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BIOASSAY OF

3,3'-IMINOBIS-1-PROPANOL DIMETHANESULFONATE (ESTER) HYDROCHLORIDE (IPD)

FOR POSSIBLE CARCINOGENICITY

CAS No. 3458-22-8

NCI-CG-TR-18

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

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CONTRIBUTORS: This report presents the results of the bioassay of 3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] for possible carcinogenicity, conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. D. P. Griswold[1], J. D. Prejean[1], E. K. Weisburger[2], and J. H. Weisburger[2,3]. Ms. J. Belzer[1] and Mr. I. Brown[1] were responsible for the administration of the chemical and the care of the laboratory animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[5]. The statistical analyses were performed by Dr. J. R. Joiner[6], using methods selected for the bioassay program by Dr. J. J. Gart[7]. Chemicals used in this bioassay were analyzed under the direction of Dr. W. J. Haggerty, Jr.[8], and the results of the analyses were reviewed by Dr. S. S. Olin[6].

This report was prepared at Tracor Jitco[6] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A.

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SUMMARY

A bioassay of 3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] for possible carcinogenicity was conducted by administering the test chemical intraperitoneally to Sprague-Dawley rats and B6C3F1 mice.

The IPD was injected three times per week to groups of 35 animals, using doses of 12, 24, or 48 mg/kg for the rats, and 20 or 40 mg/kg for the mice. Rats at 12 mg/kg were treated for 52 weeks. Because of the toxicity of the chemical, administration of IPD for the group receiving 24 mg/kg was discontinued at week 34. Rats receiving 48 mg/kg were treated until all had died at week 23 (males) and week 27 (females). Both groups of mice were treated for 52 weeks. All survivors were killed after post-administration periods that varied among groups.

With rats, untreated and vehicle-control groups, each consisting of 10 males and 10 females, were started with the high- and mid-dose groups and additional untreated and vehicle-control groups of the same size were started with the low-dose groups. With mice, untreated and vehicle-control groups each consisted of 15 males and 15 females.

The toxicity of IPD was associated with lower mean body weights and lower rates of survival of both the rats and mice. The shortened life spans, particularly in the rats, reduced the likelihood of the development of tumors.

In rats, peritonitis and fibrous adhesions, possibly, from direct irritation by the test chemical were observed in most treated rats at necropsy. Sarcoma, fibroma, or fibrosarcoma of the peritoneum occurred in two low-dose male, one mid-dose male, and three mid-dose female rats, but not in any control animals. Because of this low incidence, and because irritation by the test chemical may have been involved in the pathogenesis, these tumors may have been due to local effects of the chemical.

In mice, lymphomas were observed at the following incidences (males: controls 0/14, low-dose 0/26, high-dose 3/21; females: controls 1/15, low-dose 2/29, high-dose 6/27). The Tarone test for life-table analysis of the probability of survival without lymphoma indicated a significant positive dose-related increase of lymphomas with a probability level of 0.011 for male mice and 0.003 for female mice.

Squamous-cell carcinoma was noted in the mice (low-dose males 6/26, high-dose females 2/27). Seven of these tumors were observed in subcutaneous tissue in the inguinal region near the sites of injection. Although not statistically significant, this tumor may be associated with administration of IPD.

Tumors of the peritoneum in rats and tumors in the subcutaneous tissue in mice may have been due to local effects related to administration of the test chemical. The lymphomas in mice, although marginally significant, were too few in number to clearly be related to dosing.

Conclusions from this study are limited by early deaths and toxicity, but the appearance of tumors in the peritoneum near the injection sites in both rats and mice indicate the carcinogenic potential of IPD.

TABLE OF CONTENTS

APPENDIXES

TABLES

FIGURES

I. INTRODUCTION

3,3′-Iminobis-1-propanol dimethanesulfonate (ester) hydrochloride (CAS 3458-22-8; NCI C01547), hereinafter called IPD, was synthesized from bis(3-hydroxypropyl)amine and methanesulfonic acid anhydride (El-Merzabani and Sakurai, 1965). It was found to have antitumor activity against a number of experimental tumors that were naturally resistant to nitrogen mustard and has been used in Japan for the treatment of myelogenous leukemia (El-Merzabani and Sakurai, 1965; Hirano et al., 1972). IPD was selected for carcinogen bioassay as one agent in a series of anticancer drugs that are administered chronically in the treatment of human cancer.

II. MATERIALS AND METHODS

A. Chemical

The IPD was supplied by the Drug Development Branch, Division of Cancer Treatment (DCT), National Cancer Institute (NCI). It was purchased from Yoshitomi Pharmaceutical Industries, Limited, 35 Hiranomachi 3-chome, Higashi-ku, Osaka, Japan.

Analyses of each of two batches were provided through contracts of the Division of Cancer Treatment, NCI, and showed that the material consisted of > 99% of the designated chemical. The analytical methods included melting point, infrared and nuclear magnetic resonance spectroscopy, thin-layer chromatography (three solvent systems), and elemental analysis. No impurities were detected.

The IPD was stored at -20°C in the original glass container until used in this study.

B. Dosage Preparation

The IPD was prepared in phosphate-buffered saline as a fresh solution immediately prior to use. The actual mixing of the drug and the vehicle was performed in the animal laboratory, in a 10-ml Potter-Elvehjem tissue grinder with a Teflon pestle. The concentrations administered were 0.48%, 0.96%, or 1.92% (w/v) for rats, and 0.2% or 0.4% (w/v) for mice. The test chemical solution or the vehicle was administered to the treated animals or vehicle controls intraperitoneally, using one needle for each injection group at a constant volume of 0.25 ml/100 g body weight for rats, or 1.0 ml/100 g for mice. Unused solutions of IPD were discarded each day of administration.

C. Animals

For the subchronic studies, female Sprague-Dawley rats and Swiss mice of each sex, obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, through contracts of the Division of Cancer Treatment, NCI, were used. For the chronic studies, Sprague-Dawley rats and B6C3F1 mice, obtained from Charles River Breeding Laboratories, Inc., were used. On arrival at the laboratory, all animals were quarantined for an acclimation period (rats for 5 days, mice for 8 days), assigned to control or treated groups, and then earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C and the relative humidity was maintained at 40-60%. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) and water were supplied daily and were made 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 22; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N. Y.). Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; racks were cleaned once per week.

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

RATS

Gavage Studies

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

Intraperitoneal Injection Studies

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

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

Gavage Studies

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

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
(MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR)
(CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
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amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine
hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide
monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)

E. Subchronic Studies

Subchronic studies were conducted with female Sprague-Dawley rats and male and female Swiss mice to estimate the maximum tolerated doses of IPD, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. In these subchronic studies, IPD was administered intraperitoneally three times per week for 45 days to female rats anu mice of each sex in twofold increasing concentrations, followed by 45 days of observation. The treated groups consisted of five animals each; the vehicle controls consisted of 10 animals each. The rats received 24, 48, 96, or 192 mg/kg/dose; the mice received 40, 80, 160, or 320 mg/kg/dose.

All rats receiving doses of 96 or 192 mg/kg died. The mortality rates for rats receiving 24 or 48 mg/kg were 20% and 40%, respectively; at these doses there were no differences in mean body weight between the 90-day survivors and the controls.

All mice receiving doses of 160 or 320 mg/kg died. The mortality rate for mice receiving 80 mg/kg was 90%. At 40 mg/kg, all mice survived, but there was a 30% depression in mean body weight, compared with the controls.

Low and high doses for the chronic study were set at 24 and 48 mg/kg, respectively, for rats and 20 and 40 mg/kg for mice.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2. Originally, doses of 24 or 48 mg/kg were administered to groups of rats of each sex; however, toxicity resulted at the high dose, and low-dose groups at 12 mg/kg were started on study at week 56.

Table 1. Design of Chronic Studies of IPD in Rats

Sex and Test Group	Initial No. of Animals[a]	IPD Dose[b] (mg/kg)	Time on Study: Treated (weeks)	Time on Study: Untreated (weeks)
Male				
Low-Dose Untreated-Controls[c]	10	0		89
Mid- and High-Dose Untreated-Control	10	0		89
Low-Dose Vehicle-Controls[c]	10	0[d]	52	37
Mid- and High-Dose Vehicle-Control	10	0[d]		37
Low-Dose	35	12	52	3
Mid-Dose	35	24	34[e]	38[f]
High-Dose	35	48	23[g]	
Female				
Low-Dose Untreated-Controls[c]	10	0		89
Mid- and High-Dose Untreated-Control	10	0		89
Low-Dose Vehicle-Controls[c]	10	0[d]	52	37
Mid- and High-Dose Vehicle-Control	10	0[d]	52	37
Low-Dose	35	12	52	28
Mid-Dose	35	24	34[e]	37[f]
High-Dose	35	48	27[g]	

[a]High- and mid-dose males, with controls, were 35 days of age when placed on study; females with controls were 42 days of age. Low-dose males and females, with controls, were 55 days of age when placed on study.

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

Table 1. Design of Chronic Studies of IPD in Rats

(continued)

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[c]Ten controls were started initially with the mid- and high-dose groups, the other 10 were started concurrently with the low-dose group.

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

[e]Mid-dose male and female animals were treated only 34 weeks due to the toxicity of the chemical.

[f]Mid-dose male and female animals were observed only 38 and 37 weeks, respectively, due to the death of all animals.

[g]High-dose male and female animals were treated only 23 and 27 weeks, respectively, due to the death of all animals.

Table 2. Design of Chronic Studies of IPD in Mice

Sex and Test Group	Initial No. of Animals[a]	IPD Dose[b] (mg/kg)	Time on Study Treated (weeks)	Time on Study Untreated (weeks)
Male				
Untreated-Control	15	0		86
Vehicle-Control	15	0[c]	52	34
Low-Dose[d]	34	20	52	25
High-Dose	35	40	52	11
Female				
Untreated-Control	15	0		86
Vehicle-Control	15	0[c]	52	34
Low-Dose[d]	36	20	52	34
High-Dose	35	40	52	25

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

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

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

[d]The low-dose group consisted of 34 males and 36 females instead of 35 animals of each sex, because of missexing during the initiation of the study.

G. Clinical and Pathologic Examinations

All animals were observed twice per day for signs of toxicity, and animals that were moribund were killed and necropsied. Rats (mid- and high-dose) and mice were weighed individually each week for the first 8 weeks, once every 2 weeks for the next 72 weeks, and once per month for the remainder of the study. Low-dose rats were weighed once every 2 weeks for 66 weeks and once per month thereafter. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were taken 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 from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each treated group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

The mean body weights of rats of each sex treated with IPD were consistently lower than those of the vehicle controls (figure 1). Although the suppression of mean body weights was more marked in the males than in the females, the data indicate dose-related effects for both sexes. The growth rates of the untreated controls, not shown, were similar to those of the vehicle controls. Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variation. As the study progressed, all treated animals developed a poor physical condition; however, no other clinical signs were recorded.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats receiving IPD at the doses used in this study, together with those of the controls, are shown in figure 2. The following table shows the numbers of weeks on study at which 50% and 100% mortality occurred in the treated and control rats.

