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BIOASSAY OF

ISOPHOSPHAMIDE

FOR POSSIBLE CARCINOGENICITY

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SUMMARY

A bioassay of the anticancer drug isophosphamide for possible carcinogenicity was conducted by injecting the test chemical intraperitoneally into Sprague-Dawley rats and B6C3F1 mice.

Groups of 35 rats and 35 mice of each sex were given the injections at one of two doses three times per week for 52 weeks. Doses for rats were either 6 or 12 mg/kg, and for mice either 10 or 20 mg/kg. After the period of administration of the isophosphamide, the surviving rats were observed for 31 weeks and the mice for 28 weeks. Untreated controls as well as vehicle controls (buffered saline) were used. The matched vehicle-control groups each consisted of 10 rats or 15 mice of each sex; pooled vehicle-control groups, used for statistical evaluation, consisted of the matched vehicle controls of each species combined with 20 male and 20 female matched vehicle-control rats or 15 male and 15 female matched vehicle-control mice from a similar bioassay of another test chemical. All surviving rats were killed at 79-84 weeks, all surviving mice at 79-81 weeks.

Mean body weights of the high-dose rats of either sex were lower than those of the matched vehicle controls after approximately 25 weeks on study. Survival was low among the high-dose male and female rats, but in the low-dose groups it was adequate for meaningful statistical analyses of the incidences of tumors. Mean body weights of the mice did not show any consistent effect from the isophosphamide treatment. Survival was adequate for meaningful statistical analyses in both groups of female mice, while survival of the males was 31% for both treated groups at the end of the study.

In male rats, tumors of the hematopoietic system included six malignant lymphomas and two granulocytic leukemias. With the unadjusted analysis, these tumors showed a dose-related trend in male rats using pooled vehicle controls (controls 0/29, low-dose 3/32, high-dose 5/34, $P = 0.032$) and a higher incidence in the high-dose males than in the pooled vehicle controls ($P = 0.040$). These tumors were not significant when compared with matched

vehicle controls using adjusted analyses, and they cannot clearly be associated with treatment. However, it should be noted that five rats with these tumors were observed in the high-dose group whose median survival was only 35 weeks.

In female rats, the incidence of uterine leiomyosarcoma was significant in the low-dose group using pooled vehicle controls (controls 0/27, low-dose 15/32, $P < 0.001$). There was also a significant incidence of mammary fibroadenoma among low-dose females using pooled vehicle controls (controls 8/28, low-dose 28/33, $P < 0.001$). The incidence of each tumor was also significant when compared with matched vehicle controls using time-adjusted analyses. The low survival of the high-dose group (median time on study, 35 weeks) may explain the lower incidences of the uterine leiomyosarcoma and the mammary fibroadenoma in this group. In some rats, the leiomyosarcomas metastasized to the lungs, urinary bladder, spleen, and other abdominal sites.

In female mice, the incidence of malignant lymphoma of the hematopoietic system showed a significant dose-related trend using either matched vehicle controls (controls 0/14, low-dose 3/32, high-dose 13/34, $P = 0.001$) or pooled vehicle controls (controls 1/29, $P < 0.001$). Further, incidences of this tumor in the high-dose females were significantly higher than incidences in the matched vehicle ($P = 0.005$) or pooled vehicle ($P = 0.001$) controls.

It is concluded that under the conditions of this bioassay, isophosphamide was not carcinogenic in male Sprague-Dawley rats or in male B6C3F1 mice. However, the incidence of leiomyosarcomas of the uterus indicates that isophosphamide was carcinogenic in female Sprague-Dawley rats, and the incidence of fibroadenoma of the mammary gland in female rats was associated with treatment with isophosphamide. Isophosphamide was carcinogenic in female B6C3F1 mice, producing malignant lymphomas of the hematopoietic system.

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I. INTRODUCTION

Isophosphamide (Ifosfamide; CAS 3778-73-2, NSC 109724, NCI C016380) is a cyclophosphamide analog differing from the parent chemical by transposition of a chloroethyl group from the side chain to the ring nitrogen. Isophosphamide requires microsomal activation for cytotoxicity, which proceeds by alkylation and chain scission of DNA (Allen and Creaven, 1972). Developed and tested in Germany by the Asta-Werke Chemical Company during the late 1960's, the drug was active in leukemia L1210, Lewis lung, Ehrlich ascites, and Yoshida sarcoma test systems (Carter, 1972) and produced positive clinical results with oat-cell tumors of the lung, ovarian cancer, breast cancer, and lymphomas (Slavik and Carter, 1973). Isophosphamide has undergone phase II clinical evaluations in the United States with breast cancer and colorectal cancer (Ahmann et al., 1974; Kovach et al., 1974). Isophosphamide was one of a series of anticancer agents administered chronically in clinical practice that was selected for screening for carcinogenicity in the bioassay program.

II. MATERIALS AND METHODS

A. Chemical

Isophosphamide is the generic name for 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-1,3,2-oxazaphosphorine-2-oxide. The material used in this study was manufactured by Asta-Werke AG (Brackwede/Westphalia, Germany) and was obtained through the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (NCI). The purity of the batch used was tested at Stanford Research Institute, Menlo Park, California, and found to be 99 $\pm$ 1% pure. Elemental analyses (C, H, N, Cl, P) were correct for $C_7H_{15}O_2N_2Cl_2P$, the molecular formula for isophosphamide. Water content was $\leq$ 0.18% by Karl Fischer determination. Infrared and nuclear magnetic resonance spectra were compatible with the structural formula for isophosphamide. Gas-liquid chromatography showed one main component and two minor contaminants ($\leq$ 1%). No attempt was made to identify or quantitate the impurities. On receipt at the test laboratory, isophosphamide was stored in glass bottles at -20°C.

B. Dosage Preparation

Test solutions were prepared by dissolving the drug in a buffered saline vehicle. Four concentrations (0.48, 0.24, 0.20, and

0.10%) were prepared. Solutions were made daily and administered immediately to avoid decomposition.

C. Animals

Male Sprague-Dawley rats and male Swiss mice were used in the subchronic studies. Sprague-Dawley rats and B6C3F1 mice of both sexes were used in the chronic studies. All animals were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, through contracts of the Division of Cancer Treatment, NCI. Upon arrival at the laboratory, the animals were quarantined (rats for 5 days, mice for 12 days) as an acclimation period and were then earmarked and assigned to control and test groups.

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 40-60%. There were 15 changes of room air per hour. Air was passed through both 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 (Allied Mills, Inc., Chicago, Ill.) and water were supplied daily and were available ad libitum.

Rats were housed five per cage and mice seven per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The bottoms of the rat cages were lined with Iso-Dri® hardwood chips (Carworth, Edison, N.J.), and cage tops were covered with disposable filter bonnets beginning at week 34; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Bedding was replaced weekly, and cages, water bottles, feeders, and racks were sanitized weekly.

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

RATS

5-azacytidine (CAS 118-92-3)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
beta-2′-deoxy-6-thioguanosine monohydrate (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
acronine (CAS 7008-42-6)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate (phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate) (estradiol mustard) (CAS 22966-79-6)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine hydrochloride) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine hydrochloride) (CAS 366-70-1)

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (No CAS or NSC number available)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride (CAS 3458-22-8)

MICE

anthranilic acid (CAS 118-92-3)
pyrazinamide (CAS 98-96-4)
L-tryptophan (CAS 73-22-3)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride (CAS 3458-22-8)
5-azacytidine (CAS 320-67-2)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
beta-2′-deoxy-6-thioguanosine monohydrate (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
acronine (CAS 7008-42-6)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate (phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate) (estradiol mustard) (CAS 22966-79-6)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (CAS 136-40-3)
2-ethyl-4-pyridinecarbothioamide (ethionamide) (CAS 536-33-4)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine hydrochloride) (CAS 63-92-3)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine hydrochloride) (CAS 366-70-1)
4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (No CAS or NSC number available)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide) (CAS 1156-19-0)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of isophosphamide, on the basis of which low and high doses were determined for administration in the chronic studies. Sprague-Dawley male rats and Swiss male mice were administered isophosphamide by intraperitoneal injection three times per week for 45 days. Following treatment, all animals were observed for an additional 45 days. Five animals of each species were used at each dose, 10 animals were used as untreated controls, and 10 animals as vehicle (buffered saline) controls.

In rats 3/5 animals died at each of the highest doses (24 and 48 mg/kg). At lower doses (2.4, 6.0, and 12 mg/kg) there were no deaths, and body weight gain in these groups was no more than 15% lower than that of the controls. No gross abnormalities were seen in any animals at necropsy. The low and high doses for rats in the chronic studies were set at 6 and 12 mg/kg.

In mice 3/5 animals died at 80 mg/kg, 2/5 at 40 mg/kg, 1/5 at 20 mg/kg, and 0/5 at 10 or 4 mg/kg. Depression in weight gain occurred during the treatment period in the group receiving 80 mg/kg; however, weights were similar among surviving mice in all groups by the end of the study. The low and high doses for mice in the chronic studies were set at 10 and 20 mg/kg.

F. Designs of Chronic Studies

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

Since the numbers of animals in the matched vehicle-control groups (hereinafter referred to as "vehicle controls") were small, pooled vehicle-control groups (hereinafter referred to as "pooled controls") also were used for statistical comparisons. Vehicle controls from the current tests on isophosphamide were combined with vehicle controls from tests performed on 3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride (IPD). The pooled controls for statistical tests consisted of 30 male and 30 female rats and 30 male and 30 female mice. The tests on IPD were also conducted at Southern Research Institute and overlapped the isophosphamide study by at least 16 months for rats and 14 months for mice. The pooled controls were of the same strain and from the same supplier, they were administered the same vehicle by the same route of administration, and they were examined by the same pathologists.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and palpated for masses at each weighing. Rats and mice were weighed individually each week (rats for 20 weeks, mice for 13 weeks) and every 2 weeks for the remainder of the study. Those animals that

Table 1. Design of Chronic Studies of Isophosphamide in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Isophosphamide Dosage[b] (mg/kg)	Time on Study	
			Treated[c] (weeks)	Untreated (weeks)
Male				
Untreated-Control	10	0		83
Vehicle-Control	10	0[d]	52	30-31
Low-Dose	35	6	52	31
High-Dose	35	12	52	27
Female				
Untreated-Control	10	0		84
Vehicle-Control	10	0[d]	52	31
Low-Dose	35	6	52	30-31
High-Dose	35	12	52	27

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

[b]Isophosphamide was administered intraperitoneally in buffered saline three times per week at a volume of 0.25 ml/100g body weight. Doses were based on individual weights.

[c]All animals were placed on study on the same day.

[d]Vehicle-control groups received only buffered saline solution at the same volume as the treated animals.

Table 2. Design of Chronic Studies of Isophosphamide in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Isophosphamide Dosage[b] (mg/kg)	Time on Study: Treated[c] (weeks)	Time on Study: Untreated (weeks)
Male				
Untreated-Control	15	0		80
Vehicle-Control	15	0[d]	52	28
Low-Dose	35	10	52	28
High-Dose	35	20	52	27
Female				
Untreated-Control	15	0		81
Vehicle-Control	15	0[d]	52	28
Low-Dose	35	10	52	28
High-Dose	35	20	52	27

[a]All animals were 42 days of age when placed on study.

