)	(8)	(34)	(33)
NECROSIS, COAGULATIVE			1 (3%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#LIVER/CENTRILOBULAR	(10)	(8)	(34)	(33)
NECROSIS, NOS				1 (3%)
NECROSIS, COAGULATIVE		1 (13%)		1 (3%)
#PANCREAS	(10)	(8)	(34)	(33)
INFLAMMATION, CHRONIC			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(10)	(8)	(34)	(33)
INFLAMMATION, CHRONIC			6 (18%)	8 (24%)
ENDOCRINE SYSTEM				
#ADRENAL	(10)	(8)	(34)	(33)
ANGIECTASIS	3 (30%)	1 (13%)	12 (35%)	7 (21%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(8)	(34)	(33)
CYST, NOS	3 (30%)	1 (13%)	4 (12%)	3 (9%)
#UTERUS	(10)	(7)	(34)	(33)
HEMORRHAGE				1 (3%)
#UTERUS/ENDOMETRIUM	(10)	(7)	(34)	(33)
INFLAMMATION, SUPPURATIVE	1 (10%)	1 (14%)	6 (18%)	5 (15%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (10%)		4 (12%)	3 (9%)
HYPERPLASIA, NOS			1 (3%)	
HYPERPLASIA, CYSTIC				2 (6%)
#OVARY	(10)	(7)	(34)	(33)
CYST, NOS			1 (3%)	1 (3%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*AURICULAR CARTILAGE	(10)	(8)	(34)	(33)
DYSPLASIA, NOS			1 (3%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(8)	(34)	(33)
STEATITIS				1 (3%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1	2	1	1
AUTOLYSIS/NO NECROPSY		2	1	2

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	14	34	35
ANIMALS NECROPSIED	15	14	32	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	14	32	29
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(14)	(32)	(29)
ULCER, FOCAL			1 (3%)	
*SUBCUT TISSUE	(15)	(14)	(32)	(29)
EDEMA, NOS			1 (3%)	
INFLAMMATION, CHRONIC		1 (7%)		
INFLAMMATION, CHRONIC FOCAL			1 (3%)	
RESPIRATORY SYSTEM				
#LUNG/BRONCHIOLE	(15)	(14)	(32)	(29)
HYPERPLASIA, PLASMA CELL			1 (3%)	
#LUNG	(15)	(14)	(32)	(29)
BRONCHOPNEUMONIA SUPPURATIVE	1 (7%)			1 (3%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(14)	(13)	(30)	(26)
ATROPHY, NOS				2 (8%)
#SPLEEN	(15)	(14)	(32)	(29)
ANGIECTASIS				1 (3%)
HYPERPLASIA, LYMPHOID				1 (3%)
HEMATOPOIESIS	1 (7%)		2 (6%)	3 (10%)
#LYMPH NODE		(2)	(3)	(3)
CYST, NOS		1 (50%)		
HEMORRHAGE		1 (50%)		
#CERVICAL LYMPH NODE		(2)	(3)	(3)
HYPERPLASIA, LYMPHOID			1 (33%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE		(2)	(3)	(3)
INFLAMMATION WITH FIBROSIS			1 (33%)	
HYPERPLASIA, LYMPHOID				2 (67%)
#PELVIC LYMPH NODE		(2)	(3)	(3)
INFLAMMATION, SUPPURATIVE			1 (33%)	
ABSCESS, NOS			1 (33%)	
#THYMUS	(15)	(14)	(31)	(29)
HYPERPLASIA, LYMPHOID				1 (3%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(14)	(32)	(29)
INFLAMMATION, SUPPURATIVE	1 (7%)			1 (3%)
NECROSIS, FOCAL	1 (7%)			
NECROSIS, COAGULATIVE				1 (3%)
LIPOIDOSIS	1 (7%)			
HYPERPLASIA, NODULAR	1 (7%)	1 (7%)		
HYPERPLASIA, GRANULOCYTIC	1 (7%)			
*BILE DUCT	(15)	(14)	(32)	(29)
HYPERPLASIA, NOS	1 (7%)			
#PANCREAS	(15)	(14)	(32)	(29)
INFLAMMATION WITH FIBROSIS			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(15)	(14)	(32)	(29)
PYELONEPHRITIS SUPPURATIVE	1 (7%)			
AMYLOIDOSIS			1 (3%)	
#URINARY BLADDER	(15)	(14)	(32)	(29)
INFLAMMATION, CHRONIC			1 (3%)	
ULCER, CHRONIC				1 (3%)
ENDOCRINE SYSTEM				
#ADRENAL	(15)	(14)	(32)	(29)
AMYLOIDOSIS			1 (3%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM				
#PROSTATE	(15)	(14)	(32)	(29)
INFLAMMATION, SUPPURATIVE			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*BONE	(15)	(14)	(32)	(29)
HEALED FRACTURE				1 (3%)
CALLUS				1 (3%)
BODY CAVITIES				
*PERITONEUM	(15)	(14)	(32)	(29)
INFLAMMATION, CHRONIC FOCAL			1 (3%)	
*MESENTERY	(15)	(14)	(32)	(29)
NECROSIS, FAT	1 (7%)			
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(14)	(32)	(29)
HYPERPLASIA, GRANULOCYTIC		1 (7%)		
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	5	8	6	2
NO NECROPSY PERFORMED			1	1
AUTOLYSIS/NO NECROPSY			1	5

