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

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FOREWORD: This report presents the results of the bioassay of 1,4-dioxane conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected environmental chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: The bioassay of 1,4-dioxane was conducted by the Illinois Institute of Technology Research Institute (IITRI), Chicago, Illinois, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The NCI project officer was Dr. R. R. Bates[1,2]. The project director was Mr. A. Shefner[3]. Dr. M. E. King[3] was the principal investigator for this study, and Dr. P. Holmes[3] assembled the data. Mr. T. Kruckeberg[3] and Mr. K. Kaltenborn[3] were in charge of animal care.

Pathologic examinations were performed by Dr. A. R. Roesler[3]. Histopathologic examinations were carried out by Dr. D. A.

Willigan[4], who also prepared the interpretive pathology summary included in this report.

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] and Ms. P. L. Yong[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. A. Gray[3], with the assistance of S. Cepa[3] and V. DaPinto[3]. Further analyses were conducted under the direction of Dr. E. Murrill[8]. The results of the analytical work were reviewed by Dr. S. S. Olin[6]. The structural formula for the chemical was provided by NCI.

This report was prepared at Tracor Jitco[6] under the direction of Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[7]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman, Dr. Richard A. Griesemer, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire[9], Dr. Jerrold M. Ward.

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SUMMARY

A bioassay of 1,4-dioxane for possible carcinogenicity was conducted by administering the test chemical in the drinking water to Osborne-Mendel rats and B6C3F1 mice.

Groups of 35 rats and 50 mice of each sex were administered 1,4-dioxane at concentrations of either 0.5% or 1.0% (v/v) in the drinking water. Because of variations in intake of water, the doses of test chemical received by the high-dose groups were not precisely twice those received by the low-dose groups; in the male mice, the high dose was only slightly greater than the low dose. The rats were dosed for 110 weeks and the mice for 90 weeks. Matched controls consisted of 35 untreated rats and 50 untreated mice of each sex. All surviving rats were killed at 110-117 weeks and all surviving mice at 90-93 weeks.

The mean body weights of the rats and mice were not consistently affected by the administration of dioxane. Survival rates of the dosed groups of rats and female mice were lower than those of corresponding control groups, but sufficient numbers of animals were at risk for development of late-appearing tumors.

In rats, the incidence of squamous-cell carcinomas of the nasal turbinates was statistically significant in tests for dose-related trend in females ($P = 0.008$) and for direct comparison of high-dose with matched-control males ($P < 0.001$) and direct comparison of dosed with control females ($P \leq 0.003$) (males: controls 0/33, low-dose 12/33, high-dose 16/34; females: controls 0/34, low-dose 10/35, high-dose 8/35). In the females, but not in the males, the incidence of hepatocellular adenomas was significant ($P \leq 0.001$) in tests for dose-related trend and for direct comparison of both low- and high-dose groups with controls (controls 0/31, low-dose 10/33, high-dose 11/32).

In both male and female mice, the incidence of hepatocellular carcinomas was statistically significant ($P \leq 0.001$), both in tests for dose-related trend and direct comparison of both dosed groups with controls (males: controls 2/49, low-dose 18/50, high-

dose 24/47; females: controls 0/50, low-dose 12/48, high-dose 29/37). The incidences remained significant when hepatocellular adenomas were combined with hepatocellular carcinomas.

It is concluded that under the conditions of this bioassay, 1,4-dioxane induced hepatocellular adenomas in female Osborne-Mendel rats. 1,4-Dioxane was carcinogenic in both sexes of rats, producing squamous-cell carcinomas of the nasal turbinates, and in both sexes of B6C3F1 mice, producing hepatocellular carcinomas.

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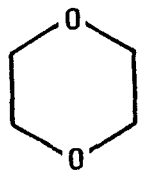
I. INTRODUCTION



1,4-DIOXANE

1,4-Dioxane (CAS 123-91-1; NCI C03689), a dimer of ethylene oxide, hereinafter called dioxane, is used extensively as an industrial solvent for lacquers, varnishes, paints, plastics, dyes, oils, waxes, resins, and cellulose acetate and as an inhibitor in chlorinated solvents (Stecher, 1968; Stanford Research Institute, 1975; Matheson, 1972). In biological and chemical laboratories, dioxane is employed as a solvent for tissue processing, liquid scintillation counting, and photochemical reactions. Nearly 18 million pounds were produced for these uses in 1973 (U. S. International Trade Commission, 1976).