[b]Isophosphamide was administered intraperitoneally in buffered saline three times per week at a volume of 1.0 ml/100g body weight. Doses were based on the mean weight of animals in a cage.

[c]All animals were placed on study on the same day.

[d]Vehicle-control groups received only buffered saline solution at the same volume as the treated animals.

were moribund at the time of clinical examination were killed and necropsied.

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. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals for which particular organs or tissues were examined microscopically varies, and does not necessarily

represent the number of animals that were placed on experiment 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, animal 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.

The data of the experiments in this bioassay program are subjected to the statistical analyses described in the subsequent paragraphs of this section. The analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival are estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals are statistically censored as of the time they are missing or are dead due to other than natural causes; animals dying from natural causes are statistically uncensored. Statistical analyses for a possible

dose-related effect on survival employ 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 are reported for all tests except the departure from linearity test, which is noted when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions is 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 include only those animals for which such sites are examined histologically. However, when macroscopic examination is required to detect lesions and when this examination is followed by histologic sampling (e.g., skin or mammary tumors), or when lesions could appear at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of the incidences of tumors is to determine whether animals receiving the test chemical develop a significantly higher proportion of tumors than do control animals. Statistical analyses of the incidences of specific types of tumors are made using the one-tailed Fisher exact test (Cox, 1970) to compare a control group with groups of treated animals at each dose. When results for a number of

treated groups (k) are compared simultaneously with those for a control group, a correction which ensures 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. When appropriate the correction is discussed in the narrative section, but it is not used 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), is 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.

An alternative analysis is applied when early deaths result from causes that are not associated with the formation of tumors. In this analysis, deaths that occur before the first tumor is observed are excluded by basing the statistical tests on animals that survive at least as long as 52 weeks, unless a tumor is found at the anatomic site of interest before week 52. When such an early tumor is found, comparisons are based exclusively on animals that survive at least as long as the animal in which the

first tumor is found. Once this reduced set of data is obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact test, Cochran-Armitage test, etc.) are followed.

When appropriate, life-table methods are applied to the incidence of tumors. Curves of the proportions surviving without a tumor being observed are computed according to Saffiotti et al. (1972). The times at which animals die naturally or are killed are entered as the time point of tumor observation. Cox's methods of comparing these curves are used for two groups, and Tarone's extension to testing for linear trend is used for three groups. The tests for the incidence of tumors using life-table methods are one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity (< 0.05, two-tailed test) are also noted.

The approximate 95% confidence interval for the relative risk between each of the treated groups and its control is calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and that in a control group is expressed

by a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk are included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of similar experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, the occurrence of 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) will also obtain. When the lower limit is less than unity and the upper limit is greater than unity, the former indicates the absence of a significant result while the latter indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights both of male and female rats administered isophosphamide were comparable with those of the vehicle controls for the first 25 weeks; thereafter, the mean body weights of the high-dose groups were slightly lower (figure 1). Weights of vehicle-control and treated male rats were less than those of untreated controls. A sudden loss of weight during weeks 32 to 34 in both control and treated groups, particularly in the males, coincided with an onset of rales. The animals were treated with oxytetracycline in the drinking water at 0.6 mg/ml in weeks 35-41, and at 0.3 mg/ml in weeks 41-45. The rales were observed in higher incidences in the treated rats than in the controls. However, no clinical signs clearly associated with treatment with isophosphamide were recorded.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for the treated male and female rats, together with those for the untreated and vehicle controls, are shown in figure 2.

In male rats, the Tarone test result for positive dose-related trend in mortality of treated animals compared with vehicle

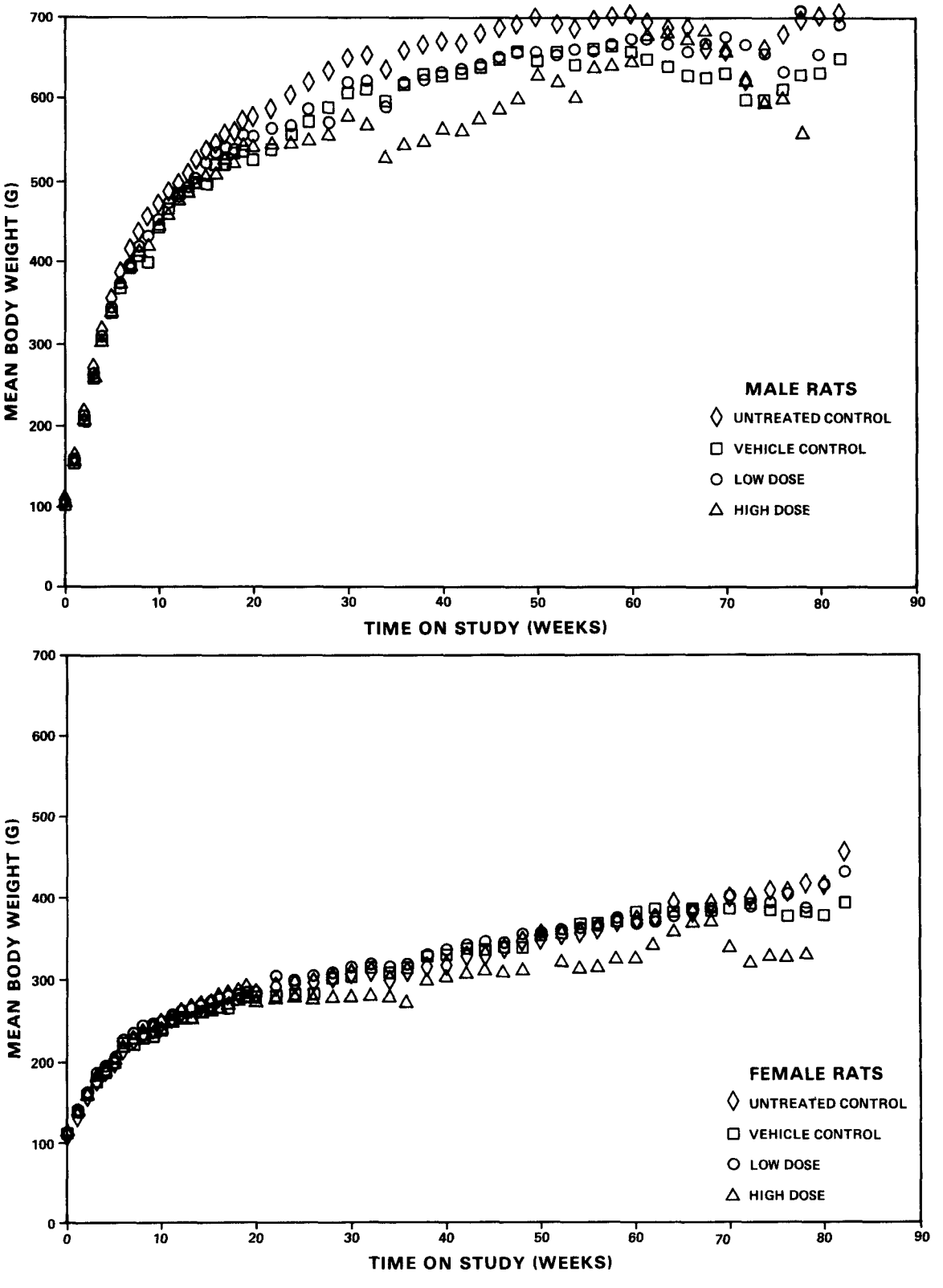

Figure 1. Growth Curves For Rats Treated With Isophosphamide

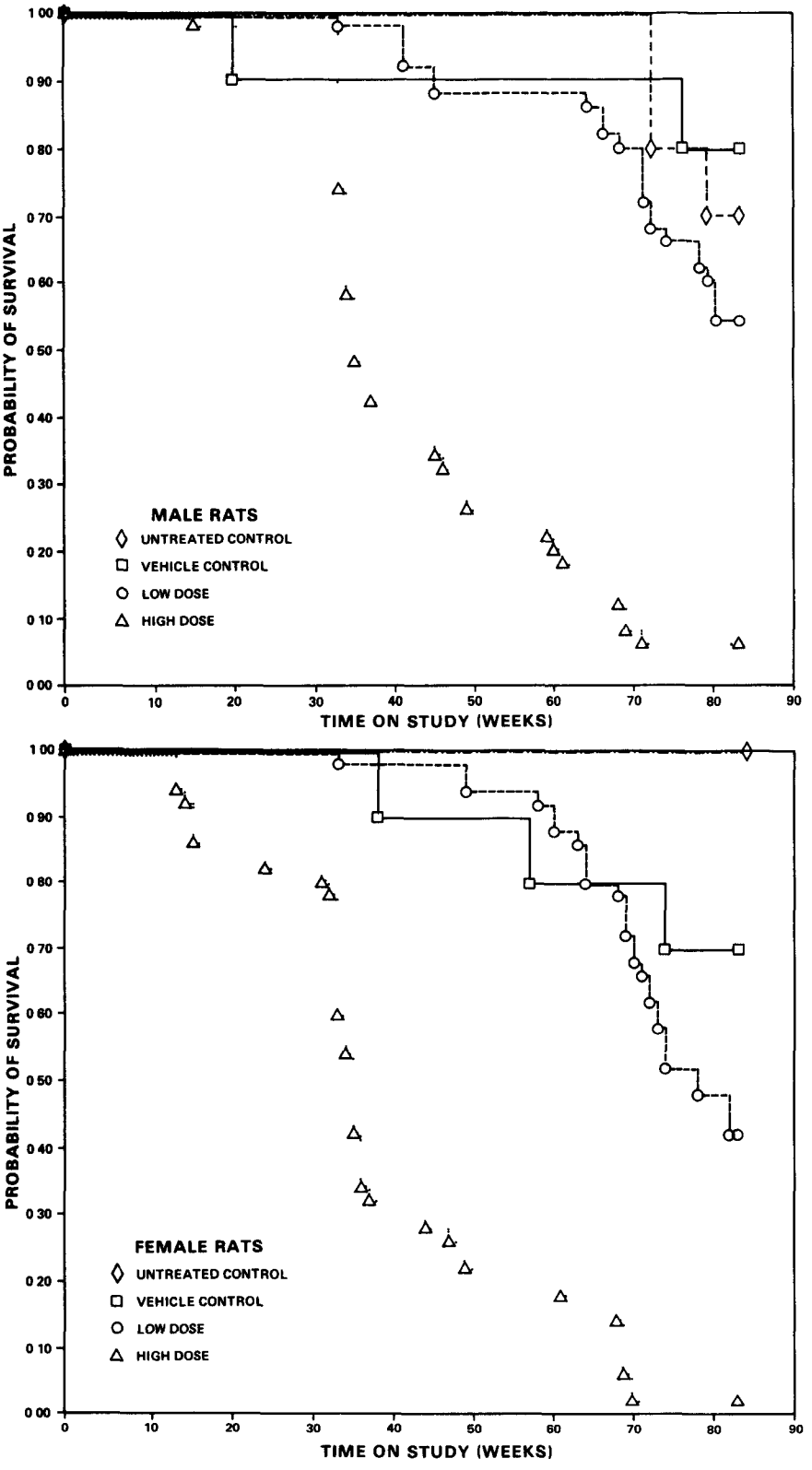

Figure 2. Survival Curves For Rats Treated With Isophosphamide

controls over the period is significant ($P < 0.001$), with a similar significant level of departure from linear trend ($P < 0.001$), due to the steep increase in the rate of mortality in the high-dose animals. The median time on study of the high-dose group was 35 weeks, and that of the low-dose group was greater than 83 weeks. Seventy percent of the untreated controls, 80% of the vehicle controls, 55% of the low-dose animals, and 6% of the high-dose animals lived to the end of the study. By week 52 of the study, only 9/35 high-dose male rats had survived; of those that died by week 52, five had developed tumors.