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	16	36	35
ANIMALS NECROPSIED	14	16	31	24
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	16	30	24
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(14)	(16)	(31)	(24)
INFLAMMATION, NECROTIZING		1 (6%)		
RESPIRATORY SYSTEM				
#LUNG/BRONCHIOLE	(14)	(16)	(31)	(24)
HYPERPLASIA, LYMPHOID			1 (3%)	
#LUNG	(14)	(16)	(31)	(24)
BRONCHOPNEUMONIA SUPPURATIVE			2 (6%)	1 (4%)
BRONCHOPNEUMONIA CHRONIC SUPPURA				1 (4%)
HYPERPLASIA, LYMPHOID		1 (6%)		
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(12)	(16)	(30)	(23)
ATROPHY, NOS	3 (25%)	2 (13%)	1 (3%)	5 (22%)
HYPERPLASIA, GRANULOCYTIC				1 (4%)
#SPLEEN	(14)	(16)	(31)	(24)
HYPERPLASIA, GRANULOCYTIC				3 (13%)
HYPERPLASIA, LYMPHOID	1 (7%)	1 (6%)		
HEMATOPOIESIS	1 (7%)	2 (13%)	1 (3%)	2 (8%)
#MESENTERIC L. NODE	(1)	(1)	(1)	(3)
CONGESTION, NOS		1 (100%)		
CIRCULATORY SYSTEM				
#MYOCARDIUM	(14)	(16)	(31)	(24)
INFLAMMATION, SUPPURATIVE			1 (3%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(14)	(16)	(30)	(24)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)	
ADHESION, NOS			1 (3%)	
HYPERPLASIA, NODULAR			1 (3%)	
ANGIECTASIS				1 (4%)
HEMATOPOIESIS		1 (6%)		
#LIVER/CENTRILOBULAR	(14)	(16)	(30)	(24)
NECROSIS, NOS			2 (7%)	
URINARY SYSTEM				
#KIDNEY	(14)	(16)	(31)	(24)
INFLAMMATION, SUPPURATIVE				1 (4%)
INFLAMMATION, CHRONIC			2 (6%)	
HYPERPLASIA, LYMPHOID		1 (6%)		
#KIDNEY/GLOMERULUS	(14)	(16)	(31)	(24)
AMYLOIDOSIS			1 (3%)	
#KIDNEY/PELVIS	(14)	(16)	(31)	(24)
HYPERPLASIA, LYMPHOID	1 (7%)			
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(14)	(16)	(31)	(24)
CYST, NOS			1 (3%)	2 (8%)
*VAGINA	(14)	(16)	(31)	(24)
INFLAMMATION, SUPPURATIVE			1 (3%)	
#UTERUS/ENDOMETRIUM	(14)	(16)	(31)	(24)
INFLAMMATION, SUPPURATIVE			1 (3%)	
INFLAMMATION, HEMORRHAGIC			2 (6%)	
HYPERPLASIA, CYSTIC	7 (50%)	12 (75%)	6 (19%)	5 (21%)
#OVARY	(14)	(16)	(31)	(24)
INFLAMMATION, HEMORRHAGIC			1 (3%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, CYSTIC			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE/LACRIMAL GLAND	(14)	(16)	(31)	(24)
HYPERPLASIA, NOS		1 (6%)		
*EAR CANAL	(14)	(16)	(31)	(24)
INFLAMMATION, SUPPURATIVE		1 (6%)		
ABSCESS, NOS		1 (6%)		
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(14)	(16)	(31)	(24)
STEATITIS	1 (7%)			
*PERITONEUM	(14)	(16)	(31)	(24)
INFLAMMATION, CHRONIC			1 (3%)	
ADHESION, NOS			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	5	2	4	1
ACCIDENTAL DEATH			1	1
NO NECROPSY PERFORMED	1			
AUTO/NECROPSY/NO HISTO			1	
AUTOLYSIS/NO NECROPSY			4	10