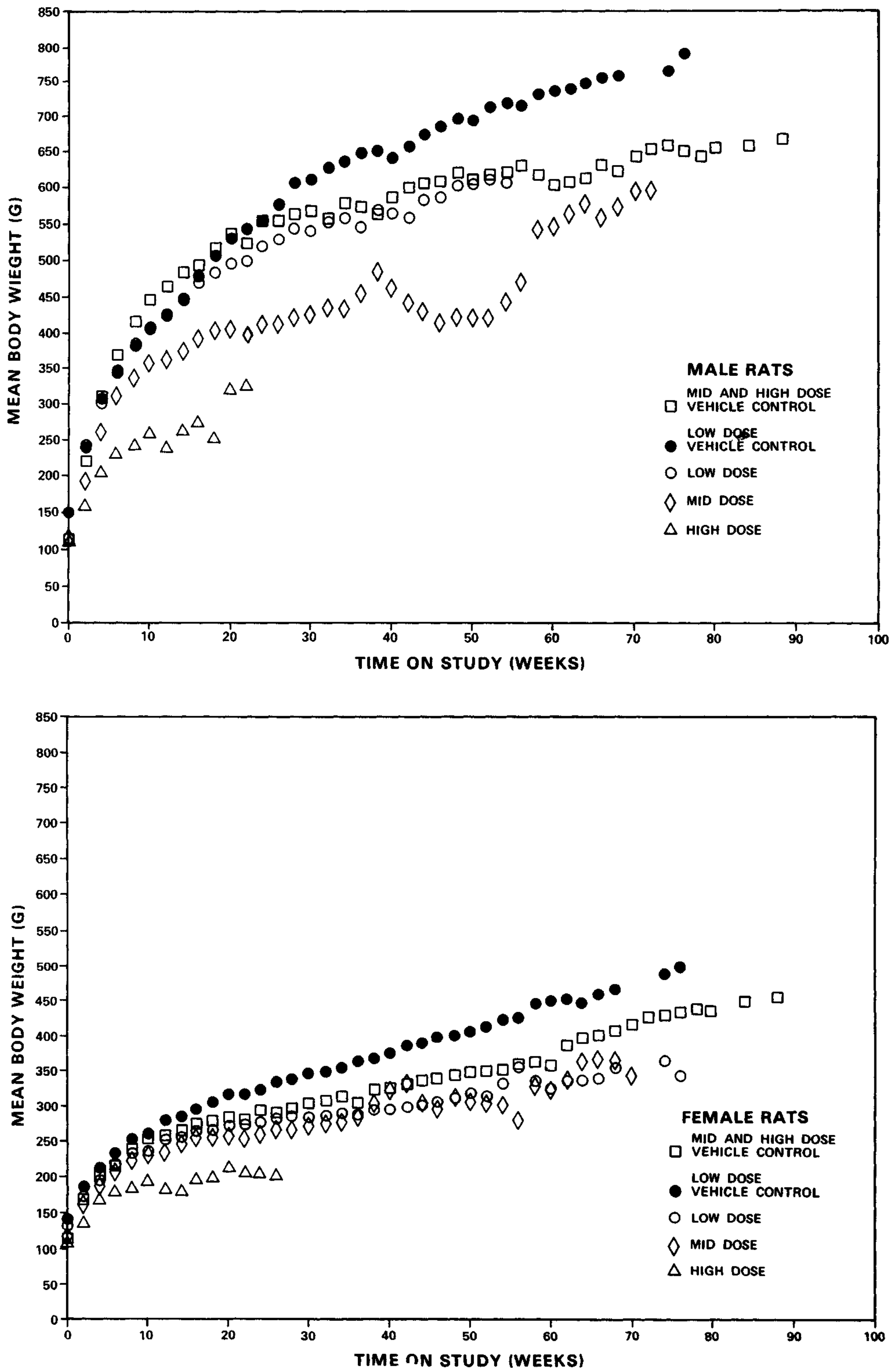


Figure 1. Growth Curves for Rats Treated with IPD

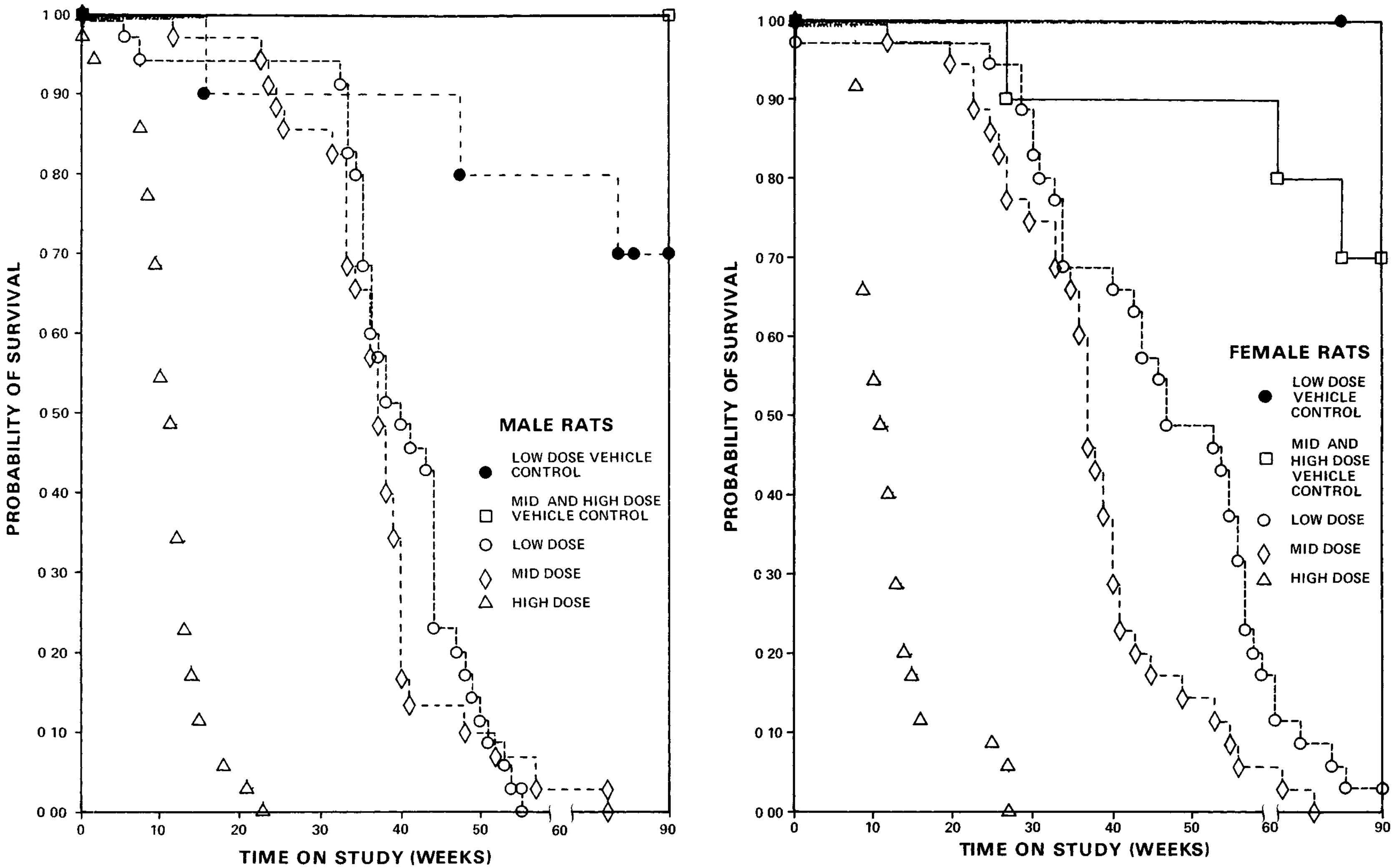


Figure 2. Survival Curves for Rats Treated with IPD

Treated Group	Time to Death (Weeks on Study)			
	50% Mortality		100% Mortality	
	Male	Female	Male	Female
High-Dose	11	11	23	27
Mid-Dose	37	37	72	71
Low-Dose	38	47	55	80
Vehicle-Control	89+	89+	89+	89+

The data show that as the dose increased, the time to death decreased. The survival curves show highly significant ($P < 0.001$) dose-related positive trends in mortality, which are not linear ($P < 0.001$). The nonlinearity is due to the steep declines in survival of the treated groups compared with the vehicle-control groups.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1-A4; findings on nonneoplastic lesions are summarized in Appendix C, tables C1-C4.

A variety of tumors occurred in both the control (untreated and vehicle) and chemical-treated groups. Some types of neoplasms occurred only, or with greater frequency, in rats of treated groups compared with those of control groups. These lesions, however, are not uncommon in this strain of rat independent of the administration of any test chemical.

A small number of spindle-cell tumors occurred in the subcutaneous and peritoneal tissues with metastasis to the lungs and mediastinal lymph nodes. The subcutaneous tumors included one fibroma (1/18 [6%] in the combined groups of vehicle-control females) and two fibrosarcomas (1/20 [5%] in the combined vehicle-control males and 1/33 [3%] in the low-dose females). The peritoneal tumors occurred in six rats: one fibroma (1/28 [4%] in the mid-dose males); two fibrosarcomas (1/28 [4%] in the mid-dose males and 1/31 [3%] in the mid-dose females); and four undifferentiated or pleomorphic spindle-cell sarcomas coded as sarcoma, NOS (not otherwise specified), (2/32 [6%] in the low-dose males and 2/31 [6%] in the mid-dose females). In one mid-dose female rat, a peritoneal fibrosarcoma had metastasized to the mediastinal lymph nodes. In another mid-dose female rat, a peritoneal sarcoma of unspecified type had metastasized to the lungs. No sarcomas were found in untreated control animals.

Both the subcutaneous and the peritoneal spindle-cell tumors varied in the degree of differentiation and had various degrees of collagenous formation. The fibromas had well-differentiated fibroblasts with ample collagen, whereas the fibrosarcomas were more pleomorphic, more anaplastic, and more variable in the amount of collagen deposited. The poorly differentiated spindle-cell tumors with little or no production of collagen were classified as sarcoma, NOS. One of the subcutaneous fibrosarcomas and two of the sarcoma, NOS, lesions were pleomorphic with formation of bizarre multinucleated giant cells. Two of the nonspecified sarcomas had extensively infiltrated the smooth muscle of the digestive tract. The confusing blend of neoplastic and nonneoplastic tissue made further classification of the sarcomas virtually impossible. The possibility of a leiomyosarcoma was not ruled out. Adenocarcinomas were observed in the large intestine in 2/28 (7%) of the mid-dose males. Both lesions were well differentiated, and one had large glandular spaces lined by columnar, cuboidal, and squamous epithelial cells. These spaces were filled by large amounts of mucin. Glands of this tumor had invaded the muscle layers.