In females, the Tarone test result is also significant ($P < 0.001$), with a similar significant level of departure from linear trend ($P < 0.001$), due to the sharp increase in the rate of mortality in the treated groups, especially in the high-dose group. One hundred percent of the untreated controls, 70% of the vehicle controls, 42% of the low-dose animals, and 3% of the high-dose animals lived to termination of the study. The median time on study of the high-dose group was only 34 weeks, and that of the low-dose group was 74 weeks. Early deaths of the treated animals were not tumor associated, since few tumors were seen in the animals that died early. By week 52 of the study, only 8/35 high-dose female rats had survived; of those that died by week 52, two had developed tumors, one as early as week 33. Due to

the numerous deaths in the high-dose groups early in the study, the statistical analysis was made using the data concerning those animals that lived longer than week 52 on study or lived beyond the week in which the first tumor appeared, with separate consideration given to each tumor site.

C. Pathology (Rats)

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

There was a spontaneous and random occurrence of a variety of tumors both in the matched-control groups (untreated and vehicle, phosphate-buffered saline) and in the treated groups. Some types of neoplasms occurred only, or with greater frequency, in rats of treated groups as compared with controls. These neoplasms, however, are not uncommon in this strain of rat independent of any treatment.

Mesenchymal stromal tumors, which included fibromas, fibrosarcomas, leiomyosarcomas, and undifferentiated spindle-cell sarcomas coded as sarcomas, NOS (not otherwise specified), occurred in 5/32 low-dose males, 3/34 high-dose males, 17/33 low-dose females, and 2/34 high-dose females. None of these lesions occurred in the control groups, 0/17 male and 0/20 female

rats. The majority of the stromal tumors in the females were poorly differentiated leiomyosarcomas which were characterized by spindle cells without collagen formation arising from the smooth muscle of the uterus. One leiomyosarcoma was observed in the pelvic subcutaneous tissues without definitive involvement of the uterus. Metastatic leiomyosarcomas occurred in the lungs, urinary bladder, spleen, and other abdominal sites.

The other stromal tumors, which were composed of spindle cells having various degrees of collagen formation and differentiation, occurred in a variety of sites in both males and females. One osteosarcoma occurred in the subcutis of a vehicle-control female rat.

The incidence of mammary fibroadenoma in female rats was highest in the low-dose group (vehicle controls 3/10, low-dose 28/33, high-dose 6/34).

A few rats had malignant lymphomas and granulocytic leukemias. These hematopoietic tumors occurred only in treated rats, but have been observed previously at a low frequency in rats of this strain.

In addition to the neoplastic lesions, a large number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the treated and control groups

(Appendix C). For the most part, these nonneoplastic lesions are commonly seen in aged rats. A high incidence of respiratory infections, especially bronchopneumonias, in the high-dose male and female rats is correlated with decreased life spans and is believed to have been a factor in the small number of neoplastic lesions which occurred in these groups. The incidence of peritonitis and peritoneal adhesions was low, indicating that irritation and infection were not major problems despite the frequent intraperitoneal injections.

In the judgment of the pathologists, the results of the study indicate that isophosphamide in phosphate-buffered saline administered intraperitoneally was responsible for a high frequency of sarcomas in male and female Sprague-Dawley rats, particularly uterine leiomyosarcomas. In addition, mammary fibroadenomas were induced in low-dose female rats.

D. Statistical Analyses of Results (Rats)

Tables E1-E4 in Appendix E contain the statistical analyses of the incidences of those specific primary tumors that were observed in at least 5% of one or more treated groups of either sex. The untreated controls are not included in these tables, since the test conditions of the vehicle controls are closer to those of the treated animals. Due to the severe mortality rates

in the treated rats of both sexes, time-adjusted analyses were performed, eliminating animals that died before week 52 on study; however, when a tumor was found at the specific site before week 52 of the study, the incidence was then based on animals that survived at least as long as the animal in which the first tumor appeared. These time-adjusted analyses are shown in tables E3 and E4. The statistical narrative below is based on the adjusted analyses.

In male rats, the results of the Cochran-Armitage test for positive dose-related trend on the time-adjusted proportions and the Fisher exact test for direct positive comparisons of time-adjusted incidences between the vehicle controls and each of the treated groups are not significant. The incidences of pituitary tumors in control male and female rats were significantly greater than in the treated animals.

In female rats, the result of the Cochran-Armitage test on the time-adjusted incidence of fibroadenoma of the mammary gland is significant (P = 0.009), and an indicated departure from linear trend is observed (P = 0.028), due to the steep increase in incidence in the treated groups. The Fisher exact test shows that the incidence in the low-dose group is significantly higher (P = 0.003) than that in the vehicle controls, and the lower limit of the 95% confidence interval of this relative risk has a

value greater than one. The statistical conclusion indicates that this incidence in the low-dose group is dose associated. Although the high-dose percentage is also high (86%), it is based on an incidence of only 6/7 animals. This small sample size reduces the power of the test, and as result, the incidence in the high-dose group is not statistically significant.

Leiomyosarcomas of the uterus occurred exclusively in the treated groups. The Fisher exact test shows that the incidence in the low-dose group is significantly higher ($P = 0.012$) than that in the vehicle controls. The incidence in the high-dose group is not significant, but the power of the test in this instance is weak because of the small sample size of the vehicle-control and high-dose groups (eight animals and seven animals, respectively).

In rats, there is no other tumor at any specific site for which the incidence is significant in the positive direction. When the incidences of types of tumors are combined for statistical analysis (as, for example, granulocytic leukemia and lymphoma of the hematopoietic system), the incidences of the individual components of the grouping are not included in the statistical analyses in the tables unless at least one group was observed to have 5% or more of such tumor; however, a list of the incidences of each type of tumor is provided in tables A1 and A2 of Appendix A.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the mice showed no consistent effect of the isophosphamide in either sex or at either dose (figure 3), and no other clinical signs of toxicity were observed. No evidence of outbreak of clinical disease was recorded.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for the treated male and female mice, together with those for the untreated and vehicle controls, are shown in figure 4.

In male mice, the Tarone test result for positive dose-related trend in mortality of treated animals compared with vehicle controls over the period is not significant at the 0.05 level. Over 90% of the untreated controls, 7% of the vehicle controls, and 31% of the treated animals lived to the end of the study. The median times on study of the high-dose, low-dose, and vehicle-control groups were 53 weeks, 44 weeks, and 29 weeks, respectively. One hepatocellular adenoma of the liver occurred as early as week 44 on study. Only three male mice in the vehicle-control group lived beyond week 44 on study, and no tumor was observed in this group. In the low-dose group, 18/35 male