(N) NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Estradiol Mustard by Gavage[a]

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	3/17 (18)	3/8 (38)	4/35 (11)	0/31 (0)
P Values[c,d]	P = 0.025(N)	P = 0.003(N)	N.S.	P = 0.006*(N) P = 0.039**(N)
Relative Risk (Pooled Vehicle Control)[f]			0.648	0.000
Lower Limit			0.127	0.000
Upper Limit			4.049	0.888
Relative Risk (Vehicle Control)[f]			0.305	0.000
Lower Limit			0.076	0.000
Upper Limit			1.821	0.408
Weeks to First Observed Tumor		86	85	--
Pituitary: Chromophobe Adenoma or Carcinoma[b]	5/17 (29)	4/8 (50)	5/35 (14)	0/31 (0)
P Values[c,d]	P = 0.003(N)	P < 0.001(N)	P = 0.046*(N)	P = 0.001*(N) P = 0.004**(N)
Relative Risk (Pooled Vehicle Control)[f]			0.486	0.000
Lower Limit			0.135	0.000
Upper Limit			1.867	0.421
Relative Risk (Vehicle Control)[f]			0.286	0.000
Lower Limit			0.099	0.000
Upper Limit			1.209	0.261
Weeks to First Observed Tumor		86	79	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	0/18 (0)	0/9 (0)	2/34 (6)	0/32 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	--
Lower Limit			0.164	--
Upper Limit			Infinite	--
Relative Risk (Vehicle Control)[f]			Infinite	--
Lower Limit			0.088	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor		--	85	--
Mammary Gland: Fibroadenoma[b]	0/18 (0)	0/9 (0)	2/35 (6)	5/33 (15)
P Values[c,d]	P = 0.043	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.159	0.724
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.085	0.391
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	85	85

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma or Adenocarcinoma, NOS[b]	0/18 (0)	0/9 (0)	3/35 (9)	5/33 (15)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.324	0.724
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.175	0.391
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	85	85
Brain: Glioma, NOS, or Astrocytoma[b]	0/18 (0)	0/9 (0)	0/33 (0)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.174
Upper Limit			--	Infinite
Relative Risk (Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.093
Upper Limit			--	Infinite
Weeks to First Observed Tumor		--	--	63

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Estradiol Mustard by Gavage[a]

(continued)

[a]Treated groups received doses of 0.62 or 1.25 mg/kg body weight.

[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 treated group is the probability level for the Fisher exact test for the comparison of that treated group with the 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 treated 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 treated group and the specified control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Estradiol Mustard by Gavage[a]

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Pituitary : Chromophobe Adenoma or Carcinoma[b]	10/16 (63)	4/6 (67)	11/31 (35)	11/33 (33)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			0.568	0.533
Lower Limit			0.314	0.294
Upper Limit			1.187	1.123
Relative Risk (Vehicle Control)[f]			0.532	0.500
Lower Limit			0.316	0.296
Upper Limit			1.766	1.670
Weeks to First Observed Tumor		73	77	66
Mammary Gland: Adenocarcinoma, NOS[b]	1/18 (6)	0/8 (0)	3/34 (9)	5/33 (15)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			1.588	2.727
Lower Limit			0.142	0.346
Upper Limit			80.808	124.585
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.163	0.354
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	77	53