In addition to the neoplastic lesions, a 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; however, the proliferative nonneoplastic lesions of the connective tissues lining the peritoneum were associated with treated groups. The incidences of these nonneoplastic lesions together with incidences of neoplastic peritoneal lesions, were as follows:

	Untreated Control	Vehicle Control	Low Dose	Mid Dose	High Dose
MALES					
Number of animals necropsied	(20)	(20)	(32)	(28)	(30)
Peritoneum					
Chronic Inflammation	0	0	30	23	7
Fibrous Adhesion, NOS	0	0	21	5	0
Fibroma	0	0	0	1	0
Fibrosarcoma	0	0	0	1	0
Sarcoma, NOS (spindle-cell)	0	0	2	0	0
FEMALES					
Number of animals necropsied	(20)	(18)	(33)	(31)	(31)
Peritoneum					
Chronic Inflammation	0	0	32	28	7
Fibrous Adhesion, NOS	0	0	30	8	0
Fibrosarcoma	0	0	0	1	0
Sarcoma, NOS (spindle-cell)	0	0	0	2	0

Chronic peritonitis and needle trauma may have had an important role in the pathogenesis of these peritoneal neoplasms. Needle injuries may also have been a factor in the induction of the subcutaneous fibromas and fibrosarcomas.

The small number of tumors observed may have been influenced by complications of severe chronic peritonitis and bone-marrow atrophy, with resulting decreased life span. With the reduced period at risk, however, the tumors that were observed may have greater importance.

Injection of rats with IPD resulted in few tumors. The majority of the neoplastic lesions appeared unrelated to administration of the chemical. Adenocarcinomas of the large intestine and peritoneal sarcomas may have significance. The effectiveness of the carcinogenesis bioassay was reduced by an associated decrease in life span resulting from bone-marrow atrophy and severe chronic peritonitis. In the judgment of the pathologist, the results of this study failed to define the carcinogenic activity of IPD in Sprague-Dawley rats.

Histologic features of the tumors referred to above are presented in Appendix G.

D. <u>Statistical Analyses of Results (Rats)</u>

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that were observed in at least 5% of one or more than one treated group of either sex. No pooled-control groups are used in the statistical analyses, since there are no controls from other studies that are suitable for pooling. The untreated-control groups are not used in the analyses, since the conditions of the vehicle-control groups are more nearly comparable to the conditions of the treated groups.

Two separate analyses using the Cochran-Armitage test for linear trend are included in the tables. The first line in the tables shows the results of the analysis of the incidences of tumors in the four groups of animals (vehicle-control, low-dose, mid-dose, and high-dose groups), while the second line shows the results of the analysis of the incidences seen in the three groups of animals (vehicle-control, low-dose, and mid-dose groups).

In each sex, neither the Cochran-Armitage tests for positive dose-related trend in proportions for the incidence of tumors at any site (using either three doses or two doses) nor any of the Fisher exact tests for the comparison of the incidences of tumors in a treated group with that in the controls in the positive direction is significant at the 0.05 level. The survival was so poor in the treated groups that little reliance can be placed on the negative results of this test. Significant results in the negative direction are observed in the incidences of mammary and pituitary tumors, which may be accounted for by the early mortalities of the treated animals.

In all of the 95% confidence intervals shown in the tables, values of one or less than one are included, indicating the absence of positive statistically significant results. It should also be noted that in each of the intervals with an upper limit greater than one, there is the theoretical possibility of the induction of that particular tumor by IPD, which could not be detected under the conditions of this test.

Time-adjusted analysis on the proportions of sarcoma, NOS, fibroma, or fibrosarcoma of the peritoneum in male rats is shown in table E2. The results of the Cochran-Armitage test are significant (P = 0.031 when control, low-, mid- and high-dose groups are used, and P = 0.045 when only control, low- and mid-dose groups are used), but the Fisher exact tests are not significant.

The time-adjusted analysis on the incidence of sarcoma, NOS, or fibrosarcoma of the peritoneum in female rats is shown in table E4. The results of the Cochran Armitage test are significant (P < 0.001), but departures from linear trend are observed (P < 0.001). These departures from a linear effect result from the higher proportion observed in the mid-dose group when compared with either the low-dosed or high-dosed groups. The Fisher exact test shows that the incidence in the mid-dose rats is significantly higher (P = 0.003) than that in the matched vehicle controls. These statistical test results suggest the possibility of dose association; however, it should be noted that the sample size used is very small, especially that in the mid-dose group, which is only 4. The zero incidence observed in the high-dose rats is probably due to the severe early mortality of this group of rats.

IV. RESULTS - MICE

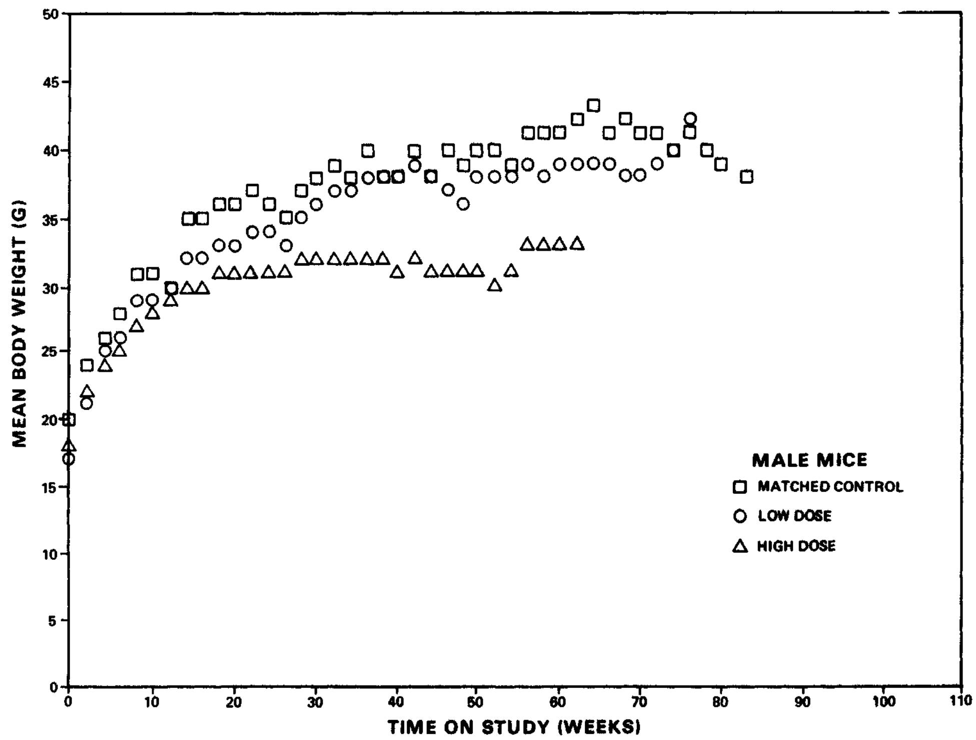
A. Body Weights and Clinical Signs (Mice)

The mean body weights of low- and high-dose male and female groups of mice were consistently lower in a generally dose-related manner than those of the vehicle controls (figure 3). The growth rates of the untreated controls, not shown, were similar to those of the vehicle controls. Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variation. No other clinical signs were recorded.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats receiving IPD at the doses used in this study, together with those of the controls, are shown in figure 4. The following table shows the numbers of weeks that elapsed before 50% and 100% mortality occurred in the treated and control mice.

	Time to Death (Weeks on Study)			
Treated Group	50% Mortality		100% Mortality	
	Male	Female	Male	Female
High-Dose	53	63	63	77
Low-Dose	63	65	77	86
Vehicle-Control	86+	86+	86+	86+