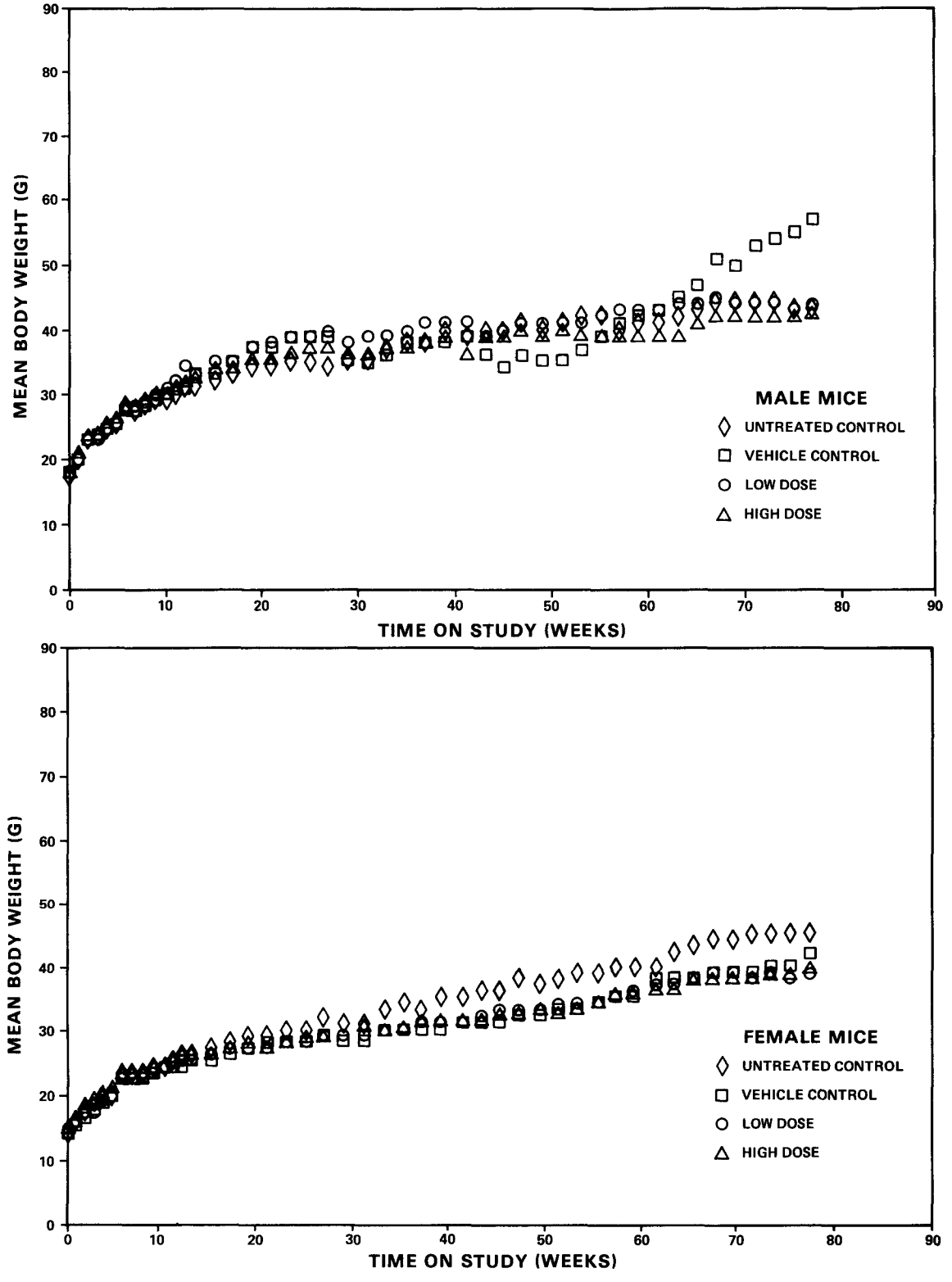

Figure 3. Growth Curves For Mice Treated With Isophosphamide

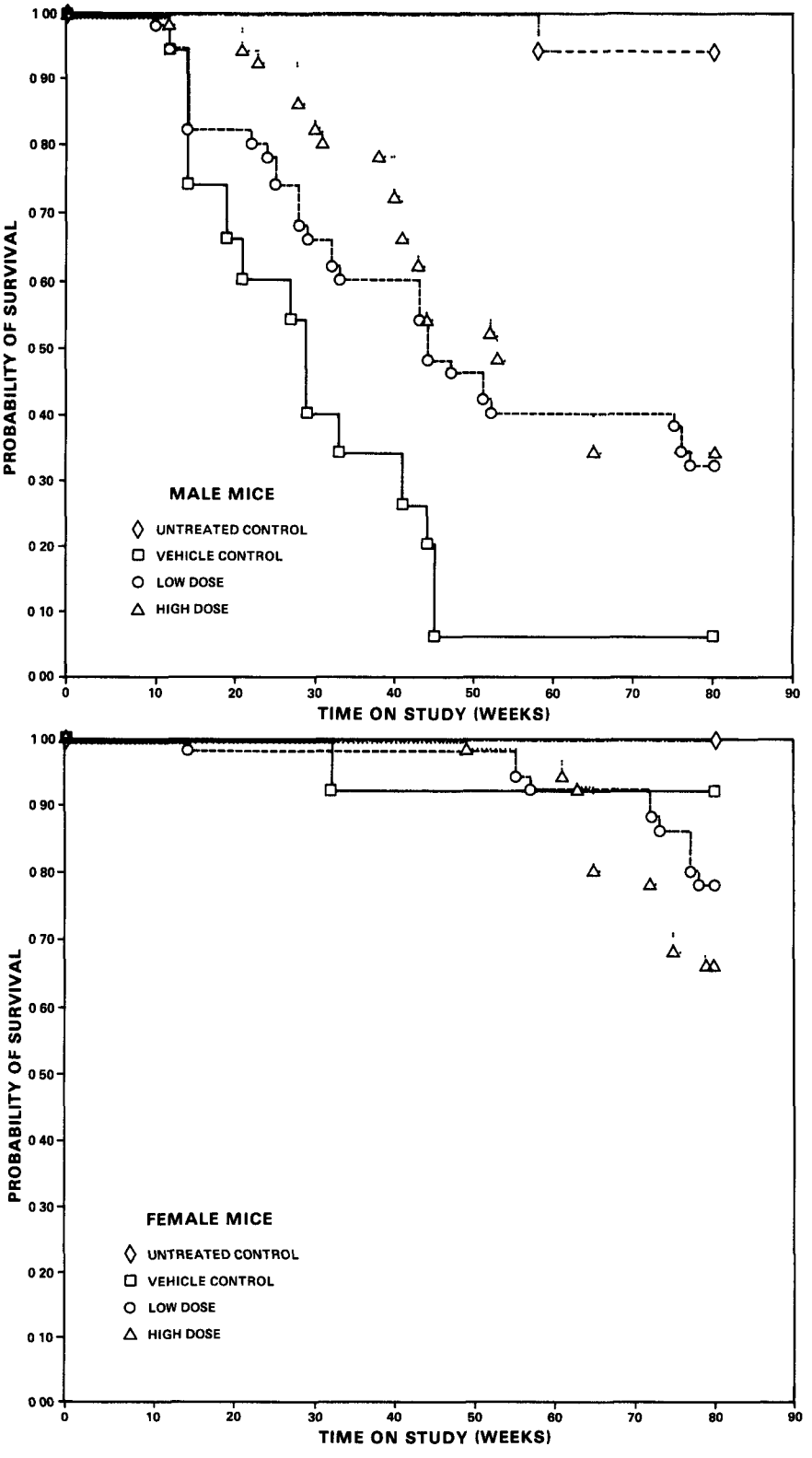

Figure 4. Survival Curves For Mice Treated With Isophosphamide

mice survived until week 44 of the study, and in the high-dose group, 22/35 male mice survived until week 44. The early deaths of the treated male mice may have suppressed the incidences of late-developing tumors, and analysis of the male mice is made using only those animals surviving 44 weeks or more. A pooled-control group is used for comparison with the treated groups in the analyses without time-adjustment.

In females, the Tarone test result of the mortality over the period is significant ($P = 0.004$), with over 90% of the controls, 77% of the low-dose group, and 66% of the high-dose group living to the end of the study, providing sufficient animals for meaningful statistical analyses of the incidence of late-developing tumors.

C. Pathology (Mice)

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

With the exception of malignant lymphomas in female mice, the neoplasms listed in Appendix B appeared with approximately equal frequency in treated and matched-control mice, or appeared in insignificant numbers.

Malignant lymphomas, histiocytic type, occurred in 3/32 low-dose and 13/34 high-dose females, but in none of the untreated or vehicle controls. In addition to the neoplastic lesions, a large number of degenerative, proliferative, and inflammatory changes were encountered in animals of the treated and control groups (Appendix D). For the most part, these nonneoplastic lesions are commonly seen in aged mice and were not associated with increased deaths or decreased life spans.

In the judgment of the pathologists, the results of the study indicate that isophosphamide in phosphate-buffered saline given intraperitoneally is responsible for an increased incidence of malignant lymphomas in female B6C3F1 mice.

D. Statistical Analyses of Results (Mice)

Tables F1-F3 in Appendix F contain the statistical analyses of the incidences of those specific primary tumors that were observed in at least 5% of one or more treated groups of either sex. The untreated controls are not included in tables F1 and F2, since the test conditions of the vehicle controls more closely resemble those of the treated groups. Due to the severe mortality rates in the treated male mice, time-adjusted analyses on the incidences of liver tumors were performed. These time-adjusted analyses are based on animals that lived at least as

long as week 44 of the study, when the first liver tumor occurred, and are shown in table F3. The statistical narrative below on male mice is based on the time-adjusted analyses.

In male mice, the results of the Cochran-Armitage test for positive dose-related trend on the time-adjusted proportions and the Fisher exact test on the direct comparisons of time-adjusted incidences of hepatocellular adenoma or carcinoma between the vehicle controls and each of the treated groups are not significant; however, it should be noted that the power of the tests is weak due to the small size of the control-group sample, only three animals. When the time-adjusted incidence in the pooled vehicle controls is used in the Fisher exact test, a probability level of 0.043 results, but this value is above the 0.025 required by the multiple-comparison criterion. The incidence of hepatocellular carcinoma is not included in the statistical tables, because the incidences in the treated groups are less than 5%; however, a list of the incidences of each type of tumor is provided in tables B1 and B2 of Appendix B.

In the analyses of the incidences of malignant lymphoma of the hematopoietic system in female mice, the Cochran-Armitage test shows highly significant positive dose-related trends of $P = 0.001$ and $P < 0.001$ using the vehicle controls and the pooled controls, respectively. The Fisher exact test shows that the

incidence in the high-dose group is significantly higher than that in either the vehicle controls (P = 0.005) or the pooled controls (P = 0.001). The statistical conclusion is that the occurrence of malignant lymphoma in female mice is associated with isophosphamide at the doses of this experiment. There is no incidence of granulocytic leukemia in female mice, and there is no other tumor at any specific site for which the statistical tests are significant.

V. DISCUSSION

In this bioassay, mean body weights of both male and female rats administered isophosphamide were comparable with those of the controls for the first 25 weeks; thereafter, the mean body weights of the high-dose groups were slightly lower. There was a positive dose-related trend in survival in both sexes of rats. In the high-dose rats, the median survival among males was 35 weeks, and only two animals survived until termination of the study at week 79. Among females, the median survival was 35 weeks, and only one animal survived until termination of the study. Inflammatory lesions of the lungs, including bronchopneumonia, were observed in higher incidences in the treated rats than in the controls, and may have been responsible, at least in part, for the lower body weights and survival rates observed in the high-dose groups.

Mean body weights of the mice did not show any consistent effect from the isophosphamide treatment, and no other clinical signs attributable to the chemical were recorded for the mice. Survival of all groups of females was sufficient (vehicle controls 93%, low-dose 77%, high-dose 66%) for meaningful statistical analyses of the incidence of tumors, while survival of the males was low (vehicle controls 7%, low-dose 31%, high-dose 31%). The median times on study of the vehicle

controls, low-dose group, and high-dose group of male mice were 44 weeks, 53 weeks, and 29 weeks, respectively.

In male rats, tumors of the hematopoietic system included six malignant lymphomas and two granulocytic leukemias. With the unadjusted analyses, these tumors showed a dose-related trend in male rats using pooled controls (controls 0/29, low-dose 3/32, high-dose 5/34, $P = 0.032$) and a higher incidence in the high-dose males than in the pooled controls ($P = 0.040$). These tumors were not significant when compared with those in the vehicle controls using time-adjusted analyses, and they cannot clearly be associated with treatment. However, it should be noted that the five rats with these tumors were observed among the high-dose animals whose median survival was only 35 weeks.

The incidence of uterine leiomyosarcoma in female rats was statistically significant in the low-dose group using pooled controls (15/32, $P < 0.001$). The incidence was also significant when compared with vehicle controls using time-adjusted analyses. The poor survival rate of the high-dose females (median time on study, 35 weeks) may account for the low incidence of this lesion at this dose. Metastatic leiomyosarcomas occurred in the lungs, urinary bladder, spleen, and other abdominal sites. These results indicate that the incidence of uterine leiomyosarcomas in female rats is related to treatment.

There was a highly significant incidence of fibroadenoma of the mammary gland in the low-dose female rats (28/33) when compared with the incidence in the vehicle controls (3/10, $P = 0.001$) and with that in the pooled controls (8/28, $P < 0.001$). The incidence was also significant when compared with matched vehicle controls using time-adjusted analyses. This tumor was found in only six high-dose animals and the low survival rate of this group must be considered when assessing the low incidence. Although fibroadenoma is a type of mammary tumor commonly found in control female Sprague-Dawley rats in the bioassay program at this laboratory (untreated controls 66/220, vehicle controls 75/245), the higher incidence in the low-dose group indicates that this tumor is associated with treatment. Other epithelial tumors of the mammary gland including adenoma, adenocarcinoma, cystadenoma, and cystadenocarcinoma were found among the animals having fibroadenoma and in one additional low-dose and vehicle-control female, respectively.

In mice, five low-dose males had hepatocellular adenoma or carcinoma of the liver. In comparison with the pooled vehicle controls, a probability level of 0.043 resulted using time-adjusted analysis, but this value is above the 0.025 required by the multiple-comparison criterion and these tumors are not clearly related to treatment. Malignant lymphomas of the

hematopoietic system in female mice showed a significant dose-related incidence (vehicle controls 0/14, P = 0.001; pooled controls 1/29, P < 0.001; low-dose 3/32, high-dose 13/34). The incidence of malignant lymphomas in the high-dose females was significantly higher than that in the vehicle controls (P = 0.005) or pooled controls (P = 0.001) using unadjusted analyses. These tumors were not found in males.