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	8/18 (44)	4/8 (50)	16/34 (47)	16/33 (48)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			1.059	1.091
Lower Limit			0.556	0.574
Upper Limit			2.327	2.384
Relative Risk (Vehicle Control)[f]			0.941	0.970
Lower Limit			0.480	0.495
Upper Limit			3.105	3.182
Weeks to First Observed Tumor		74	67	63
Mammary Gland: Adenoma, NOS, Adenocarcinoma, NOS, or Fibroadenoma[b]	9/18 (50)	4/8 (50)	20/34 (59)	20/33 (61)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			1.176	1.212
Lower Limit			0.682	0.703
Upper Limit			2.301	2.350
Relative Risk (Vehicle Control)[f]			1.176	1.212
Lower Limit			0.621	0.640
Upper Limit			3.701	3.780
Weeks to First Observed Tumor		74	67	53

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Estradiol Mustard by Gavage[a]

(continued)

[a]Treated groups received doses of 0.62 or 1.25 mg/kg body weight.

[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 treated group is the probability level for the Fisher exact test for the comparison of that treated group with the 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 treated 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 treated group and the specified control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED ESTRADIOL MUSTARD BY GAVAGE

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/28 (0)	0/14 (0)	6/32 (19)	1/29 (3)
P Values[c,d]	N.S.	N.S.	P = 0.018**	N.S.
Departure from Linear Trend[e]	P = 0.005	P = 0.015		
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.437	0.053
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.754	0.027
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	72	83

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	2/28 (7)	2/14 (14)	12/32 (38)	5/29 (17)
P Values[c,d]	N.S.	N.S.	P = 0.006**	N.S.
Departure from Linear Trend[e]	P = 0.006	P = 0.037		
Relative Risk (Pooled Vehicle Control)[f]			5.250	2.414
Lower Limit			1.321	0.436
Upper Limit			44.443	23.631
Relative Risk (Vehicle Control)[f]			2.625	1.207
Lower Limit			0.717	0.236
Upper Limit			21.934	11.666
Weeks to First Observed Tumor		84	59	68

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Lymphocytic Leukemia[b]	0/28 (0)	0/14 (0)	6/32 (19)	17/29 (59)
P Values[c,d]	P < 0.001	P < 0.001	P = 0.018**	P < 0.001* P < 0.001**
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.437	5.435
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.754	2.848
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	41	25
Myocardium: Sarcoma, NOS[b]	0/28 (0)	0/14 (0)	6/32 (19)	2/29 (7)
P Values[c,d]	N.S.	N.S.	P = 0.018**	N.S.
Departure from Linear Trend[e]	P = 0.016	P = 0.039		
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.437	0.292
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.754	0.152
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	54	81

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Stomach: Squamous-cell Carcinoma[b]	0/28 (0)	0/14 (0)	0/32 (0)	2/29 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.292
Upper Limit			--	Infinite
Relative Risk (Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.152
Upper Limit			--	Infinite
Weeks to First Observed Tumor		--	--	81
Liver: Hepatocellular Carcinoma[b]	0/28 (0)	0/14 (0)	2/32 (6)	0/29 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	--
Lower Limit			0.264	--
Upper Limit			Infinite	--
Relative Risk (Vehicle Control)[f]			Infinite	--
Lower Limit			0.138	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor		--	78	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	6/28 (21)	2/14 (14)	5/32 (16)	1/29 (3)
P Values[c,d]	P = 0.037(N)	N.S.	N.S.	P = 0.046**(N)
Relative Risk (Pooled Vehicle Control)[f]			0.729	0.161
Lower Limit			0.198	0.004
Upper Limit			2.559	1.208
Relative Risk (Vehicle Control)[f]			1.094	0.241
Lower Limit			0.214	0.004
Upper Limit			10.641	4.322
Weeks to First Observed Tumor		84	78	83

[a]Treated groups received doses of 15 or 30 mg/kg body weight.

[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 treated group is the probability level for the Fisher exact test for the comparison of that treated group with the 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 F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/31 (0)	0/16 (0)	4/31 (13)	0/24 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.007	P = 0.020		
Relative Risk (Pooled Vehicle Control)[f]			Infinite	--
Lower Limit			0.946	--
Upper Limit			Infinite	--
Relative Risk (Vehicle Control)[f]			Infinite	--
Lower Limit			0.508	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor		--	64	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/31 (3)	0/16 (0)	7/31 (23)	1/24 (4)
P Values[c,d]	N.S.	N.S.	P = 0.042* P = 0.026**	N.S.
Departure from Linear Trend[e]	P = 0.007	P = 0.008		
Relative Risk (Pooled Vehicle Control)[f]			7.000	1.292
Lower Limit			0.984	0.017
Upper Limit			302.728	97.132
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.068	0.037
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	64	40