The carcinogenicity of dioxane has been studied extensively. (Argus et al., 1965; Hoch-Ligeti et al., 1970; Argus et al., 1973; Kociba et al., 1974). Dioxane was selected for testing along with a series of chlorinated dibenzo-p-dioxins, some of which are highly toxic contaminants of certain herbicides and pentachlorophenol microbicides.

II. MATERIALS AND METHODS

A. Chemical

The chemical tested was reagent-grade dioxane supplied by J. T. Baker Chemical Co., Phillipsburg, New Jersey. Lots No. 45468 and 43475 were used during the chronic studies and were analyzed to confirm their identity and purity. The analysis of Lot No. 43475 was performed several months after completion of the bioassay. Vapor phase chromatography showed Lot No. 45468 to be at least 99.9% dioxane. Spectra were consistent with the structure of dioxane. Both lots were also analyzed by polarography for the presence of sodium diethyldithiocarbamate, an inhibitor of peroxide formation, stated by the manufacturer to be present at a level of 0.001%. Lot No. 43475 could not be analyzed for the inhibitor because of an interfering substance. In Lot No. 45468, less than 0.0002% sodium diethyldithiocarbamate was detected. The presence of peroxide was measured by titration with titanium tetrachloride or sodium iodide. Lot No. 45468 had very low levels of peroxide, less than 0.001% peroxide, while Lot No. 43475, in contrast, had a level of 0.109% peroxide (calculated as dioxane hydroperoxide). Argus et al. (1973) analyzed their 10% dioxane stock solutions and tap water dilutions used in a dosed water study for peroxides, but could detect none (< 0.0002%).

B. Dosage Preparation

The dioxane solutions for this study were prepared in tap water twice per week and stored in polyethylene containers. These were then used to supply the water bottles for the dosed animals.

C. Animals

Osborne-Mendel rats and B6C3F1 mice of both sexes were used in the chronic studies. All animals were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, under a contract with the Division of Cancer Treatment, NCI. Rats and mice were received at the test laboratory at approximately 4 weeks of age. They were quarantined for 1 week. Animals having no visible signs of disease were then earmarked and assigned to control or dosed groups according to a series of random numbers.

D. Animal Maintenance

Animals were housed in temperature- and humidity-controlled rooms. The temperature was maintained at 22-23°C and the relative humidity at 40-50%. Fluorescent lighting was provided for 12 hours each day. Room air was changed 22 times per hour and exchanged through fiberglass filters.

Rats were housed 4 per cage and mice 10 per cage in suspended

polypropylene cages (Maryland Plastics, Federalsburg, Maryland), covered with a wire mesh screen and a polyester filter. A wood-chip bedding (Absorb-Dri®, Lab Products, Garfield, N. J.) was used in the cages. Dosed water or tap water in glass water bottles with sipper tubes was available to respective groups of animals ad libitum; bottles were refilled twice per week. Animals were fed Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Illinois). Diets were available ad libitum and were supplied once per week.

Cages, cage lids, and water bottles were sanitized at 82°C once per week. Bedding was replaced once per week. Rats and mice were housed in separate rooms. Untreated controls were housed in the same room with the dosed animals. Rats and mice dosed with dioxane were housed in the same room with rats and mice fed dibenzodioxin (CAS 262-12-4), 2,7-dichlorodibenzodioxin (CAS 33857-26-0), and 1,2,3,4,6,7,8,9-octachlorodibenzodioxin (CAS 3268-87-9).

E. Designs of Chronic Studies

In this study, dioxane was administered to rats and mice at concentrations of either 0.5% or 1.0% in drinking water. These concentrations were chosen on the basis of doses administered in previous studies (Argus et al., 1965). During the second year of the study, fluid intake was measured for 1 week out of every

month. This permitted an estimation of the average daily dioxane intake, shown in tables 1 and 2. Decreased fluid consumption was observed in the high-dose male mice, in which the average daily intake of the test chemical was only slightly higher than that of the low-dose group and did not reflect the twofold difference in concentration between the low and high doses.