Since isophosphamide is an anticancer drug, most of the testing has been in clinical studies with humans (Carter 1972; Ahmann et al., 1974; Kovach et al., 1974). Animal studies have been focused upon chemotherapeutic effects rather than carcinogenic effects (Finklestein, 1975).

It is concluded that under the conditions of this bioassay, isophosphamide was not carcinogenic in male Sprague-Dawley rats or in male B6C3F1 mice. However, the incidence of leiomyosarcomas of the uterus indicates that isophosphamide was carcinogenic in female Sprague-Dawley rats, and the incidence of fibroadenoma of the mammary gland in female rats was associated with treatment with isophosphamide. Isophosphamide was carcinogenic in female B6C3F1 mice, producing malignant lymphomas of the hematopoietic system.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH ISOPHOSPHAMIDE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ISOPHOSPHAMIDE

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	8	9	32	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	8	9	32	34
INTEGUMENTARY SYSTEM				
*SKIN	(8)	(9)	(32)	(34)
SEBACECUS ADENOMA			1 (3%)	
*SUBCUT TISSUE	(8)	(9)	(32)	(34)
SARCOMA, NOS			1 (3%)	
FIBROMA			2 (6%)	
RESPIRATORY SYSTEM				
#LUNG	(7)	(9)	(32)	(34)
ADENOCARCINOMA, NOS, METASTATIC		1 (11%)		
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (6%)	
FIBPOSARCOMA, METASTATIC				1 (3%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(8)	(9)	(32)	(34)
MALIG.LYMPHOMA, UNDIFFER-TYPE			2 (6%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				2 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (3%)
GRANULOCYTIC LEUKEMIA			1 (3%)	1 (3%)
#BONE MARROW	(8)	(8)	(31)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (3%)
CIRCULATORY SYSTEM				
#HEART	(7)	(8)	(29)	(34)
FIBROSARCOMA			1 (3%)	
DIGESTIVE SYSTEM				
NONE				

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
URINARY SYSTEM				
#KIDNEY	(7)	(9)	(32)	(34)
ADENOCARCINOMA, NOS		1 (11%)		
#URINARY BLADDER	(8)	(9)	(28)	(30)
TRANSITIONAL-CELL CARCINOMA	1 (13%)			
ENDOCRINE SYSTEM				
#PITUITARY	(8)	(7)	(29)	(32)
CHROMOPHOBE ADENOMA	1 (13%)	3 (43%)	3 (10%)	
CHROMOPHOBE CARCINOMA	1 (13%)	1 (14%)	1 (3%)	
#ADRENAL	(8)	(9)	(31)	(34)
CORTICAL ADENOMA	1 (13%)		2 (6%)	2 (6%)
PHEOCHROMOCYTOMA	1 (13%)		2 (6%)	2 (6%)
#PANCREATIC ISLETS	(8)	(9)	(32)	(33)
ISLET-CELL ADENOMA	1 (13%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(8)	(9)	(32)	(34)
FIBROADENOMA		1 (11%)		
#TESTIS	(8)	(9)	(30)	(33)
INTERSTITIAL-CELL TUMOR	1 (13%)			
NERVOUS SYSTEM				
#BRAIN/MENINGES	(7)	(8)	(30)	(33)
SARCOMA, NOS			1 (3%)	1 (3%)
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
BODY CAVITIES				
*MEDIASTINUM	(8)	(9)	(32)	(34)
FIBROSARCOMA				1 (3%)
*PERITONEUM	(8)	(9)	(32)	(34)
FIBROSARCOMA				1 (3%)
*MESENTERY	(8)	(9)	(32)	(34)
LIPOMA		1 (11%)		
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(8)	(9)	(32)	(34)
FIBROSARCOMA			1 (3%)	
MESOTHELIOMA, MALIGNANT			1 (3%)	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@	3	2	8	11
MORIBUND SACRIFICE			7	22
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	7	8	20	2
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	5	17	12
TOTAL PRIMARY TUMORS	7	7	21	12
TOTAL ANIMALS WITH BENIGN TUMORS	4	4	11	4
TOTAL BENIGN TUMORS	5	5	12	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	2	9	8
TOTAL MALIGNANT TUMORS	2	2	9	8
TOTAL ANIMALS WITH SECONDARY TUMORS#		1		1
TOTAL SECONDARY TUMORS		1		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ISOPHOSPHAMIDE

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	33	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	33	34
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(10)	(10)	(33)	(34)
SQUAMOUS CELL CARCINOMA			1 (3%)	
FIBROMA			1 (3%)	
LEIOMYOSARCOMA			1 (3%)	
OSTEOSARCOMA		1 (10%)		
RESPIRATORY SYSTEM				
#LUNG	(9)	(10)	(33)	(33)
LEIOMYOSARCOMA, METASTATIC			1 (3%)	
OSTEOSARCOMA, METASTATIC		1 (10%)		
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(10)	(33)	(34)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)	
#BONE MARROW	(10)	(9)	(32)	(32)
GRANULOCYTIC SARCOMA			2 (6%)	
#SPLEEN	(10)	(10)	(33)	(34)
LEIOMYOSARCOMA, METASTATIC			1 (3%)	
HEMANGIOSARCOMA			1 (3%)	1 (3%)
CIRCULATORY SYSTEM				
#ENDOCARDIUM	(9)	(9)	(33)	(33)
FIBROSARCOMA			1 (3%)	
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(33)	(33)
HEPATOCELLULAR ADENOMA			1 (3%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
URINARY SYSTEM				
#URINARY BLADDER	(10)	(10)	(26)	(32)
SQUAMOUS CELL CARCINOMA			1 (4%)	
TRANSITIONAL-CELL PAPILLOMA		1 (10%)		
TRANSITIONAL-CELL CARCINOMA			1 (4%)	
LEIOMYOSARCOMA, METASTATIC			1 (4%)	
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(10)	(31)	(34)
CHROMOPHOBE ADENOMA	5 (50%)	3 (30%)	7 (23%)	
CHROMOPHOBE CARCINOMA	1 (10%)	4 (40%)	1 (3%)	1 (3%)
#ADRENAL	(10)	(10)	(33)	(34)
CORTICAL ADENOMA	3 (30%)	3 (30%)	8 (24%)	1 (3%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(33)	(34)
ADENOMA, NOS			2 (6%)	
ADENOCARCINOMA, NOS	1 (10%)	1 (10%)	2 (6%)	2 (6%)
CYSTADENOMA, NOS	1 (10%)		3 (9%)	1 (3%)
CYSTADENOCARCINOMA, NOS			2 (6%)	
FIBROSARCOMA			1 (3%)	
FIBROADENOMA	3 (30%)	3 (30%)	28 (85%)	6 (18%)
*VAGINA	(10)	(10)	(33)	(34)
SQUAMOUS CELL CARCINOMA				1 (3%)
#UTERUS	(9)	(9)	(32)	(34)
SQUAMOUS CELL CARCINOMA			1 (3%)	
LEIOMYOSARCOMA			14 (44%)	1 (3%)
ENDOMETRIAL STROMAL POLYP	1 (11%)		1 (3%)	
#UTERUS/ENDOMETRIUM	(9)	(9)	(32)	(34)
LEIOMYOSARCOMA			1 (3%)	
NERVOUS SYSTEM				
#BRAIN	(10)	(10)	(31)	(34)
SARCOMA, NOS, INVASIVE			1 (3%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ASTROCYTOMA		1 (10%)		
#CEREBELLUM	(10)	(10)	(31)	(34)
HAMARTOMA			1 (3%)	
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*MUSCLE OF HEAD	(10)	(10)	(33)	(34)
SARCOMA, NOS			1 (3%)	
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(10)	(33)	(34)
SARCOMA, NOS				1 (3%)
LEIOMYOSARCOMA, METASTATIC			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@			9	15
MORIBUND SACRIFICE		3	11	19
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	10	7	15	1
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	9	32	9
TOTAL PRIMARY TUMORS	15	17	84	15
TOTAL ANIMALS WITH BENIGN TUMORS	7	7	31	7
TOTAL BENIGN TUMORS	13	10	52	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	6	23	6
TOTAL MALIGNANT TUMORS	2	7	32	7
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	4	
TOTAL SECONDARY TUMORS		1	5	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH ISOPHOSPHAMIDE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ISOPHOSPHAMIDE

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	14	14	30	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	14	29	27
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(14)	(14)	(29)	(27)
ADENOCARCINOMA, NOS, UNC PRIM OR ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (3%)	1 (4%)
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(14)	(14)	(29)	(27)
HEPATOCELLULAR ADENOMA	2 (14%)		4 (14%)	2 (7%)
HEPATOCELLULAR CARCINOMA			1 (3%)	
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#ADRENAL	(14)	(14)	(29)	(27)
PHEOCHROMOCYTOMA			1 (3%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(14)	(14)	(30)	(27)
ADENOMA, NOS				1 (4%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(14)	(30)	(27)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@	1	10	24	16
MORIBUND SACRIFICE		4		8
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	14	1	11	11
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2		6	4
TOTAL PRIMARY TUMORS	2		7	4
TOTAL ANIMALS WITH BENIGN TUMORS	2		5	3
TOTAL BENIGN TUMORS	2		5	3
TOTAL ANIMALS WITH MALIGNANT TUMORS			1	1
TOTAL MALIGNANT TUMORS			1	1
TOTAL ANIMALS WITH SECONDARY TUMORS#			1	
TOTAL SECONDARY TUMORS			1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			1	
TOTAL UNCERTAIN TUMORS			1	

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ISOPHOSPHAMIDE

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS MISSING			1	
ANIMALS NECROPSIED	15	14	32	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	14	32	34
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(14)	(32)	(34)
ADENOSQUAMOUS CARCINOMA			1 (3%)	
KERATOACANTHOMA			1 (3%)	
*SUBCUT TISSUE	(15)	(14)	(32)	(34)
SARCOMA, NOS			1 (3%)	
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(14)	(32)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			3 (9%)	12 (35%)
#SPLEEN	(15)	(14)	(32)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (3%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(14)	(32)	(33)
HEPATOCELLULAR CARCINOMA			1 (3%)	
#STOMACH	(15)	(14)	(32)	(34)
LEIOMYOSARCOMA				1 (3%)
URINARY SYSTEM				
NONE				

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#UTERUS	(14)	(14)	(32)	(33)
LEIOMYOSARCOMA			1 (3%)	1 (3%)
#OVARY	(14)	(14)	(32)	(31)
HEMANGIOMA				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 B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@		1	5	6
MORIBUND SACRIFICE			3	6
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED		1		
TERMINAL SACRIFICE	15	13	26	23
ANIMAL MISSING			1	
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*			7	15
TOTAL PRIMARY TUMORS			8	16
TOTAL ANIMALS WITH BENIGN TUMORS			1	1
TOTAL BENIGN TUMORS			1	1
TOTAL ANIMALS WITH MALIGNANT TUMORS			7	14
TOTAL MALIGNANT TUMORS			7	15
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				

@ INCLUDES AUTOLYZED ANIMALS

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS TREATED WITH ISOPHOSPHAMIDE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ISOPHOSPHAMIDE