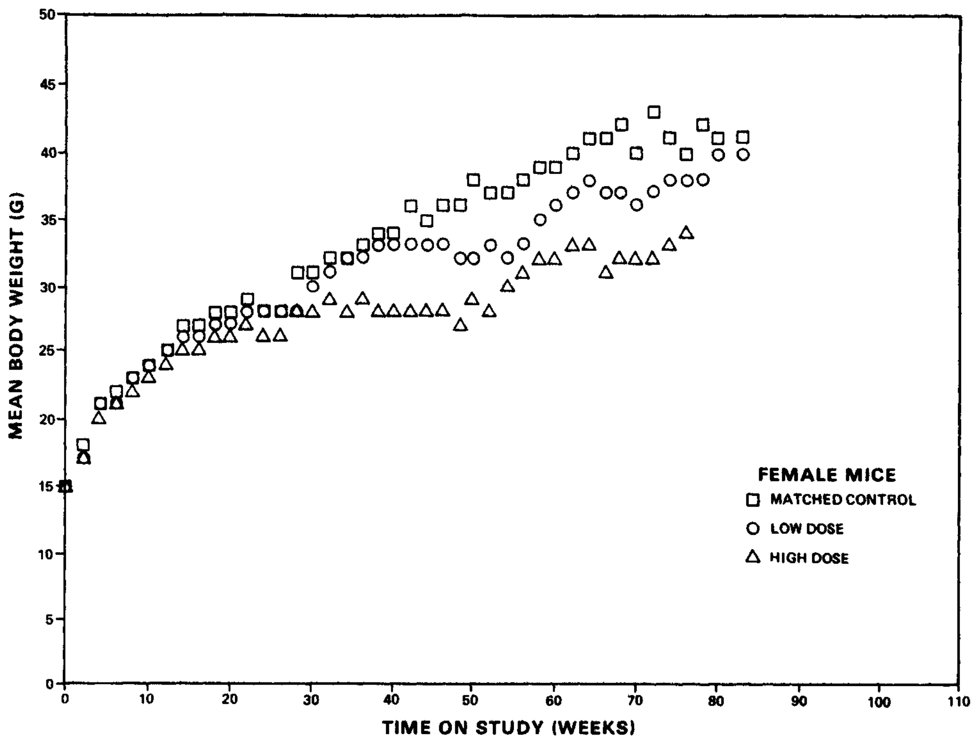


Figure 3. Growth Curves for Mice Treated with IPD

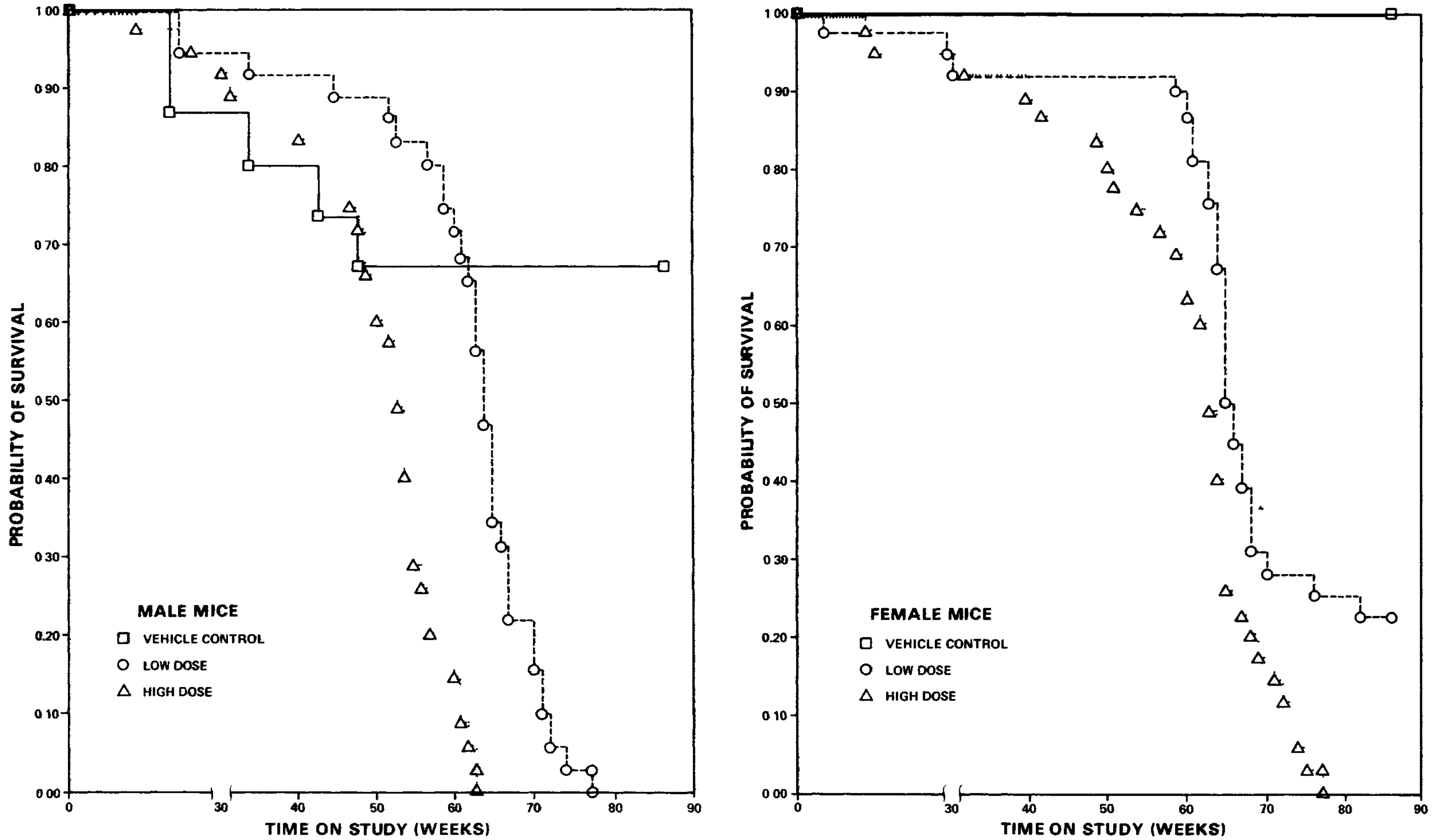


Figure 4. Survival Curves for Mice Treated with IPD

The data show that as the dose increased, the time to death decreased.

As in rats, the results of the Tarone test for mice showed a highly significant dose-related trend in mortality ($P < 0.001$ for each sex). Neither males nor females showed a significant departure from linear trend in relation to dose.

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 squamous-cell carcinomas and malignant lymphomas, the neoplasms listed in Appendix B appeared with approximately equal frequency in the treated and control mice, or appeared in insignificant numbers. Most of the squamous-cell carcinomas were located in the inguinal region and were believed to have originated from the preputial glands. Since the region where these arose was also near the sites of injection, local factors may have been involved in the pathogenesis.

Squamous-cell carcinomas in eight mice were recorded. The incidences were 6/26 (23%) in the low-dose males and 2/27 (7%) in the high-dose females. Seven of the squamous-cell carcinomas, (in the six low-dose males and in one of the high-dose females) occurred in subcutaneous tissue in the inguinal area near the sites of injections. The presence of cyst walls in organization of the tissues suggests that these tumors arose from the epithelium of preputial glands. The lesions were similar in appearance, with abundant well-differentiated keratin-forming pearls in the center and poorly organized pleomorphic squamous cells in the walls. Some of the lesions were atypical with bizarre giant tumor cells. Several lesions had numerous mitotic figures. One lesion had invaded a perineural lymphatic. The eighth tumor was a squamous-cell carcinoma of the sebaceous glands in the ear canal of a female mouse. Neoplastic squamous cells had replaced the sebaceous glands and resulted in epithelial atypism and hypercellularity. (A tissue mass from another male mouse consisted cnly of keratin and probably represented a ninth squamous-cell carcinoma; however, this lesion was not included in the summary tables.)

Malignant lymphomas involved one or more organs in 13 mice. The majority of the affected mice were female (males: high-dose 3/21 [14%]; females: untreated controls 1/13 [8%], vehicle controls 1/15 [7%], low-dose 2/29 [7%], high-dose 6/27 [22%]). The organs with lymphoid tumors were kidney, liver, spleen, thymus, mesenteric and mediastinal lymph nodes, heart, lungs, stomach, adrenal, ovary, uterus, urinary bladder, and bone marrow. One malignant lymphoma, grossly identified as an abdominal mass, probably originated in the mesenteric lymph node. Seven mice had disseminated malignant lymphomas involving multiple organs. Of the 13 malignant lymphomas, nine were of the well-differentiated lymphocytic type; the remaining four included two undifferentiated lymphomas and two types unspecified. Lesions classified as a lymphocytic type had a uniform population of small cells with little cytoplasm and small round to oval nuclei having small inconspicuous nucleoli and coarse, dark chromatin. Cells of the lymphoblastic type were similar to cells of the lymphocytic type, with an increased amount of basophilic cytoplasm and larger, more varied nuclei having finer reticulated chromatin and more distinct nucleoli. The unspecified lymphomas had cellular distortion which prevented further classification.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the treated and control groups (Appendix D). For the most part these nonneoplastic lesions were similar to those commonly observed in aged mice. The chronic fibrous peritonitis that was observed in rats given IPD was also present to a lesser extent in the mice. Two of these mice had peritoneal osseous metaplasia. Both the peritonitis and osseous metaplasia appeared to be related to intraperitoneal injection, since these lesions also occurred in the vehicle-control group. In addition to the peritonitis, respiratory infections and bone-marrow injury may also have had a role in reducing the life spans of mice during this study. The extent to which reduced life spans influenced the number of tumors observed could not be determined.

In the judgment of the pathologist, the results of this study indicate that IPD given intraperitoneally to B6C3F1 mice was responsible for squamous-cell carcinomas of the inguinal region and an increased frequency of malignant lymphomas.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that were observed in 5% of one or more than one treated group of either sex. No pooled-control groups are used in the statistical analyses, since there are no controls from other studies that are suitable for pooling. The untreated-control groups are not used in the analyses, since the conditions of the vehicle controls are more nearly comparable to the conditions of the treated groups.

In male mice, the Cochran-Armitage test for positive dose-related trend in proportions for malignant lymphomas has a probability level of 0.045, with an incidence of 3/21 (14%) in the high-dose group and none in the low-dose or vehicle-control groups. The female mice also show a larger proportion of this tumor in the high-dose group; however, due to the relatively small numbers in the groups, the results of the Fisher exact test do not show a significant difference between the high-dose and control groups in either sex. The results of the Cochran-Armitage test on the incidence in female mice are not significant. The time-adjusted analyses on the incidence of malignant lymphoma in both male and female mice are shown in tables F2 and F4, respectively. After adjustment, the male mice show an incidence of 3/20 (15%), 0/24, and 0/12 in the high-dose, low-dose, and vehicle-control groups, respectively. In female mice, the time-adjusted incidence becomes 1/15 (7%) in the vehicle-control group, 2/29 (7%) in the low-dose group, and 6/26 (23%) in the high-dose group. The results of the statistical tests using time-adjusted data are not significant. The life-table method was performed, using as an adjustment the week on study at which each malignant lymphoma was observed. Based on these data, figure 5 shows the Kaplan and Meier estimate of the probability of survival without the observation of a tumor. The Tarone test results indicated a significant positive dose-related trend for both males and females with a probability level of 0.011 for male mice and 0.003 for female mice. In neither sex is departure from linear trend indicated.

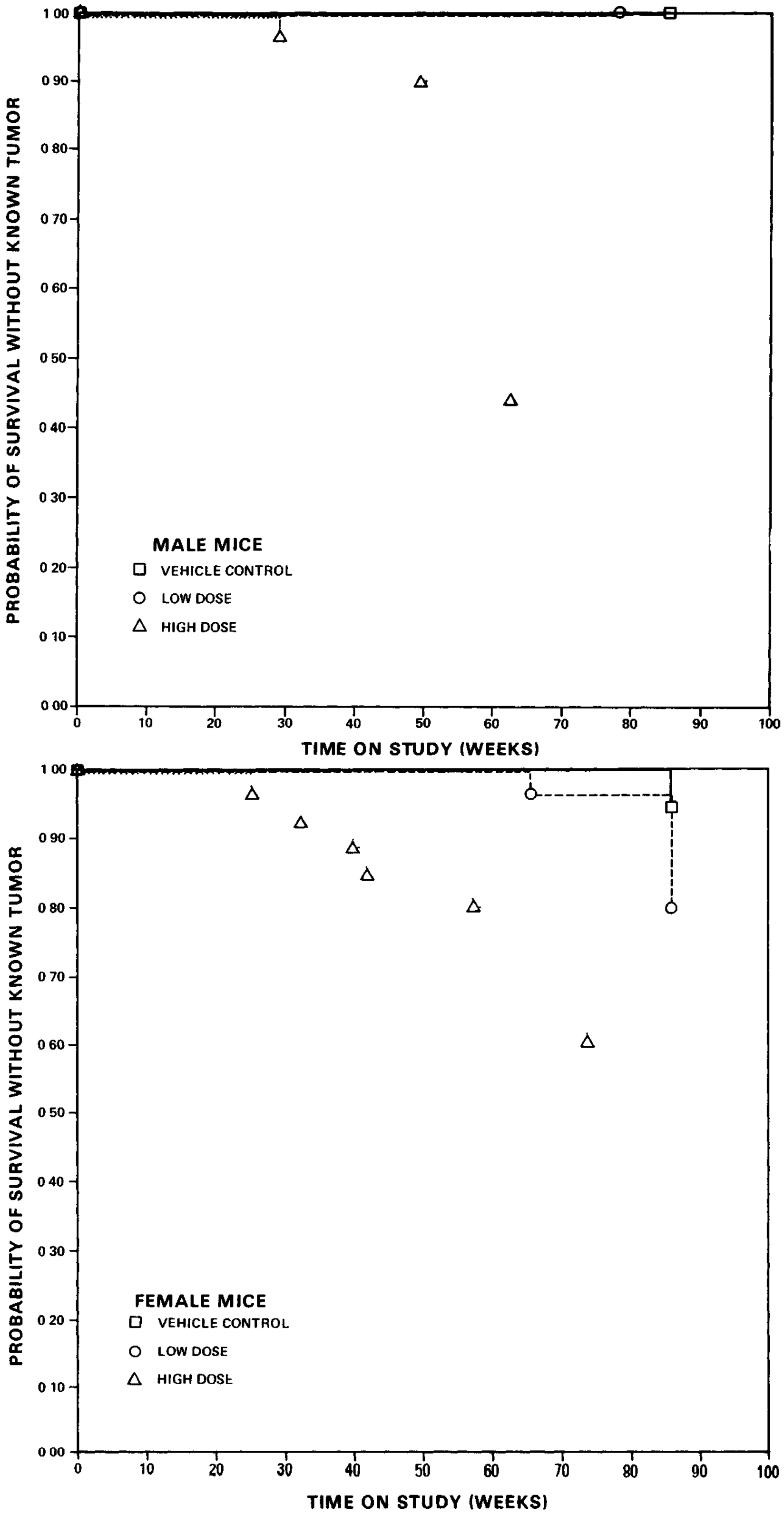


Figure 5. Life Table for Mice Treated with IPD: Malignant Lymphoma

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

V. DISCUSSION

The doses of IPD used in the bioassay were toxic, as shown by the lowered mean body weights and rates of survival of the treated rats and mice. The shortened life spans, particularly in the rats, reduced the likelihood of the development of tumors. High-dose rats of both sexes had all died by week 27. It should also be noted that animals were treated for a maximum of only 52 weeks, which is a shorter period of time than used in other current bioassays.