F. Clinical and Pathologic Examinations

Animals were observed twice daily. Body weights were measured every 2 weeks for the first 12 weeks and every month during the rest of the study. Measurement of food and water consumption was begun during the second year of the study, and was done once per month using 20% of the animals of each group as a representative sample of the population.

Animals that were moribund were killed. All animals were necropsied whether they died or were killed, except for those lost through cannibalization or autolysis. The following tissues were taken at necropsy: mammary gland, trachea, lungs and bronchi, heart, bone marrow, liver, gall bladder (mice) and bile duct, spleen, pancreas, kidney, esophagus, thyroid, adrenal, gonads, brain, stomach, nasal septum, skin, and tissue masses. At 105 weeks from the earliest starting date, a new necropsy protocol was instituted. This affected the male controls and high-dose

Table 1. Design of Chronic Studies of 1,4-Dioxane in Rats

Sex and Test Group	Initial No. of Animals[a]	1,4-Dioxane in Drinking Water (%,v/v)	Average Dose (mg/kg/day)[b]	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male					
Matched-Control[c]	35	0	0	110	0
Low-Dose	35	0.5	240(130-380)	110	0
High-Dose[c]	35	1.0	530(290-780)	110	0
Female					
Matched-Control[d]	35	0	0	110	6-7
Low-Dose	35	0.5	350(200-580)	110	0-1
High-Dose	35	1.0	640(500-940)	110	0-1

[a]All animals were 5 weeks of age when placed on study.

[b]The mean consumption of dioxane solution per week was determined at intervals during the second year of the bioassay. The average doses were calculated with the use of the following formula:

$$\text{mg/kg/day} = \frac{\text{mean ml solution consumed/wk} \times \text{\% dioxane} \times \text{density of dioxane} \times 10}{\text{mean kg body weight} \times 7}$$

[c]These groups were placed on study 1 year after the study began, to replace two original groups of male rats that died during an air-conditioning failure.

[d]Untreated female controls were placed on study 5 weeks later than the dosed groups.

Table 2. Design of Chronic Studies of 1,4-Dioxane in Mice

Sex and Test Group	Initial No. of Animals[a]	1,4-Dioxane in Drinking Water (%,v/v)	Average Dose (mg/kg/day)[b]	Time on Study[c] Dosed (weeks)	Time on Study[c] Observed (weeks)
Male					
Matched-Control	50	0	0	90	2-3
Low-Dose	50	0.5	720(530-990)	90	1-2
High-Dose	50	1.0	830(680-1150)	90	1
Female					
Matched-Control	50	0	0	90	1-2
Low-Dose	50	0.5	380(180-620)	90	1-2
High-Dose	50	1.0	860(450-1560)	90	0-1

[a]Mice were 5 weeks of age when placed on study.

[b]The mean consumption of dioxane solution per week was determined at intervals during the second year of the bioassay. The average doses were calculated with the use of the following formula:

$$\text{mg/kg/day} = \frac{\text{mean ml solution consumed/wk} \times \text{\% dioxane} \times \text{density of dioxane} \times 10}{\text{mean kg body weight} \times 7}$$

[c]Groups were placed on study not more than 7 weeks apart.

groups of rats which were started a year later than the original groups of rats and mice. The tissues taken after that time included skin, mandibular lymph node, salivary gland, mammary gland, bone marrow, thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, colon, mesenteric lymph node, liver, pancreas, spleen, kidney, urinary bladder, adrenal, gonads, nasal cavity, brain, pituitary, spinal cord, skeletal muscle, sciatic nerve, and tissue masses. Tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All tissues were examined microscopically by the pathologist.

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.

G. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data

System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

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

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the

narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

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

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

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

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

of unity represent the condition of a larger proportion in the dosed group than in the control.

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

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of the low-dose males were higher than those of the matched controls, particularly during the second year of the bioassay, while those of the low-dose females were comparable throughout the test period (figure 1). The weights of the high-dose animals of both sexes were lower than those of the controls, particularly during the second year of the bioassay. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No clinical signs other than those of altered body weights were reported.