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	8	9	32	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	8	9	32	34
INTEGUMENTARY SYSTEM				
*SKIN	(8)	(9)	(32)	(34)
INFLAMMATION, CHRONIC			1 (3%)	
INFLAMMATION, GRANULOMATOUS			1 (3%)	
*SUBCUT TISSUE	(8)	(9)	(32)	(34)
ABSCESS, NOS			1 (3%)	
PERIARTERITIS			1 (3%)	
RESPIRATORY SYSTEM				
#TRACHEA	(8)	(8)	(30)	(31)
INFLAMMATION, NOS	1 (13%)		1 (3%)	17 (55%)
INFLAMMATION, SUPPURATIVE				3 (10%)
INFLAMMATION, ACUTE/CHRONIC	3 (38%)	5 (63%)	20 (67%)	
INFLAMMATION, CHRONIC			1 (3%)	3 (10%)
#LUNG/BRONCHUS	(7)	(9)	(32)	(34)
BRONCHIECTASIS	1 (14%)	1 (11%)	5 (16%)	7 (21%)
INFLAMMATION, NOS			1 (3%)	4 (12%)
INFLAMMATION, CHRONIC			3 (9%)	1 (3%)
#LUNG	(7)	(9)	(32)	(34)
BRONCHOPNEUMONIA, NOS	1 (14%)		5 (16%)	21 (62%)
INFLAMMATION, NOS				1 (3%)
INFLAMMATION, INTERSTITIAL			1 (3%)	
ABSCESS, NOS			1 (3%)	2 (6%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(8)	(8)	(31)	(34)
ATROPHY, NOS				2 (6%)
#LYMPH NODE	(2)	(5)	(9)	(20)
ATROPHY, NOS				1 (5%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
#THYMUS	(7)	(6)	(23)	(22)
ATROPHY, NOS				1 (5%)
CIRCULATORY SYSTEM				
#HEART	(7)	(8)	(29)	(34)
PERIARTERITIS			1 (3%)	
DIGESTIVE SYSTEM				
#LIVER	(8)	(9)	(32)	(34)
HEMORRHAGIC CYST				1 (3%)
NECROSIS, NOS	1 (13%)			
#STOMACH	(7)	(9)	(31)	(33)
ULCER, NOS		1 (11%)		1 (3%)
ULCER, CHRONIC				1 (3%)
#COLON	(8)	(9)	(31)	(33)
HEMORRHAGE				1 (3%)
INFLAMMATION, ACUTE				1 (3%)
URINARY SYSTEM				
#KIDNEY	(7)	(9)	(32)	(34)
INFLAMMATION, CHRONIC	3 (43%)	1 (11%)	5 (16%)	3 (9%)
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#PROSTATE	(8)	(9)	(31)	(33)
INFLAMMATION, SUPPURATIVE	1 (13%)			
INFLAMMATION, CHRONIC	1 (13%)			
*SEMINAL VESICLE	(8)	(9)	(32)	(34)
INFLAMMATION, ACUTE/CHRONIC	1 (13%)			
NERVOUS SYSTEM				
NONE				

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(8)	(9)	(32)	(34)
INFLAMMATION, CHRONIC			1 (3%)	
BODY CAVITIES				
*PERITONEUM	(8)	(9)	(32)	(34)
ABSCESS, NOS				1 (3%)
INFLAMMATION, CHRONIC			2 (6%)	2 (6%)
METAPLASIA, OSSEOUS			1 (3%)	
*PLEURA	(8)	(9)	(32)	(34)
INFLAMMATION, NOS				2 (6%)
INFLAMMATION, ACUTE/CHRONIC				1 (3%)
INFLAMMATION, CHRONIC				2 (6%)
*EPICARDIUM	(8)	(9)	(32)	(34)
INFLAMMATION, CHRONIC				1 (3%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1	2	1	
AUTOLYSIS/NO NECROPSY	2	1	3	1

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF ISOPHOSPHAMIDE

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	33	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	33	34
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(33)	(34)
INFLAMMATION, GRANULOMATOUS		1 (10%)		
RESPIRATORY SYSTEM				
*LARYNX	(10)	(10)	(33)	(34)
INFLAMMATION, SUPPURATIVE				1 (3%)
INFLAMMATION, ACUTE/CHRONIC	1 (10%)			
#TRACHEA	(9)	(10)	(29)	(29)
INFLAMMATION, NOS				6 (21%)
INFLAMMATION, SUPPURATIVE				1 (3%)
INFLAMMATION, ACUTE				2 (7%)
INFLAMMATION, ACUTE/CHRONIC	6 (67%)	4 (40%)	12 (41%)	8 (28%)
INFLAMMATION, CHRONIC				2 (7%)
#LUNG/BRONCHUS	(9)	(10)	(33)	(33)
BRONCHIECTASIS	1 (11%)	2 (20%)	4 (12%)	6 (18%)
INFLAMMATION, NOS				4 (12%)
#LUNG	(9)	(10)	(33)	(33)
EDEMA, NOS				1 (3%)
BRONCHOPNEUMONIA, NOS		1 (10%)	6 (18%)	13 (39%)
INFLAMMATION, INTERSTITIAL				4 (12%)
INFLAMMATION, NECROTIZING				1 (3%)
ABSCESS, NOS			1 (3%)	
INFLAMMATION, CHRONIC	1 (11%)			1 (3%)
PLASMACYTOSIS				1 (3%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(10)	(9)	(32)	(32)
FIBROSIS			1 (3%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ATROPHY, NOS		1 (11%)		6 (19%)
#SPLEEN	(10)	(10)	(33)	(34)
NECROSIS, NOS			1 (3%)	
#LYMPH NODE		(6)	(24)	(30)
INFLAMMATION, NECROTIZING				1 (3%)
PLASMACYTOSIS				1 (3%)
#THYMUS	(6)	(10)	(27)	(28)
PLASMACYTOSIS				1 (4%)
CIRCULATORY SYSTEM				
#HEART	(9)	(9)	(33)	(33)
PERIARTERITIS		1 (11%)		1 (3%)
PLASMACYTOSIS				1 (3%)
#ENDOCARDIUM	(9)	(9)	(33)	(33)
FIBROSIS			1 (3%)	
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(33)	(33)
HEMORRHAGE				1 (3%)
NECROSIS, COAGULATIVE				1 (3%)
#PANCREAS	(10)	(10)	(31)	(33)
HEMORRHAGE				1 (3%)
INFLAMMATION, INTERSTITIAL				1 (3%)
ATROPHY, NOS				1 (3%)
#STOMACH	(10)	(10)	(33)	(34)
ULCER, NOS			2 (6%)	
#GASTRIC SUBMUCOSA	(10)	(10)	(33)	(34)
HEMORRHAGE				1 (3%)
#CECUM	(9)	(10)	(33)	(34)
HEMORRHAGIC CYST				1 (3%)
URINARY SYSTEM				
#KIDNEY	(10)	(10)	(33)	(34)
INFLAMMATION, CHRONIC			1 (3%)	1 (3%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
#KIDNEY/PELVIS	(10)	(10)	(33)	(34)
INFLAMMATION, CHRONIC				1 (3%)
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(33)	(34)
HYPERPLASIA, CYSTIC	1 (10%)			2 (6%)
#UTERUS	(9)	(9)	(32)	(34)
HEMORRHAGE			1 (3%)	
HEMORRHAGE, CHRONIC				1 (3%)
PYOMETRA	3 (33%)		3 (9%)	
ANGIECTASIS			1 (3%)	
#UTERUS/ENDOMETRIUM	(9)	(9)	(32)	(34)
INFLAMMATION, NOS			1 (3%)	
#OVARY/OVIDUCT	(9)	(9)	(32)	(34)
INFLAMMATION, NOS				1 (3%)
#OVARY	(7)	(10)	(21)	(26)
CYST, NOS			1 (5%)	
HEMORRHAGE				1 (4%)
INFLAMMATION, SUPPURATIVE				1 (4%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*JOINT	(10)	(10)	(33)	(34)
INFLAMMATION, CHRONIC SUPPURATIV				2 (6%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
BODY CAVITIES				
*PERITONEUM	(10)	(10)	(33)	(34)
HEMORRHAGE				2 (6%)
INFLAMMATION, FIBRINOUS				2 (6%)
INFLAMMATION, CHRONIC				2 (6%)
*PLEURA	(10)	(10)	(33)	(34)
INFLAMMATION, NOS				2 (6%)
INFLAMMATION, CHRONIC				2 (6%)
*PERICARDIUM	(10)	(10)	(33)	(34)
INFLAMMATION, CHRONIC				1 (3%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(10)	(10)	(33)	(34)
PLASMACYTOSIS				1 (3%)
HYPERPLASIA, LYMPHOID				1 (3%)
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED			1	
AUTOLYSIS/NO NECROPSY			2	1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH ISOPHOSPHAMIDE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ISOPHOSPHAMIDE

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	14	14	30	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	14	29	27
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(14)	(30)	(27)
INFLAMMATION, SUPPURATIVE				1 (4%)
ABSCESS, NOS				1 (4%)
RESPIRATORY SYSTEM				
#LUNG	(14)	(14)	(29)	(27)
INFLAMMATION, INTERSTITIAL	1 (7%)		1 (3%)	2 (7%)
BRONCHOPNEUMONIA SUPPURATIVE	1 (7%)			
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)	
HEMATOPOIETIC SYSTEM				
#SPLEEN	(14)	(14)	(28)	(24)
ATROPHY, NOS				1 (4%)
HEMATOPOIESIS	1 (7%)	1 (7%)	2 (7%)	4 (17%)
#MEDIASTINAL L.NODE		(1)	(1)	(5)
ATROPHY, NOS				1 (20%)
#PANCREATIC L.NODE		(1)	(1)	(5)
HYPERPLASIA, LYMPHOID				1 (20%)
#MESENTERIC L. NODE		(1)	(1)	(5)
HYPERPLASIA, LYMPHOID				1 (20%)
#THYMUS	(14)	(14)	(29)	(26)
ATROPHY, NOS				1 (4%)
CIRCULATORY SYSTEM				
NONE				

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(14)	(14)	(29)	(27)
HYPERPLASIA, NODULAR			1 (3%)	1 (4%)
#LIVER/CENTRILOBULAR	(14)	(14)	(29)	(27)
DEGENERATION, NOS				1 (4%)
URINARY SYSTEM				
#KIDNEY	(14)	(14)	(29)	(27)
INFLAMMATION, NOS				1 (4%)
INFLAMMATION, SUPPURATIVE		1 (7%)		1 (4%)
PYELONEPHRITIS SUPPURATIVE		2 (14%)		
INFLAMMATION, CHRONIC SUPPURATIV				1 (4%)
HYPERPLASIA, LYMPHOID				2 (7%)
#KIDNEY/PELVIS	(14)	(14)	(29)	(27)
INFLAMMATION, NOS				1 (4%)
#URINARY BLADDER	(14)	(14)	(29)	(27)
INFLAMMATION, SUPPURATIVE		1 (7%)		
HYPERPLASIA, EPITHELIAL				1 (4%)
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#PROSTATE	(14)	(14)	(29)	(27)
INFLAMMATION, SUPPURATIVE		1 (7%)	1 (3%)	2 (7%)
INFLAMMATION, CHRONIC				1 (4%)
INFLAMMATION, CHRONIC SUPPURATIV				1 (4%)
*SEMINAL VESICLE	(14)	(14)	(30)	(27)
HEMORRHAGE				1 (4%)
LYMPHOCYTIC INFILTRATE				1 (4%)
INFLAMMATION, SUPPURATIVE				2 (7%)
GRANULOMA, NOS				1 (4%)
NECROSIS, NOS				1 (4%)
HYPERPLASIA, LYMPHOID				1 (4%)
NERVOUS SYSTEM				
NONE				