In rats, peritonitis and fibrous adhesions of the peritoneum were observed in most of the treated animals at necropsy. Sarcoma, fibroma, or fibrosarcoma of the peritoneum occurred only in treated animals. Metastases to the lung or the lymph nodes occurred in two of the mid-dose females. Using time-adjusted analyses of the incidences of these tumors in mid- and low-dose animals surviving at least 44 weeks (male) and 52 weeks (female), there were significant dose-related trends ($P = 0.045$ in males, and $P = 0.001$ in females), and the incidence in the mid-dose females was significantly higher ($P = 0.003$) than that in the vehicle controls. However, these significant results were based on tumors in only six animals (two low-dose males, one mid-dose male, and three mid-dose females). Because of this low incidence and because irritation by the test chemical and chronic peritonitis may have been involved in the pathogenesis, these tumors may have been due to local effects of the injection of IPD. Therefore, these tumors are not considered as evidence of carcinogenicity of the test chemical.

Atrophy of the bone marrow was observed in all of the high-dose animals of both sexes that were necropsied and examined histopathologically.

In mice, lymphomas were observed at the following incidences (males: controls 0/14, low-dose 0/26, high-dose 3/21; females: controls 1/15, low-dose 2/29, high-dose 6/27). The results of the unadjusted and time adjusted analysis are not significant; however, the Tarone test for life-table analysis of the probability of survival without lymphoma indicated a significant positive dose-related increase of lymphomas with a probability level of 0.011 for male mice and 0.003 for female mice. These significant results are based on tumors in only three male and eight treated female mice.

Squamous-cell carcinomas occurred in 6/26 low-dose male and 2/27 high-dose female mice, but in no other group. Seven of these carcinomas occurred in subcutaneous tissue in the inguinal area near the sites of injection and probably arose from the epithelium of the preputial glands; the eighth, in a female, was in the sebaceous glands of the ear canal. None of the statistical tests for these tumors was significant; however, since most tumors arose near the sites of injection, they may have been related to the repeated intraperitoneal injections of the test chemical, irritation by the test chemical, or both.

IPD is an antitumor agent that has immunosuppressive properties, as shown by reduction of leukocytes in Donryu rats (Tsukagoshi and Sakurai, 1970), reduction of spleen and bone-marrow cells in CDF1 mice (Vadlamudi et al., 1971a), and suppression of hemagglutinin synthesis in CDF1 mice (Vadlamudi et al., 1971b). These immunosuppressive properties may, in turn, be responsible for the apparent increase in tumors of the lymphoid system in mice, which was observed in the present bioassay. Results from a pulmonary tumor test system have shown that intraperitoneal injections of IPD into strain A mice at doses of 46 mg/kg three times per week for 8 weeks induced statistically significant numbers of pulmonary tumors (Stoner et al., 1973).

Tumors of the peritoneum in rats and tumors in the subcutaneous tissue in mice may have been due to local effects related to administration of the test chemical. The lymphomas in mice, although statistically significant, were too few in number to be clearly related to dosing.

Conclusions from this study are limited by early deaths and toxicity, but the appearance of tumors in the peritoneum near the injection sites in both rats and mice indicate the carcinogenic potential of IPD.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD

TABLE A1

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD (CONTROL GROUPS)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	10	10
ANIMALS NECROPSIED	10	10	10	10
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	10	10
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(10)	(10)
KERATOACANTHOMA			1 (10%)	
*SUBCUT TISSUE	(10)	(10)	(10)	(10)
FIBROSARCOMA				1 (10%)
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(8)	(10)	(8)
CHROMOPHOBE CARCINOMA	1 (10%)		1 (10%)	

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

TABLE A1 CONTROL MALE RATS: NEOPLASMS (CONTINUED)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(10)	(10)
ADENOCARCINOMA, NOS				1 (10%)
FIBROADENOMA				1 (10%)
#TESTIS	(9)	(10)	(10)	(10)
INTERSTITIAL-CELL TUMOR		1 (10%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(10)	(10)	(10)	(10)
SQUAMOUS CELL CARCINOMA		1 (10%)		
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	10	10
NATURAL DEATH@	1			1
MORIBUND SACRIFICE				2
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	9	10	10	7
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 CONTROL MALE RATS: NEOPLASMS (CONTINUED)

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

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

TABLE A2

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	32	28	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	32	28	30
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#COLON	(32)	(28)	(24)
ADENOCARCINOMA, NOS		1 (4%)	
#CECUM	(32)	(28)	(24)
MUCINOUS ADENOCARCINOMA		1 (4%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			

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

TABLE A2 TREATED MALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
#BRAIN	(29)	(28)	(22)
ASTROCYTOMA	1 (3%)		1 (5%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(32)	(28)	(30)
KERATOACANTHOMA		1 (4%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(32)	(28)	(30)
SARCOMA, NOS	2 (6%)		
FIBROMA		1 (4%)	
FIBROSARCOMA		1 (4%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	35	35	35
NATURAL DEATH@	17	16	22
MORIBUND SACRIFICE	18	19	13
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE			
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 TREATED MALE RATS: NEOPLASMS (CONTINUED)

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

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

TABLE A3

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD (CONTROL GROUPS)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	10	10
ANIMALS NECROPSIED	10	10	8	10
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	8	10
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(10)	(10)	(8)	(10)
FIBROMA			1 (13%)	
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(6)	(8)	(10)
CHROMOPHOBE ADENOMA		1 (17%)	3 (38%)	5 (50%)
#ADRENAL	(10)	(10)	(8)	(10)
CORTICAL ADENOMA			1 (13%)	

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

TABLE A3 CONTROL FEMALE RATS: NEOPLASMS (CONTINUED)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
#THYROID	(5)	(7)	(8)	(10)
C-CELL CARCINOMA	1 (20%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(8)	(10)
ADENOMA, NOS				1 (10%)
ADENOCARCINOMA, NOS	1 (10%)			1 (10%)
FIBROADENOMA	2 (20%)	3 (30%)	3 (38%)	2 (20%)
#UTERUS	(10)	(10)	(8)	(10)
ENDOMETRIAL STROMAL POLYP	1 (10%)		1 (13%)	
NERVOUS SYSTEM				
#BRAIN	(10)	(9)	(8)	(10)
ASTROCYTOMA			1 (13%)	
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MESENTERY	(10)	(10)	(8)	(10)
LIPOMA	1 (10%)			
ALL OTHER SYSTEMS				
NONE				

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

TABLE A3 CONTROL FEMALE RATS: NEOPLASMS (CONTINUED)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	10	10
NATURAL DEATH@	1		3	
MORIBUND SACRIFICE	1	1		
SCHEDULED SACRIFICE			1	
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	8	9	6	10
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	4	6	6
TOTAL PRIMARY TUMORS	6	4	10	9
TOTAL ANIMALS WITH BENIGN TUMORS	4	4	6	6
TOTAL BENIGN TUMORS	4	4	9	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	2		1	1
TOTAL MALIGNANT TUMORS	2		1	1
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE A4

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	33	31	31
ANIMALS EXAMINED HISTOPATHOLOGICALLY	33	31	31
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(33)	(31)	(31)
FIBROSARCOMA	1 (3%)		
RESPIRATORY SYSTEM			
#LUNG	(33)	(31)	(30)
SARCOMA, NOS, METASTATIC		1 (3%)	
HEMATOPOIETIC SYSTEM			
#MEDIASTINAL L.NODE	(2)	(9)	(12)
FIBROSARCOMA, METASTATIC		1 (11%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(33)	(31)	(31)
HEPATOCELLULAR ADENOMA		1 (3%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(29)	(27)	(22)
CHROMOPHOBE ADENOMA	2 (7%)		

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

TABLE A4 TREATED FEMALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(33)	(31)	(31)
ADENOMA, NOS	2 (6%)		
ADENOCARCINOMA, NOS	1 (3%)		
FIBROADENOMA	4 (12%)		
HEMANGIOMA		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(33)	(31)	(31)
SARCOMA, NOS		2 (6%)	
FIBROSARCOMA		1 (3%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	35	35	35
NATURAL DEATH@	8	15	21
MORIBUND SACRIFICE	26	20	14
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	1		
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A4 TREATED FEMALE RATS: NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	3	
TOTAL PRIMARY TUMORS	10	5	
TOTAL ANIMALS WITH BENIGN TUMORS	7	2	
TOTAL BENIGN TUMORS	8	2	
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	3	
TOTAL MALIGNANT TUMORS	2	3	
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF IPD

TABLE B1

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

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	34	35
ANIMALS MISSING			1	
ANIMALS NECROPSIED	14	14	26	21
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	14	26	21
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(14)	(14)	(26)	(21)
SQUAMOUS CELL CARCINOMA			6 (23%)	
RESPIRATORY SYSTEM				
#LUNG	(14)	(14)	(26)	(22)
CARCINOMA,NOS			1 (4%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(14)	(26)	(21)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				1 (5%)
#MEDIASTINAL L.NODE		(2)	(1)	(3)
CARCINOMA, NOS, METASTATIC			1 (100%)	
#THYMUS	(11)	(14)	(22)	(20)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				2 (10%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#STOMACH	(14)	(14)	(23)	(22)
SQUAMOUS CELL PAPILLOMA				1 (5%)
URINARY SYSTEM				
NONE				

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

TABLE B1 MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
#PANCREATIC ISLETS	(13)	(14)	(24)	(21)
ISLET-CELL ADENOMA		1 (7%)		
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	34	35
NATURAL DEATH@	3	5	20	26
MORIBUND SACRIFICE			13	9
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	12	10		
ANIMAL MISSING			1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*		1	6	4
TOTAL PRIMARY TUMORS		1	7	4
TOTAL ANIMALS WITH BENIGN TUMORS		1		1
TOTAL BENIGN TUMORS		1		1
TOTAL ANIMALS WITH MALIGNANT TUMORS			6	3
TOTAL MALIGNANT TUMORS			7	3
TOTAL ANIMALS WITH SECONDARY TUMORS#			1	
TOTAL SECONDARY TUMORS			1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE B2