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System:				
Lymphoma[b]	0/31 (0)	0/16 (0)	9/31 (29)	11/24 (46)
P Values[c,d]	P < 0.001	P = 0.002	P = 0.015* P = 0.001**	P = 0.001* P < 0.001**
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			2.692	4.437
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.448	2.384
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	36	27

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography:	Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Myocardium:	Sarcoma, NOS[b]	0/31 (0)	0/16 (0)	8/31 (26)	1/24 (4)
P Values[c,d]		N.S.	N.S.	P = 0.025* P = 0.002**	N.S.
Departure from Linear Trend[e]		P < 0.001	P = 0.004		
Relative Risk (Pooled Vehicle Control)[f]				Infinite	Infinite
	Lower Limit			2.338	0.070
	Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]				Infinte	Infinite
	Lower Limit			1.257	0.037
	Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor			--	52	60

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Stomach: Squamous-cell Carcinoma[b]	0/31 (0)	0/16 (0)	2/31 (6)	2/24 (8)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.301	0.389
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.161	0.208
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	76	50
Mammary Gland: Adenocarcinoma or Carcinoma, NOS[b]	0/31 (0)	0/16 (0)	0/31 (0)	3/24 (13)
P Values[c,d]	P = 0.022	P = 0.044	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.794
Upper Limit			--	Infinite
Relative Risk (Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.426
Upper Limit			--	Infinite
Weeks to First Observed Tumor		--	--	40

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

[a]Treated groups received doses of 15 or 30 mg/kg body weight.

[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 treated group is the probability level for the Fisher exact test for the comparison of that treated group with the 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 treated 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 treated group and the specified control group.

Table F3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (52)[b]	0/28 (0)	0/13 (0)	6/30 (20)	1/24 (4)
P Values[c,d]	N.S.	N.S.	P = 0.015**	N.S.
Departure from Linear Trend[e]	P = 0.006	P = 0.020		
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.534	0.063
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.753	0.031
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	72	83

Table F3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (52)[b]	2/28 (7)	2/13 (15)	12/30 (40)	5/24 (21)
P Values[c,d]	N.S.	N.S.	P = 0.004**	N.S.
Departure from Linear Trend[e]	P = 0.008			
Relative Risk (Pooled Vehicle Control)[f]			5.600	2.917
Lower Limit			1.415	0.530
Upper Limit			47.024	28.113
Relative Risk (Vehicle Control)[f]			2.600	1.354
Lower Limit			0.721	0.270
Upper Limit			21.512	12.864
Weeks to First Observed Tumor		84	59	68

Table F3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Myocardium:				
Sarcoma, NOS (52)[b]	0/28 (0)	0/13 (0)	6/30 (20)	2/24 (8)
P Values[c,d]	N.S.	N.S.	P = 0.015**	N.S.
Departure from Linear Trend[e]	P = 0.020			
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.534	0.353
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.753	0.172
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	54	81
Liver: Hepatocellular				
Carcinoma (52)[b]	0/28 (0)	0/13 (0)	2/30 (7)	0/24 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	--
Lower Limit			0.282	--
Upper Limit			Infinite	--
Relative Risk (Vehicle Cotrol)[f]			Infinite	--
Lower Limit			0.138	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor		--	78	--

Table F3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma (52)[b]	6/28 (21)	2/13 (15)	5/30 (17)	1/24 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			0.778	0.194
Lower Limit			0.212	0.004
Upper Limit			2.715	1.440
Relative Risk (Vehicle Control)[f]			1.083	0.271
Lower Limit			0.215	0.005
Upper Limit			10.475	4.791
Weeks to First Observed Tumor		84	78	83

[a]Treated groups received doses of 15 or 30 mg/kg body weight.