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(14)	(14)	(30)	(27)
INFLAMMATION, NOS				1 (4%)
INFLAMMATION, SUPPURATIVE		1 (7%)	1 (3%)	
*MESENTERY	(14)	(14)	(30)	(27)
NECROSIS, FAT		1 (7%)		
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	10	9	17	13
AUTO/NECROPSY/NO HISTO			1	
AUTOLYSIS/NO NECROPSY	1	1	5	8

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF ISOPHOSPHAMIDE

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS MISSING			1	
ANIMALS NECROPSIED	15	14	32	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	14	32	34
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG/BRONCHIOLE	(15)	(14)	(32)	(34)
HYPERPLASIA, PLASMA CELL			1 (3%)	
HYPERPLASIA, LYMPHOID	1 (7%)		1 (3%)	1 (3%)
#LUNG	(15)	(14)	(32)	(34)
INFLAMMATION, INTERSTITIAL		1 (7%)	1 (3%)	1 (3%)
BRONCHOPNEUMONIA SUPPURATIVE		1 (7%)	3 (9%)	1 (3%)
HYPERPLASIA, LYMPHOID	2 (13%)	1 (7%)	2 (6%)	1 (3%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(14)	(14)	(29)	(34)
ATROPHY, NOS	1 (7%)			
#SPLEEN	(15)	(14)	(32)	(34)
HYPERPLASIA, RETICULUM CELL				1 (3%)
HYPERPLASIA, LYMPHOID			2 (6%)	
HEMATOPOIESIS	1 (7%)		11 (34%)	5 (15%)
#MEDIASTINAL L.NODE			(2)	(7)
ATROPHY, NOS				1 (14%)
#MESENTERIC L. NODE			(2)	(7)
HYPERPLASIA, LYMPHOID			1 (50%)	
#THYMUS	(15)	(14)	(31)	(34)
ATROPHY, NOS				2 (6%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM				
#MYOCARDIUM	(15)	(14)	(32)	(34)
INFLAMMATION, SUPPURATIVE		1 (7%)		
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
#KIDNEY	(14)	(14)	(32)	(34)
HYPERPLASIA, LYMPHOID	2 (14%)		1 (3%)	
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(14)	(32)	(34)
HYPERPLASIA, CYSTIC				1 (3%)
#UTERUS	(14)	(14)	(32)	(33)
METAPLASIA, SQUAMOUS				1 (3%)
#UTERUS/ENDOMETRIUM	(14)	(14)	(32)	(33)
INFLAMMATION, SUPPURATIVE				1 (3%)
HYPERPLASIA, NOS				4 (12%)
HYPERPLASIA, CYSTIC	10 (71%)	11 (79%)	15 (47%)	25 (76%)
#OVARY	(14)	(14)	(32)	(31)
CYST, NOS			2 (6%)	
THROMBOSIS, NOS			1 (3%)	
INFLAMMATION, SUPPURATIVE		1 (7%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	CONTROL (VEH)	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(14)	(32)	(34)
INFLAMMATION, CHRONIC				1 (3%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(14)	(32)	(34)
INFLAMMATION, GRANULOMATOUS				1 (3%)
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	3	1	5	2
ANIMAL MISSING/NO NECROPSY			1	
ACCIDENTAL DEATH		1		
AUTOLYSIS/NO NECROPSY			2	1

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS TREATED WITH ISOPHOSPHAMIDE

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

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b]	0/9 (0.00)	0/29 (0.00)	2/32 (0.06)	0/34 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.045		
Relative Risk (Matched Vehicle Control)[f]			Infinite	
Lower Limit			0.093	
Upper Limit			Infinite	
Relative Risk (Pooled Vehicle Control)[f]			Infinite	
Lower Limit			0.272	
Upper Limit			Infinite	
Weeks to First Observed Tumor	--		72	--

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/9 (0.00)	0/29 (0.00)	2/32 (0.06)	0/34 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.041		
Relative Risk (Matched Vehicle Control)[f]			Infinite	
Lower Limit			0.093	
Upper Limit			Infinite	
Relative Risk (Pooled Control)[f]			Infinite	
Lower Limit			0.272	
Upper Limit			Infinite	
Weeks to First Observed Tumor	--		79	--

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Hematopoietic System:				
Malignant Lymphoma[b]	0/9 (0.00)	0/29 (0.00)	2/32 (0.06)	4/34 (0.12)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.071	0.200
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.233	0.056
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		79	34
Hematopoietic System:				
Leukemia or Lymphoma[b]	0/9 (0.00)	0/29 (0.00)	3/32 (0.09)	5/34 (0.15)
P Values[c,d]	N.S.	P = 0.032	N.S.	P = 0.040**
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.140	0.270
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.417	1.099
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		45	34

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	3/7 (0.43)	4/25 (0.16)	3/29 (0.10)	0/32 (0.00)
P Values[c,d]	P = 0.002 (N)	P = 0.024 (N)	N.S.	P = 0.003* (N) P = 0.032** (N
Relative Risk (Matched Vehicle Control)[f]			0.241	0.000
Lower Limit			0.049	0.000
Upper Limit			1.361	0.344
Relative Risk (Pooled Vehicle Control)[f]			0.647	0.000
Lower Limit			0.120	0.000
Upper Limit			3.215	0.825
Weeks to First Observed Tumor	83		79	--
Pituitary: Chromophobe Carcinoma[b]	1/7 (0.14)	1/25 (0.04)	1/29 (0.03)	0/32 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			0.241	0.000
Lower Limit			0.007	0.000
Upper Limit			8.740	4.069
Relative Risk (Pooled Vehicle Control)[f]			0.862	0.000
Lower Limit			0.024	0.000
Upper Limit			31.358	13.482
Weeks to First Observed Tumor	80		83	--

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	4/7 (0.57)	5/25 (0.20)	4/29 (0.14)	0/32 (0.00)
P Values[c,d]	P < 0.001 (N)	P = 0.012 (N)	P = 0.030* (N)	P < 0.001* (N) P = 0.013** (N
Relative Risk (Matched Vehicle Control)[f]			0.241	0.000
Lower Limit			0.080	0.000
Upper Limit			1.059	0.219
Relative Risk (Pooled Vehicle Control)[f]			0.690	0.000
Lower Limit			0.166	0.000
Upper Limit			2.738	0.605
Weeks to First Observed Tumor	--		79	--
Adrenal: Cortical Adenoma[b]	0/9 (0.00)	0/27 (0.00)	2/31 (0.06)	2/34 (0.06)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.077	0.071
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.217	0.200
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		79	33

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Adrenal: Phenochromocytoma[b]	0/9 (0.00)	0/27 (0.00)	2/31 (0.06)	2/34 (0.06)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.077	0.071
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.217	0.200
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		74	45
Mammary Gland: Fibroadenoma[b]	1/9 (0.11)	2/29 (0.07)	0/32 (0.00)	0/34 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			0.000	0.000
Lower Limit			0.000	0.000
Upper Limit			5.218	4.919
Relative Risk (Pooled Vehicle Control)[f]			0.000	0.000
Lower Limit			0.000	0.000
Upper Limit			3.015	2.847
Weeks to First Observed Tumor	83		--	--

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

(continued)

[a]Treated groups received doses of 6 or 12 mg/kg.

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

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

[d]A negative trend (N) indicates a lower incidence in a 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 Treated with Isophosphamide[a]

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b]	0/10 (0.00)	1/28 (0.04)	1/33 (0.03)	0/34 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	
Lower Limit			0.018	
Upper Limit			Infinite	
Relative Risk (Pooled Vehicle Control)[f]			0.849	0.000
Lower Limit			0.023	0.000
Upper Limit			30.876	15.225
Weeks to First Observed Tumor	--		64	--
Hematopoietic System:				
Malignant Lymphoma[b]	0/10 (0.00)	0/30 (0.00)	1/33 (0.03)	0/34 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	
Lower Limit			0.018	
Upper Limit			Infinite	
Relative Risk (Pooled Vehicle Control)[f]			Infinite	
Lower Limit			0.053	
Upper Limit			Infinite	
Weeks to First Observed Tumor	--		33	--

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	3/10 (0.30)	11/28 (0.39)	7/31 (0.23)	0/34 (0.00)
P Values[c,d]	P = 0.003 (N)	P < 0.001 (N)	N.S.	P = 0.009* (N) P < 0.001** (N
Relative Risk (Matched Vehicle Control)[f]			0.753	0.000
Lower Limit			0.228	0.000
Upper Limit			3.431	0.472
Relative Risk (Pooled Vehicle Control)[f]			0.575	0.000
Lower Limit			0.226	0.000
Upper Limit			1.390	0.242
Weeks to First Observed Tumor	57		60	--

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Carcinoma[b]	4/10 (0.40)	4/28 (0.14)	1/31 (0.03)	1/34 (0.03)
P Values[c,d]	P = 0.004 (N)	N.S.	P = 0.009* (N)	P = 0.007* (N)
Departure from Linear Trend[e]	P = 0.008			
Relative Risk (Matched Vehicle Control)[f]			0.081	0.074
Lower Limit			0.003	0.003
Upper Limit			0.681	0.622
Relative Risk (Pooled Vehicle Control)[f]			0.226	0.206
Lower Limit			0.010	0.009
Upper Limit			2.013	1.839
Weeks to First Observed Tumor	74		80	70

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	7/10 (0.70)	15/28 (0.54)	8/31 (0.26)	1/34 (0.03)
P Values[c,d]	P < 0.001 (N)	P < 0.001 (N)	P = 0.017* (N) P = 0.027** (N)	P < 0.001* (N) P < 0.001** (N
Relative Risk (Matched Vehicle Control)[f]			0.369	0.042
Lower Limit			0.208	0.002
Upper Limit			0.923	0.271
Relative Risk (Pooled Vehicle Control)[f]			0.482	0.055
Lower Limit			0.218	0.003
Upper Limit			1.014	0.333
Weeks to First Observed Tumor	57		60	70
Adrenal: Cortical Adenoma[b]	3/10 (0.30)	4/28 (0.14)	8/33 (0.24)	1/34 (0.03)
P Values[c,d]	P = 0.009 (N)	N.S. (N)	N.S.	P = 0.032* (N)
Departure from Linear Trend[e]		P = 0.024		
Relative Risk (Matched Vehicle Control)[f]			0.808	0.098
Lower Limit			0.264	0.004
Upper Limit			4.147	0.978
Relative Risk (Pooled Vehicle Control)[f]			1.697	0.206
Lower Limit			0.515	0.009
Upper Limit			6.917	1.839
Weeks to First Observed Tumor	57		60	35