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

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	36	35
ANIMALS MISSING	1			
ANIMALS NECROPSIED	13	15	29	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	15	29	26
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(13)	(15)	(29)	(27)
SQUAMOUS CELL CARCINOMA				1 (4%)
FIBROSARCOMA				1 (4%)
RESPIRATORY SYSTEM				
#LUNG	(13)	(15)	(29)	(24)
ALVEOLAR/BRONCHIOLAR ADENOMA				2 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (7%)	2 (7%)	
ADENOSQUAMOUS CARCINOMA, METASTA				1 (4%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(13)	(15)	(29)	(27)
MALIGNANT LYMPHOMA, NOS				2 (7%)
MALIG. LYMPHOMA, UNDIFFER-TYPE				2 (7%)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE	1 (8%)			1 (4%)
*ABDOMINAL CAVITY	(13)	(15)	(29)	(27)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)	
#LIVER	(13)	(15)	(29)	(24)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (7%)		
#KIDNEY	(13)	(15)	(28)	(24)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (4%)	
#THYMUS	(13)	(15)	(25)	(19)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				1 (5%)
CIRCULATORY SYSTEM				
NONE				

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

TABLE B2 FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
[IGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NCNE				
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(13)	(15)	(29)	(27)
ADENOCARCINOMA, NOS				1 (4%)
#OVARY	(8)	(14)	(25)	(18)
ADENOMA, NOS				1 (6%)
PAPILLARY ADENOMA				1 (6%)
CYSTADENOMA, NOS		1 (7%)	9 (36%)	
TUBULAR ADENOMA			1 (4%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(13)	(15)	(29)	(27)
SQUAMOUS CELL CARCINOMA				1 (4%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(13)	(15)	(29)	(27)
CARCINOMA, NOS				1 (4%)

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

TABLE B2 FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
MUCINOUS ADENOCARCINOMA			1 (3%)	
*PELVIS	(13)	(15)	(29)	(27)
MESOTHELIOMA, MALIGNANT			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	36	35
NATURAL DEATH@	1		14	24
MORIBUND SACRIFICE	1		14	11
SCHEDULED SACRIFICE			1	
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	12	15	7	
ANIMAL MISSING	1			

@ INCLUDES AUTOLYZED ANIMALS

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	3	13	14
TOTAL PRIMARY TUMORS	1	3	16	15
TOTAL ANIMALS WITH BENIGN TUMORS		1	10	4
TOTAL BENIGN TUMORS		1	10	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	2	5	10
TOTAL MALIGNANT TUMORS	1	2	6	11
TOTAL ANIMALS WITH SECONDARY TUMORS#				1
TOTAL SECONDARY TUMORS				1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD

TABLE C1

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD (CONTROL GROUPS)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	10	10
ANIMALS NECROPSIED	10	10	10	10
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	10	10
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(10)	(10)
INFLAMMATION, CHRONIC SUPPURATIV				1 (10%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (10%)	
INFLAMMATION WITH FIBROSIS		1 (10%)		
HYPERKERATOSIS		1 (10%)		
PARAKERATOSIS		1 (10%)		
*SUBCUT TISSUE	(10)	(10)	(10)	(10)
EPIDERMAL INCLUSION CYST				1 (10%)
RESPIRATORY SYSTEM				
#TRACHEA	(9)	(10)	(9)	(10)
INFLAMMATION, CHRONIC				2 (20%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (10%)		
#LUNG	(10)	(10)	(10)	(10)
PNEUMONIA, CHRONIC MURINE	1 (10%)		1 (10%)	1 (10%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(10)	(10)	(9)	(10)
ATROPHY, NOS		5 (50%)		3 (30%)
#SPLEEN	(10)	(10)	(10)	(10)
HEMATOPOIESIS				1 (10%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				

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

TABLE C1 CONTROL MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
URINARY SYSTEM				
#KIDNEY	(10)	(10)	(10)	(10)
HYDRONEPHROSIS		1 (10%)		
INFLAMMATION, INTERSTITIAL	1 (10%)			
INFLAMMATION, CHRONIC	3 (30%)	6 (60%)	2 (20%)	3 (30%)
ENDOCRINE SYSTEM				
#THYROID	(4)	(8)	(10)	(7)
CYSTIC FOLLICLES				1 (14%)
#PARATHYROID	(2)	(6)	(6)	(2)
HYPERPLASIA, NOS		1 (17%)		
REPRODUCTIVE SYSTEM				
NONE				
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 C1 CONTROL MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	4	2	6	2

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

TABLE C2

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	32	28	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	32	28	30
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(32)	(28)	(30)
EDEMA, NOS			1 (3%)
HEMORRHAGE	4 (13%)	1 (4%)	
INFLAMMATION, HEMORRHAGIC	1 (3%)		
INFLAMMATION, CHRONIC			1 (3%)
RESPIRATORY SYSTEM			
#TRACHEA	(30)	(27)	(22)
INFLAMMATION, ACUTE/CHRONIC			1 (5%)
#LUNG/BRONCHUS	(31)	(28)	(25)
ULCER, NOS			1 (4%)
#LUNG	(31)	(28)	(25)
PNEUMONIA, CHRONIC MURINE	1 (3%)	7 (25%)	9 (36%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(30)	(27)	(29)
ATROPHY, NOS	1 (3%)	8 (30%)	28 (97%)
#SPLEEN	(32)	(28)	(23)
HEMATOPOIESIS	2 (6%)	1 (4%)	
#LYMPH NODE		(8)	(8)
HYPERPLASIA, PLASMA CELL			1 (13%)
#MEDIASTINAL L.NODE		(8)	(8)
HYPERPLASIA, PLASMA CELL			2 (25%)
#MESENTERIC L. NODE		(8)	(8)
INFLAMMATION, HEMORRHAGIC			1 (13%)

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

TABLE C2 TREATED MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#MYOCARDIUM	(31)	(28)	(23)
INFLAMMATION, INTERSTITIAL		1 (4%)	
#CARDIAC VALVE	(31)	(28)	(23)
FIBROSIS	1 (3%)		
DEGENERATION, NOS	1 (3%)		
*PULMONARY ARTERY	(32)	(28)	(30)
THROMBUS, ORGANIZED		1 (4%)	
DIGESTIVE SYSTEM			
#LIVER	(32)	(27)	(27)
HEMORRHAGE			2 (7%)
HEMATOMA, NOS			3 (11%)
NECROSIS, FOCAL			2 (7%)
NECROSIS, COAGULATIVE		1 (4%)	1 (4%)
#LIVER/CENTRILOBULAR	(32)	(27)	(27)
DEGENERATION, NOS			1 (4%)
NECROSIS, NOS			1 (4%)
#PANCREAS	(32)	(27)	(23)
INFLAMMATION, INTERSTITIAL		1 (4%)	
#COLON	(32)	(28)	(24)
HEMORRHAGE	1 (3%)		1 (4%)
ULCER, NOS	1 (3%)		1 (4%)
#CECUM	(32)	(28)	(24)
HEMORRHAGE	1 (3%)		
URINARY SYSTEM			
#KIDNEY	(32)	(28)	(24)
HEMORRHAGE			1 (4%)
PYELONEPHRITIS, NOS		1 (4%)	
INFLAMMATION, CHRONIC	1 (3%)		
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(32)	(28)	(23)
HYPERPLASIA, NODULAR		2 (7%)	

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

TABLE C2 TREATED MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
#PROSTATE	(32)	(27)	(23)
INFLAMMATION, ACUTE SUPPURATIVE		1 (4%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(32)	(28)	(30)
INFLAMMATION, SUPPURATIVE	3 (9%)	4 (14%)	
INFLAMMATION, HEMORRHAGIC			1 (3%)
INFLAMMATION, CHRONIC	30 (94%)	23 (82%)	7 (23%)
ADHESION, NOS	21 (66%)	5 (18%)	
HYPERPLASIA, MESOTHELIAL		1 (4%)	
*PLEURA	(32)	(28)	(30)
INFLAMMATION, SUPPURATIVE	1 (3%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	3	
NO NECROPSY PERFORMED	1		
AUTOLYSIS/NO NECROPSY	2	7	5

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

TABLE C3

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD (CONTROL GROUPS)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	10	10	10	10
ANIMALS NECROPSIED	10	10	8	10
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	8	10
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(10)	(8)	(10)
INFLAMMATION, ACUTE/CHRONIC	1 (10%)		1 (13%)	
INFLAMMATION, CHRONIC				1 (10%)
INFLAMMATION, CHRONIC SUPPURATIV				1 (10%)
#LUNG	(10)	(10)	(8)	(10)
PNEUMONIA, CHRONIC MURINE	1 (10%)			
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(9)	(10)	(8)	(9)
ATROPHY, NOS		4 (40%)		4 (44%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#PANCREAS	(10)	(10)	(8)	(10)
NECROSIS, NOS	1 (10%)			
URINARY SYSTEM				
#KIDNEY	(9)	(10)	(8)	(10)
HYDRONEPHROSIS	1 (11%)			

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

TABLE C3 CONTROL FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MID- AND HIGH-DOSE UNTREATED CONTROL	LOW-DOSE UNTREATED CONTROL	MID- AND HIGH-DOSE VEHICLE CONTROL	LOW-DOSE VEHICLE CONTROL
INFLAMMATION, CHRONIC				2 (20%)
#URINARY BLADDER	(10)	(10)	(8)	(10)
HYPERPLASIA, EPITHELIAL	1 (10%)			
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(6)	(8)	(10)
HYPERPLASIA, CHROMOPHOBE-CELL		2 (33%)		2 (20%)
#ADRENAL	(10)	(10)	(8)	(10)
ANGIECTASIS		2 (20%)		
REPRODUCTIVE SYSTEM				
#UTERUS/ENDOMETRIUM	(10)	(10)	(8)	(10)
INFLAMMATION, SUPPURATIVE		3 (30%)	2 (25%)	
INFLAMMATION, CHRONIC SUPPURATIV		1 (10%)		1 (10%)
#OVARY	(8)	(10)	(8)	(10)
INFLAMMATION, SUPPURATIVE			1 (13%)	
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 C3 CONTROL FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