[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 the parenthesis, 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 treated group is the probability level for the Fisher exact test for the comparison of that treated group with the vehicle-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

Table F3. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Administered Estradiol Mustard by Gavage[a]

(continued)

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

Table F4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (52)[b]	0/28 (0)	0/13 (0)	4/26 (15)	0/13 (0)
P Values[c,d]	N.S.	N.S.	P = 0.047**	N.S.
Departure from Linear Trend[e]	P = 0.012	P = 0.037		
Relative Risk (Pooled Vehicle Control)[f]			Infinite	--
Lower Limit			1.026	--
Upper Limit			Infinite	--
Relative Risk (Vehicle Control)[f]			Infinite	--
Lower Limit			0.503	
Upper Limit			Infinite	--
Weeks to First Observed Tumor		--	64	--

Table F4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (40)[b]	1/28 (4)	0/13 (0)	7/27 (26)	1/18 (6)
P Values[c,d]	N.S.	N.S.	P = 0.048* P = 0.022**	N.S.
Departure from Linear Trend[e]	P = 0.008	P = 0.012		
Relative Risk (Pooled Vehicle Control)[f]			7.259	1.556
Lower Limit			1.032	0.021
Upper Limit			311.223	115.144
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.018	0.041
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	64	40

Table F4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (27)[b]	0/29 (0)	0/14 (0)	9/30 (30)	11/23 (48)
P Values[c,d]	P < 0.001	P = 0.003	P = 0.020* P = 0.001**	P = 0.002* P < 0.001**
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			2.612	4.353
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.326	2.206
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	36	27
Myocardium: Sarcoma, NOS (52)[b]	0/28 (0)	0/13 (0)	8/27 (30)	1/12 (8)
P Values[c,d]	N.S.	N.S.	P = 0.029* P = 0.002**	N.S.
Departure from Linear Trend[e]	P = 0.004	P = 0.015		
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			2.443	0.127
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.199	0.061
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	52	60

Table F4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

Topography: Morphology	Pooled Vehicle Control	Vehicle Control	Low Dose	High Dose
Stomach: Squamous-cell Carcinoma (50)[b]	0/28 (0)	0/13 (0)	2/26 (8)	2/14 (14)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.326	0.608
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.159	0.297
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	76	50
Mammary Gland: Adenocarcinoma or Carcinoma, NOS (40)[b]	0/28 (0)	0/13 (0)	0/27 (0)	3/18 (17)
P Values[c,d]	P = 0.015	P = 0.035	N.S.	N.S.
Relative Risk (Pooled Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.964
Upper Limit			--	Infinite
Relative Risk (Vehicle Control)[f]			--	Infinite
Lower Limit			--	0.472
Upper Limit			--	Infinite
Weeks to First Observed Tumor		--	--	40

Table F4. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Mice Administered Estradiol Mustard by Gavage[a]

(continued)

[a]Treated groups received doses of 15 or 30 mg/kg body weight.

[b]Number of tumor-bearing animals/number of animals examined at site (percent), based on number of 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 treated group is the probability level for the Fisher exact test for the comparison of that treated group with the vehicle-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

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

Review of the Bioassay of Estradiol Mustard* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

April 26, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/ Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Estradiol Mustard for carcinogenicity.

The primary reviewer noted that the compound is an anti-neoplastic agent targeted against tumors thought to have estrogen receptors. She agreed with the conclusion in the report that Estradiol Mustard was carcinogenic in both sexes of mice. Although the report concludes that Estradiol Mustard was not carcinogenic in rats, the primary reviewer noted that mammary tumors were observed in 8/68 treated males while only 6/215 mammary tumors have been reported in historic male control rats. Given these findings, she recommended that the report give greater emphasis to the possible significance of the mammary tumors in treated male rats. Although the small control group size and the high mortality rate reduced the sensitivity of the study, she said that the shortcomings did not invalidate the conclusion on the carcinogenicity of Estradiol Mustard. Based on the bioassay, the primary reviewer concluded that Estradiol Mustard could pose a carcinogenic risk to humans.

A Program staff pathologist commented that 5 of the mammary tumors in the treated male rats were fibroadenomas. A Subgroup member noted that a number of mammary tumors also were found in the treated female rats. He pointed out that the Sprague-Dawley strain is particularly sensitive to the induction of mammary tumors. Another Subgroup member opined that the mammary tumors may have been due to the estrogen component of the Estradiol Mustard.

A motion was approved unanimously that the report on the bioassay of Estradiol Mustard be accepted with the proviso that greater emphasis be given to the possible significance of the mammary tumors found in treated rats.

Members present were:

Michael Shimkin (Acting Chairman), University of California at San Diego
Joseph Highland, Environmental Defense Fund
George Roush, Jr., Monsanto Company
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation

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

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