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Mammary Gland: Adenoma or Adenocarcinoma, NOS[b]	1/10 (0.10)	3/28 (0.11)	3/33 (0.09)	2/34 (0.06)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			0.606	0.588
Lower Limit			0.045	0.044
Upper Limit			16.771	16.279
Relative Risk (Pooled Vehicle Control)[f]			0.566	0.549
Lower Limit			0.068	0.066
Upper Limit			3.998	3.882
Weeks to First Observed Tumor	83		72	69
Mammary Gland: Cystadenoma, NOS[b]	0/10 (0.00)	0/28 (0.00)	3/33 (0.09)	1/34 (0.03)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.147	0.017
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.400	0.048
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		80	68

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Mammary Gland:				
Cystadenocarcinoma, NOS[b]	0/10 (0.00)	0/28 (0.00)	2/33 (0.06)	0/34 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.050		
Relative Risk (Matched Vehicle Control)[f]			Infinite	
Lower Limit			0.077	
Upper Limit			Infinite	
Relative Risk (Pooled Vehicle Control)[f]			Infinite	
Lower Limit			0.215	
Upper Limit			Infinite	
Weeks to First Observed Tumor	--		60	--

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Mammary Gland: Cystadenoma or Cystadenocarcinoma, NOS[b]	0/10 (0.00)	0/28 (0.00)	5/33 (0.15)	1/34 (0.03)
P Values[c,d]	N.S.	N.S.	P = 0.040**	N.S.
Departure from Linear Trend[e]	P = 0.041	P = 0.009		
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.303	0.017
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.099	0.048
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		60	68

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	3/10 (0.30)	8/28 (0.29)	28/33 (0.85)	6/34 (0.18)
P Values[c,d]	P = 0.004 (N)	N.S.	P = 0.002* P < 0.001**	N.S.
Departure from Linear Trend[e]	P < 0.001	P < 0.001		
Relative Risk (Matched Vehicle Control)[f]			2.828	0.588
Lower Limit			1.245	0.165
Upper Limit			9.795	2.778
Relative Risk (Pooled Vehicle Control)[f]			2.970	0.618
Lower Limit			1.677	0.208
Upper Limit			5.030	1.768
Weeks to First Observed Tumor	57		63	68

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

(continued)

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Uterus: Leiomyosarcoma[b]	0/9 (0.00)	0/27 (0.00)	15/32 (0.47)	1/34 (0.03)
P Values[c,d]	N.S.	N.S.	P = 0.009* P < 0.001**	N.S.
Departure from Linear Trend[e]	P < 0.001	P < 0.001		
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.513	0.016
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			4.113	0.043
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		64	69

[a]Treated groups received doses of 6 or 12 mg/kg.

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

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

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

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b] (52)	0/8 (0)	2/31 (6)	0/9 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	--
Lower Limit		0.087	--
Upper Limit		Infinite	--
Lung: Alveolar/Bronchiolar Adenoma[b] (52)	0/8 (0)	2/31 (6)	0/9 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	--
Lower Limit		0.087	--
Upper Limit		Infinite	--

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma[b] (34)	0/8 (0)	2/32 (6)	4/26 (15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.084	0.329
Upper Limit		Infinite	Infinite
Hematopoietic System: Leukemia or Lymphoma[b] (34)	0/8 (0)	3/32 (9)	5/26 (19)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.173	0.450
Upper Limit		Infinite	Infinite

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b] (52)	4/6 (67)	4/27 (15)	0/8 (0)
P Values[c,d]	P = 0.005(N)	P = 0.020(N)	P = 0.015(N)
Relative Risk (Matched Vehicle Control)[f]		0.222	0.000
Lower Limit		0.094	0.000
Upper Limit		0.941	0.661
Adrenal: Cortical Adenoma[b] (33)	0/8 (0)	2/31 (6)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.087	0.079
Upper Limit		Infinite	Infinite

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b] (52)	3/6 (50)	3/27 (11)	0/8 (0)
P Values[c,d]	P = 0.018(N)	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		0.222	0.000
Lower Limit		0.055	0.000
Upper Limit		1.388	1.044
Pituitary: Chromophobe Carcinoma[b] (52)	3/6 (50)	1/27 (4)	0/8 (0)
P Values[c,d]	P = 0.008(N)	P = 0.014(N)	N.S.
Departure from Linear Trend[e]	P = 0.027		
Relative Risk (Matched Vehicle Control)[f]		0.074	0.000
Lower Limit		0.002	0.000
Upper Limit		0.789	1.044

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Adrenal: Pheochromocytoma[b] (45)	0/8 (0)	2/31 (6)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.087	0.079
Upper Limit		Infinite	Infinite
Mammary Gland: Fibroadenoma[b] (52)	1/8 (13)	0/31 (0)	0/9 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		4.780	15.254

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

(continued)

[a]Treated groups received doses of 6 or 12 mg/kg.

[b]Number of tumor-bearing animals/number of animals examined at site (percent), based upon animals that survived at least as long as 52 weeks, unless a tumor was found at the specific site before 52 weeks. In such an instance, it is based upon animals that survived at least as long as the animal in which the first tumor was found. The week to first observed tumor is indicated in the parentheses after footnote b.

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

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

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

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b] (52)	0/9 (0)	1/32 (3)	0/7 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	--
Lower Limit		0.016	--
Upper Limit		Infinite	--
Hematopoietic System: Malignant Lymphoma[b] (33)	0/9 (0)	1/32 (3)	0/7 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	--
Lower Limit		0.016	--
Upper Limit		Infinite	--

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b] (52)	3/9 (33)	7/30 (23)	0/7 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		0.700	0.000
Lower Limit		0.225	0.000
Upper Limit		3.650	1.781
Pituitary: Chromophobe Carcinoma[b] (52)	4/9 (44)	1/30 (3)	1/7 (14)
P Values[c,d]	P = 0.049(N)	P = 0.007(N)	N.S.
Departure from Linear Trend[e]	P = 0.013		
Relative Risk (Matched Vehicle Control)[f]		0.075	0.321
Lower Limit		0.002	0.008
Upper Limit		0.658	2.307

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b] (52)	7/9 (78)	8/30 (27)	1/7 (14)
P Values[c,d]	P = 0.006(N)	P = 0.009(N)	P = 0.020(N)
Relative Risk (Matched Vehicle Control)[f]		0.343	0.184
Lower Limit		0.218	0.008
Upper Limit		0.837	0.958
Adrenal: Cortical Adenoma[b] (35)	3/10 (30)	8/32 (25)	1/18 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		0.833	0.185
Lower Limit		0.272	0.004
Upper Limit		4.253	1.997

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Mammary Gland: Adenoma or Adenocarcinoma, NOS[b] (52)	1/9 (11)	3/32 (9)	2/7 (29)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		0.844	2.571
Lower Limit		0.084	0.166
Upper Limit		42.831	124.556
Mammary Gland: Cystadenoma, NOS[b] (52)	0/9 (0)	3/32 (9)	1/7 (14)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.191	0.076
Upper Limit		Infinite	Infinite

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Mammary Gland:			
Cystadenocarcinoma, NOS[b] (52)	0/9 (0)	2/32 (6)	0/7 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	--
Lower Limit		0.093	--
Upper Limit		Infinite	--
Mammary Gland: Cystadenoma or			
Cystadenocarcinoma, NOS[b] (52)	0/9 (0)	5/32 (16)	1/7 (14)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.402	0.076
Upper Limit		Infinite	Infinite

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

(continued)

Topography: Morphology	Matched Vehicle Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b] (52)	3/9 (33)	28/32 (88)	6/7 (86)
P Values[c,d]	P = 0.009	P = 0.003	N.S.
Departure from Linear Trend[e]	P = 0.028		
Relative Risk (Matched Vehicle Control)[f]		2.625	2.571
Lower Limit		1.188	0.860
Upper Limit		8.163	4.393
Uterus: Leiomyosarcoma[b] (52)	0/8 (0)	15/31 (48)	1/7 (14)
P Values[c,d]	N.S.	P = 0.012	N.S.
Departure from Linear Trend[e]	P = 0.006		
Relative Risk (Matched Vehicle Control)[f]		Infinite	Infinite
Lower Limit		1.417	0.068
Upper Limit		Infinite	Infinite

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

(continued)

[a]Treated groups received doses of 6 or 12 mg/kg.

[b]Number of tumor-bearing animals/number of animals examined at site (percent), based upon animals that survived at least as long as 52 weeks, unless a tumor was found at the specific site before 52 weeks. In such an instance, it is based upon animals that survived at least as long as the animal in which the first tumor was found. The week to first observed tumor is indicated in the parentheses after footnote b.

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE TREATED WITH ISOPHOSPHAMIDE

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

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma[b]	0/14 (0.00)	0/28 (0.00)	4/29 (0.14)	2/27 (0.07)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.481	0.164
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.916	0.313
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		44	79
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/14 (0.00)	0/28 (0.00)	5/29 (0.17)	2/27 (0.07)
P Values[c,d]	N.S.	N.S.	P = 0.028**	N.S.
Departure from Linear Trend[e]		P = 0.032		
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.656	0.164
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.247	0.313
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		44	79

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

(continued)

[a]Treated groups received doses of 10 or 20 mg/kg.

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

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

[d]A negative trend (N) indicates a lower incidence in a 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 Treated with Isophosphamide[a]

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/14 (0.00)	0/29 (0.00)	1/32 (0.03)	0/33 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	
Lower Limit			0.025	
Upper Limit			Infinite	
Relative Risk (Pooled Vehicle Control)[f]			Infinite	
Lower Limit			0.049	
Upper Limit			Infinite	
Weeks to First Observed Tumor	--		77	--
Hematopoietic System: Malignant Lymphoma[b]	0/14 (0.00)	1/29 (0.03)	3/32 (0.09)	13/34 (0.38)
P Values[c,d]	P = 0.001	P < 0.001	N.S.	P = 0.005* P = 0.001**
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.282	1.786
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			2.719	11.088
Lower Limit			0.234	1.853
Upper Limit			139.098	449.268
Weeks to First Observed Tumor	--		73	63

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

(continued)

[a]Treated groups received doses of 10 or 20 mg/kg.

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

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

[d]A negative trend (N) indicates a lower incidence in a 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 Treated with Isophosphamide[a]

Topography: Morphology	Matched Vehicle Control	Pooled Vehicle Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma[b]	0/3 (0)	0/14 (0)	4/18 (22)	2/22 (9)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.240	0.062
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.780	0.201
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	44	79
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/3 (0)	0/14 (0)	5/18 (28)	2/22 (9)
P Values[c,d]	N.S.	N.S.	P = 0.043**	N.S.
Departure from Linear Trend[e]		P = 0.018		
Relative Risk (Matched Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.326	0.062
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.065	0.201
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	44	79

Table F3. Time-Adjusted Analyses of the Incidence of Primary Tumors in Male Mice Treated with Isophosphamide[a]

(continued)

[a]Treated groups received doses of 10 or 20 mg/kg.

[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 44 weeks on study.

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

[d]A negative trend (N) indicates a lower incidence in a 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.

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