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

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

TABLE C4

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	33	31	31
ANIMALS EXAMINED HISTOPATHOLOGICALLY	33	31	31
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(33)	(31)	(31)
EDEMA, NOS		1 (3%)	
HEMORRHAGE			1 (3%)
RESPIRATORY SYSTEM			
#TRACHEA	(32)	(31)	(25)
INFLAMMATION, ACUTE/CHRONIC		2 (6%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (3%)		
#LUNG	(33)	(31)	(30)
EDEMA, NOS			2 (7%)
HEMORRHAGE			4 (13%)
PNEUMONIA, CHRONIC MURINE		4 (13%)	15 (50%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(32)	(31)	(31)
ATROPHY, NOS		2 (6%)	30 (97%)
#SPLEEN	(33)	(31)	(31)
PERIARTERITIS			1 (3%)
ATROPHY, NOS			2 (6%)
HEMATOPOIESIS	5 (15%)		1 (3%)
#MEDIASTINAL L.NODE	(2)	(9)	(12)
HYPERPLASIA, PLASMA CELL			2 (17%)
#MESENTERIC L. NODE	(2)	(9)	(12)
HEMORRHAGE			1 (8%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE C4 TREATED FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(33)	(31)	(31)
HEMATOMA, NOS			3 (10%)
NECROSIS, NOS			1 (3%)
NECROSIS, FOCAL			1 (3%)
NECROSIS, COAGULATIVE		2 (6%)	2 (6%)
HEMATOPOIESIS	1 (3%)		
#LIVER/CENTRILOBULAR	(33)	(31)	(31)
DEGENERATION, NOS			1 (3%)
NECROSIS, NOS		1 (3%)	3 (10%)
#ILEUM	(33)	(28)	(31)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
INFLAMMATION, CHRONIC			1 (3%)
URINARY SYSTEM			
#KIDNEY/TUBULE	(33)	(31)	(31)
NEPHROPATHY			1 (3%)
ENDOCRINE SYSTEM			
#ADRENAL	(33)	(31)	(31)
ANGIECTASIS	3 (9%)		
#THYROID	(27)	(27)	(10)
INFLAMMATION, CHRONIC		1 (4%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(33)	(28)	(30)
CYST, NOS		1 (4%)	
#UTERUS/ENDOMETRIUM	(33)	(28)	(30)
INFLAMMATION, SUPPURATIVE	1 (3%)		
INFLAMMATION, ACUTE SUPPURATIVE		1 (4%)	
#OVARY	(33)	(22)	(25)
CYST, NOS	2 (6%)		
INFLAMMATION, SUPPURATIVE	1 (3%)		
NERVOUS SYSTEM			
NONE			

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

TABLE C4 TREATED FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(33)	(31)	(31)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
MUSCULOSKELETAL SYSTEM			
*JOINT	(33)	(31)	(31)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
INFLAMMATION, CHRONIC			1 (3%)
BODY CAVITIES			
*PERITONEUM	(33)	(31)	(31)
INFLAMMATION, SUPPURATIVE	1 (3%)	5 (16%)	
INFLAMMATION, CHRONIC	32 (97%)	28 (90%)	7 (23%)
ADHESION, NOS	30 (91%)	8 (26%)	
*MESENTERY	(33)	(31)	(31)
HEMORRHAGE	1 (3%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	
AUTOLYSIS/NO NECROPSY	2	4	4

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF IPD

TABLE D1

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

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	34	35
ANIMALS MISSING			1	
ANIMALS NECROPSIED	14	14	26	21
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	14	26	21
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(14)	(14)	(26)	(22)
INFLAMMATION, INTERSTITIAL	5 (36%)	2 (14%)	2 (8%)	
BRONCHOPNEUMONIA SUPPURATIVE		2 (14%)		1 (5%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(9)	(13)	(21)	(19)
ATROPHY, NOS				2 (11%)
#SPLEEN	(14)	(14)	(24)	(20)
HEMATOPOIESIS			2 (8%)	
#MESENTERIC L. NODE		(2)	(1)	(3)
HYPERPLASIA, LYMPHOID		1 (50%)		
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(14)	(14)	(25)	(20)
NECROSIS, COAGULATIVE			1 (4%)	
HYPERPLASIA, NODULAR	1 (7%)		1 (4%)	
ANGIECTASIS	1 (7%)	1 (7%)		
URINARY SYSTEM				
NONE				

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

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*SEMINAL VESICLE	(14)	(14)	(26)	(21)
HYPERPLASIA, LYMPHOID		1 (7%)		
#TESTIS	(13)	(14)	(22)	(20)
ATROPHY, NOS				1 (5%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(14)	(14)	(26)	(21)
INFLAMMATION, SUPPURATIVE			1 (4%)	
INFLAMMATION, FIBRINOUS				1 (5%)
INFLAMMATION, CHRONIC		1 (7%)	2 (8%)	5 (24%)
METAPLASIA, OSSEOUS		1 (7%)		
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	9	7	13	9

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

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY			1	
NO NECROPSY PERFORMED				1
AUTOLYSIS/NO NECROPSY	1	1	7	13

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 IPD

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	36	35
ANIMALS MISSING	1			
ANIMALS NECROPSIED	13	15	29	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	15	29	26
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(13)	(15)	(29)	(24)
INFLAMMATION, INTERSTITIAL	2 (15%)	6 (40%)	3 (10%)	3 (13%)
BRONCHOPNEUMONIA SUPPURATIVE			1 (3%)	2 (8%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(13)	(15)	(26)	(24)
HEMATOPOIESIS			1 (4%)	
#MESENTERIC L. NODE		(3)		(2)
INFLAMMATION, GRANULOMATOUS		1 (33%)		
#THYMUS	(13)	(15)	(25)	(19)
ATROPHY, NOS				1 (5%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(13)	(15)	(29)	(24)
HYPERPLASIA, NODULAR			2 (7%)	
#STOMACH	(13)	(15)	(29)	(25)
ULCER, NOS				1 (4%)
URINARY SYSTEM				
NONE				

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

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
#THYROID FOLLICLE	(12)	(13)	(23)	(18)
HYPERPLASIA, PAPILLARY		1 (8%)		
REPRODUCTIVE SYSTEM				
#UTERUS/ENDOMETRIUM	(13)	(15)	(29)	(23)
INFLAMMATION, SUPPURATIVE		1 (7%)		
HYPERPLASIA, CYSTIC	9 (69%)	8 (53%)	10 (34%)	
#OVARY	(8)	(14)	(25)	(18)
ATROPHY, NOS				1 (6%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*FEMUR	(13)	(15)	(29)	(27)
OSTEOPOROSIS			1 (3%)	
FIBROUS OSTEODYSTROPHY			1 (3%)	
BODY CAVITIES				
*PERITONEUM	(13)	(15)	(29)	(27)
INFLAMMATION, CHRONIC			2 (7%)	6 (22%)
METAPLASIA, OSSEOUS			1 (3%)	
ALL OTHER SYSTEMS				
ANIMAL MISSING/NO NECROPSY	1			

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

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	3	3	12	8
NECROPSY PERF/NO HISTO PERFORMED				1
NO NECROPSY PERFORMED	1		1	
AUTOLYSIS/NO NECROPSY			6	8

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD

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

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose	High Dose
Peritoneum: Sarcoma, NOS, Fibroma, or Fibrosarcoma[b]	0/20 (0)	2/32 (6)	1/28 (4)	0/30 (0)
P Values (Control, Low, Mid, High Dose)[c,d]	N.S.	N.S.	N.S.	N.S.
P Values (Control, Low, Mid Dose)[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		Infinite	Infinite	
Lower Limit		0.192	0.039	
Upper Limit		Infinite	Infinite	
Weeks to First Observed Tumor	--	44	72	--

[a]Treated groups received doses of 12, 24, or 48 mg/kg three times per week.

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

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

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

Table E2. Time-adjusted Analyses of the Peritoneal Tumors in Male Rats Given Intraperitoneal Injections of IPD[a]

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose	High Dose
Peritoneum: Sarcoma, NOS, Fibroma, or Fibrosarcoma[b]	0/19 (0)	2/14 (14)	1/3 (33)	0/0 (0)
P Values (Control, Low, Mid, High Dose)[c,d]	P = 0.031	N.S.	N.S.	--
P Values (Control, Low, Mid Dose)[c,d]	P = 0.045	N.S.	N.S.	
Relative Risk (Vehicle Control)[f]		Infinite	Infinite	
Lower Limit		0.421	0.345	
Upper Limit		Infinite	Infinite	

[a]Treated groups received doses of 12, 24, or 48 mg/kg, three times per week.

[b]Number of tumor-bearing animals/number of animals examined at site (percent) that survived at least 44 weeks of study.

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

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

Table E3. Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of IPD[a]

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose	High Dose
Peritoneum: Sarcoma, NOS or Fibrosarcoma[b]	0/18 (0)	0/33 (0)	3/31 (10)	0/31 (0)
P Values (Control, Low, Mid, High Dose)[c,d]	N.S.	N.S.	N.S.	N.S.
P Values (Control, Low, Mid Dose)[c,d]	N.S.	N.S.	N.S.	
Relative Risk (Vehicle Control)[f]			Infinite	
Lower Limit			0.367	
Upper Limit			Infinite	
Weeks to First Observed Tumor	--	--	56	--
Mammary Gland: Fibroadenoma[b]	5/18 (28)	4/33 (12)	0/31 (0)	0/31 (0)
P Values (Control, Low, Mid, High Dose)[c,d]	P = 0.001(N)	N.S.	P = 0.004(N)	P = 0.004(N)
P Values (Control, Low, Mid Dose)[c,d]	P = 0.003(N)	N.S.	P = 0.004(N)	
Relative Risk (Vehicle Control)[f]		0.436	0.000	0.000
Lower Limit		0.102	0.000	0.000
Upper Limit		1.800	0.446	0.446
Weeks to First Observed Tumor	79	47	--	--

Table E3. Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of IPD[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose	High Dose
Mammary Gland: Adenoma, NOS[b]	1/18 (6)	2/33 (6)	0/31 (0)	0/31 (0)
P Values (Control, Low, Mid, High Dose)[c,d]	N.S.	N.S.	N.S.	N.S.
P Values (Control, Low, Mid Dose)[c,d]	N.S.	N.S.	N.S.	
Relative Risk (Vehicle Control)[f]		1.091	0.000	0.000
Lower Limit		0.062	0.000	0.000
Upper Limit		62.383	10.726	10.726
Weeks to First Observed Tumor	80	29	--	--
Mammary Gland: Adenoma, NOS or Fibroadenoma[b]	6/18 (33)	6/33 (18)	0/31 (0)	0/31 (0)
P Values (Control, Low, Mid, High Dose)[c,d]	P < 0.001(N)	N.S.	P = 0.001(N)	P = 0.001(N)
P Values (Control, Low, Mid Dose)[c,d]	P = 0.001(N)	N.S.	P = 0.001(N)	
Relative Risk (Vehicle Control)[f]		0.545	0.000	0.000
Lower Limit		0.178	0.000	0.000
Upper Limit		1.774	0.351	0.351
Weeks to First Observed Tumor	79	29	--	--

Table E3. Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of IPD[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	8/18 (44)	2/29 (7)	0/27 (0)	0/22 (0)
P Values (Control, Low, Mid, High Dose)[c,d]	P < 0.001(N)	P = 0.004(N)	P < 0.001(N)	P = 0.001(N)
P Values (Control, Low, Mid Dose)[c,d]	P < 0.001(N)	P = 0.004(N)	P < 0.001(N)	
Departure from Linear Trend (Control, Mid, High Dose)[e]	P = 0.001			
Relative Risk (Vehicle Control)[f]		0.155	0.000	0.000
Lower Limit		0.019	0.000	0.000
Upper Limit		0.675	0.279	0.338
Weeks to First Observed Tumor	80	58	--	--

[a]Treated groups received doses of 12, 24, or 48 mg/kg three times per week.

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

Table E3. Analyses of the Incidence of Primary Tumors in Female Rats Given Intraperitoneal Injections of IPD[a]

(continued)

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

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

Table E4. Time-adjusted Analyses of Peritoneal Tumors in Female Rats Given Intraperitoneal Injections of IPD[a]

Topography: Morphology	Vehicle Control	Low Dose	Mid Dose	High Dose
Peritoneum: Sarcoma NOS or Fibrosarcoma[b]	0/18 (0)	0/16 (0)	3/4 (75)	0/0 (0)
P Values (Control, Low, Mid, High Dose)[c,d]	$P < 0.001$	N.S.	$P = 0.003$	N.S.
Departure from Linear Trend[e]	$P < 0.001$			
P Values (Control, Low, Mid Dose)[c,d]	$P = 0.001$	N.S.	$P = 0.003$	
Departure from Linear Trend[e]	$P < 0.001$			
Relative Risk (Vehicle Control)[f]			Infinite	
Lower Limit			3.119	
Upper Limit			Infinite	

[a]Treated groups received doses of 12, 24, 48 mg/kg three times per week.

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

Table E4. Time-adjusted Analyses of Peritoneal Tumors in Female Rats Given Intraperitoneal Injections of IPD[a]

(continued)

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF IPD

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

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Squamous-cell Carcinoma[b]	0/14 (0)	6/26 (24)	0/21 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.003		
Relative Risk (Vehicle Control)[f]		Infinite	--
Lower Limit		0.931	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	64	--
Hematopoietic System: Malignant Lymphoma[b]	0/14 (0)	0/26 (0)	3/21 (14)
P Values[c,d]	P = 0.045	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		--	Infinite
Lower Limit		--	0.431
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	29

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

(continued)

[a]Treated groups received doses of 20 or 40 mg/kg three times per week.

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

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

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

Table F2. Time-adjusted Analyses of Hematopoietic Tumors in Male Mice Given Intraperitoneal Injections of IPD[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma[b]	0/12 (0)	0/24 (0)	3/20 (15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			Infinite
Lower Limit			0.395
Upper Limit			Infinite

[a]Treated groups received doses of 20 or 40 mg/kg three times per week.

[b]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 29 weeks of study.

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

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

Table F3. Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of IPD[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue:			
Squamous-cell Carcinoma[b]	0/15 (0)	0/29 (0)	1/27 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		--	Infinite
Lower Limit		--	0.031
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	63
Hematopoietic System:			
Malignant Lymphoma[b]	1/15 (7)	2/29 (7)	6/27 (22)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.034	3.333
Lower Limit		0.060	0.473
Upper Limit		58.874	146.288
Weeks to First Observed Tumor	86	65	25

Table F3. Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of IPD[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/15 (7)	2/29 (7)	2/24 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relaitve Risk (Vehicle Control)[f]		1.034	1.250
Lower Limit		0.060	0.073
Upper Limit		58.874	70.551
Weeks to First Observed Tumor	86	84	64
Ovary: Cystadenoma[b]	1/14 (7)	9/25 (36)	0/18 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.002		
Relative Risk (Vehicle Control)[f]		5.040	0.000
Lower Limit		0.838	0.000
Upper Limit		207.589	14.053
Weeks to First Observed Tumor	86	65	--

Table F3. Analyses of the Incidence of Primary Tumors in Female Mice Given Intraperitoneal Injections of IPD[a]

(continued)

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[a]Treated groups received doses of 20 or 40 mg/kg three times per week.

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

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

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

Table F4. Time-adjusted Analyses of Hematopoietic Tumors in Female Mice Given Intraperitoneal Injections of IPD[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma[b]	1/15 (7)	2/29 (7)	6/26 (23)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.034	3.462
Lower Limit		0.060	0.429
Upper Limit		58.874	151.557

[a]Treated groups received doses of 20 or 40 mg/kg three times per week.

[b]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 25 weeks of study.

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

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

APPENDIX G

HISTOLOGIC FEATURES OF TUMORS OBSERVED IN SPRAGUE-DAWLEY RATS GIVEN INTRAPERITONEAL INJECTIONS OF IPD IN BUFFERED SALINE THREE TIMES PER WEEK FOR ONE YEAR

Appendix G

Histologic Features of Tumors Observed in Sprague-Dawley Rats Given Intraperitoneal Injections of IPD in Buffered Saline Three Times per Week for One Year

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Skin and Ear Canal - Three epithelial neoplasms were observed in male rats. Two lesions had diagnoses of keratoacanthomas; one was on the back and the other involved the external ear canal. Another related lesion was a papillomatous squamous-cell carcinoma with extensive keratinization and focal secondary suppurative inflammation. Each tumor occurred in a rat from a different group: the untreated-controls, the vehicle-controls, and the mid-dose group.

Subcutis - Three pelvic mesenchymal tumors occurred in the pelvic tissues of three rats, two females and one male. All were spindle-cell tumors of various degrees of differentiation. One was classified as a well-differentiated fibroma and a second as well-differentiated fibrosarcoma. The latter had an extensive ulcerative epidermitis over the neoplastic tissue. The third lesion was a pleomorphic fibrosarcoma with bizarre multinucleated giant cells.

Mammary Glands - One mid-dose female had a hemangioma composed of large cavernous blood spaces filled with erythrocytes and lined by low cuboidal or squamoid cells. No glandular tissues were involved. The adenomas, adenocarcinomas, and fibroadenomas of the mammary gland were typical of those previously described by Davis et al. (1956) and Thompson et al. (1961).

Liver - A hepatocellular lesion was observed in one mid-dose female rat. This lesion was termed a hepatocellular adenoma and consisted of a focal area of pleomorphic hepatocytes with great variation in their size and shape, as well as macronucleosis. The hepatocytic plates were disrupted and thickened. Marked fatty changes were observed in the hepatocytes of the affected area. Following the classification of Squire and Levitt (1975), the lesion would be classified as a neoplastic nodule.

Large Intestines - Two adenocarcinomas were observed in the gastrointestinal tracts of two mid-dose male rats. One lesion had large glandular spaces lined by columnar, cuboidal, and squamoid epithelial cells and was filled by large amounts of mucin. Glands of this tumor had invaded the muscular layers.

Peritoneum - Six rats, three males and three females, had eight spindle-cell mesenchymal tumors which were classified as one fibroma, three fibrosarcomas, and sarcomas, type unspecified.

Similar or related lesions have been termed also as peritoneal sarcomas (Dunning and Curtis, 1946), malignant fibrous histiocytomas (Pradham et al., 1974) and malignant mesotheliomas (Dunning and Curtis, 1946; and Robbins, 1967). They occurred only in low- and mid-dose rats. These rats had chronic peritonitis with extensive adhesions involving the abdominal viscera. Two rats had active suppurative inflammation. One rat had three peritoneal masses: one mass classified as a fibroma with well-differentiated fibroblasts and collagen, and two masses as fibrosarcomas. In another rat, multiple well-differentiated spindle-cell tumors (fibrosarcomas) were observed. These lesions involved the liver, stomach, intestines, and pancreas with metastasis to the mediastinal lymph nodes.

The four lesions given the diagnosis of sarcoma, NOS were similar in many respects to the above fibrosarcomas. One rat had multiple abdominal masses. One mass consisted of mature spindle-cells which had infiltrated the muscular wall from the serosa and blended into the small muscle, making identification of the neoplastic cells difficult. Much of this tumor resembled a fibrosarcoma, but a leiomyosarcoma could not be ruled out. Another rat had multifocal areas of spindle-cell proliferation involving the spleen, liver, adrenals, kidneys and pancreas. The muscle layers of the stomach wall were bisected by neoplastic tissues with a confusing mixture of neoplastic and nonneoplastic cells. Unusual cells, probably regenerative and reactive leiomyocytes, were seen with fibroblasts and collagen. A pleomorphic spindle-cell sarcoma occurred in two animals. In the former, attached to the serosal of the speen, was a mass composed of spindle-cells and bizarre giant cells. The latter had a poorly differentiated sarcoma, with some areas resembling histiocytes; other areas had stromal spindle-cells. The lesions had a mixed population of small and large cells having various amounts of cytoplasm and round to oval nuclei with small nucleoli and delicate chromatin. A few areas had epithelioid cells. The liver, pancreas, large intestine, uterus, and mesenteric lymph nodes were involved. A vascular embolus of large vacuolated histiocytes was present in the lungs. No invasion of the pulmonary parenchyma was seen.

A lipoma was also seen in the mesentery of one rat. This lesion consisted of normal-appearing, well-differentiated lipocytes.

Endocrine glands - Tumors of the pituitary, adrenals, and thyroid were similar to those previously observed in Sprague-Dawley rats (Davis et al., 1956; Thompson et al., 1961; and Prejean et al., 1973).

Reproductive tract - Multiple interstitial-cell adenomas were seen in the testes of one untreated male. These were typical of those described by Jacobs and Huseby (1967) and by Davey and Moloney (1970). Stromal polyps in the uteri of two rats were similar to those described by Jacobs and Huseby (1967), and Davey and Moloney, 1970.

Brain - Three astrocytomas of the cerebral cortex were observed; one in a vehicle-control female, one in a low-dose male, and one in a high-dose male. Two were classified as astrocytoma, NOS, and one as a gemistocytic astrocytoma. In the latter lesion, a large number of large neuron-like cells were localized around a blood-filled space. The lesion resembled a hyperplastic basal ganglion; however, the number of cells, their size, and orientation around a vascular space supports a diagnosis of gemistocytic astrocytoma. The other lesions were well-differentiated highly cellular neoplasms composed of a homogenous population of small astrocytes having round to oval nuclei with delicate chromatin and small nucleoli. These cells tended to blend into the adjacent neural tissues.

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Review of the Bioassay of 3,3'-Iminobis-1-Propanol Dimethane Sulfonate (Ester) Hydrochloride* [IPD] by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

November 28, 1977

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

The bioassay of IPD was part of a program to study the carcinogenicity of a number of cancer chemotherapeutic drugs. The primary reviewer briefly described the experimental design. As shortcomings, the reviewer said the control groups were too small and the dose levels tested were too high. Many of the treated animals died early in the study due to the severe toxicity of IPD. Pointed out was a number of tumor types seen in the treated animals, including squamous-cell carcinomas, but absent in the controls. The significance of the tumors, however, were confounded by the poor survival of animals and relatively short duration of the study. Despite the problem of determining the significance of the tumors, the reviewer opined that the conclusion in the report that "no clear carcinogenic effects of IPD were demonstrated in either species" was not consistent with the findings. The reviewer suggested that a more appropriate conclusion would include a statement noting the increased tumor incidence in treated animals, experimental shortcomings, and the need to retest IPD to clearly define its carcinogenic potential.

The secondary reviewer said that no conclusion could be drawn from the study. He suggested that the study be repeated at more appropriate dosages. Another Subgroup member proposed that IPD could be effectively tested in the Strain-A pulmonary adenoma induction system, since the drug is an alkylating agent.

A motion was made that, because of the inadequate experimental design and conditions of test, only limited conclusions can be drawn from the bioassay of IPD. However, the high incidence of tumors at the injection site in both species and sexes and the dose-related incidence of lymphomas in teated mice suggest that IPD may be carcinogenic. The motion was seconded and accepted by all present except Mr. Garfinkel, who opposed it based on the small animal groups and high early mortality.

Members present were:

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

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons.

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