B. Survival (Rats)

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

In each sex, the Tarone test result for positive dose-related trend in mortality is significant ($P < 0.001$). Departures from linear trend are observed ($P = 0.010$ in males, $P = 0.030$ in females), due to the relatively steep decrease in survival

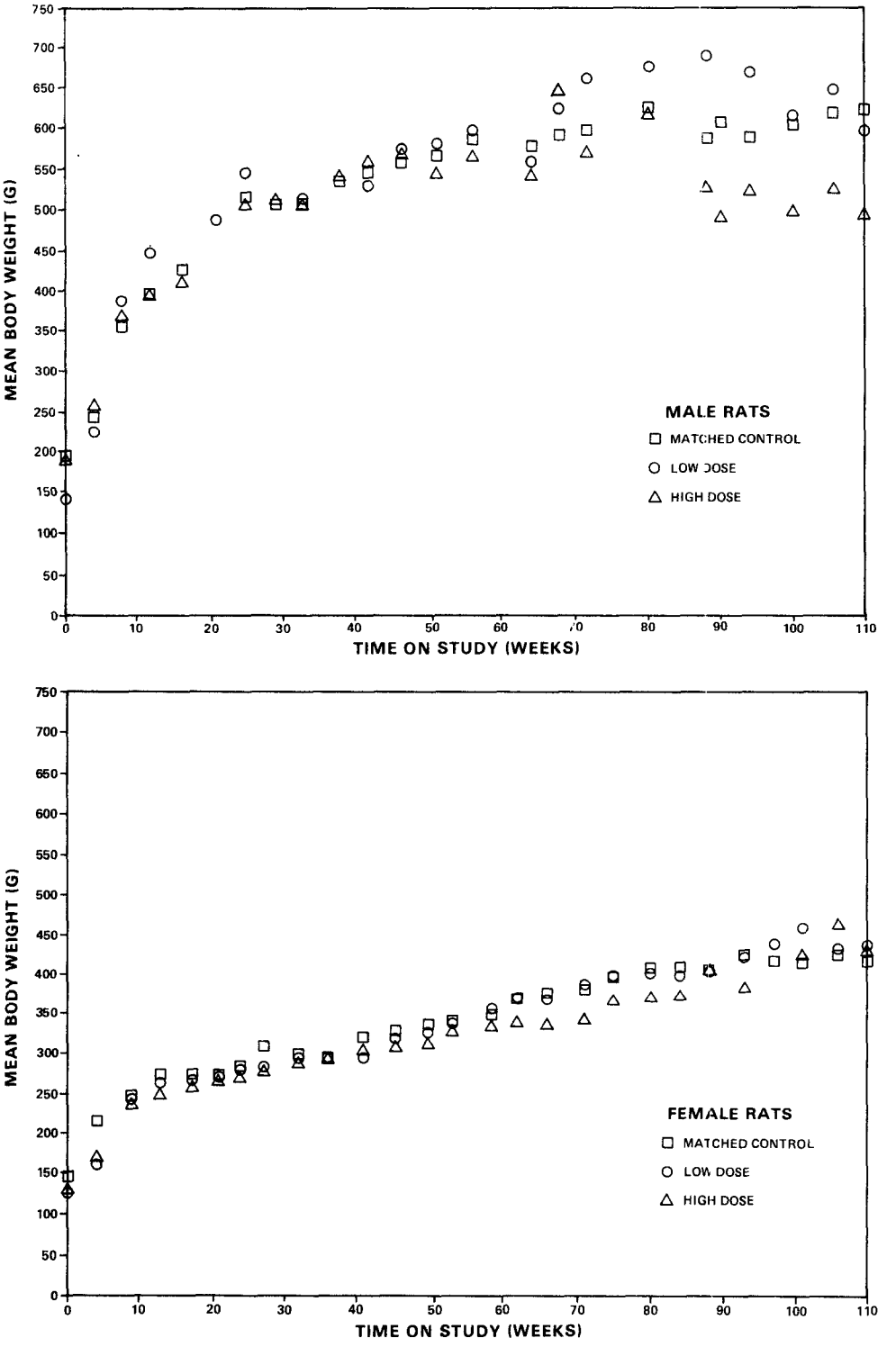


Figure 1. Growth Curves For Rats Administered 1,4-Dioxane in the Drinking Water

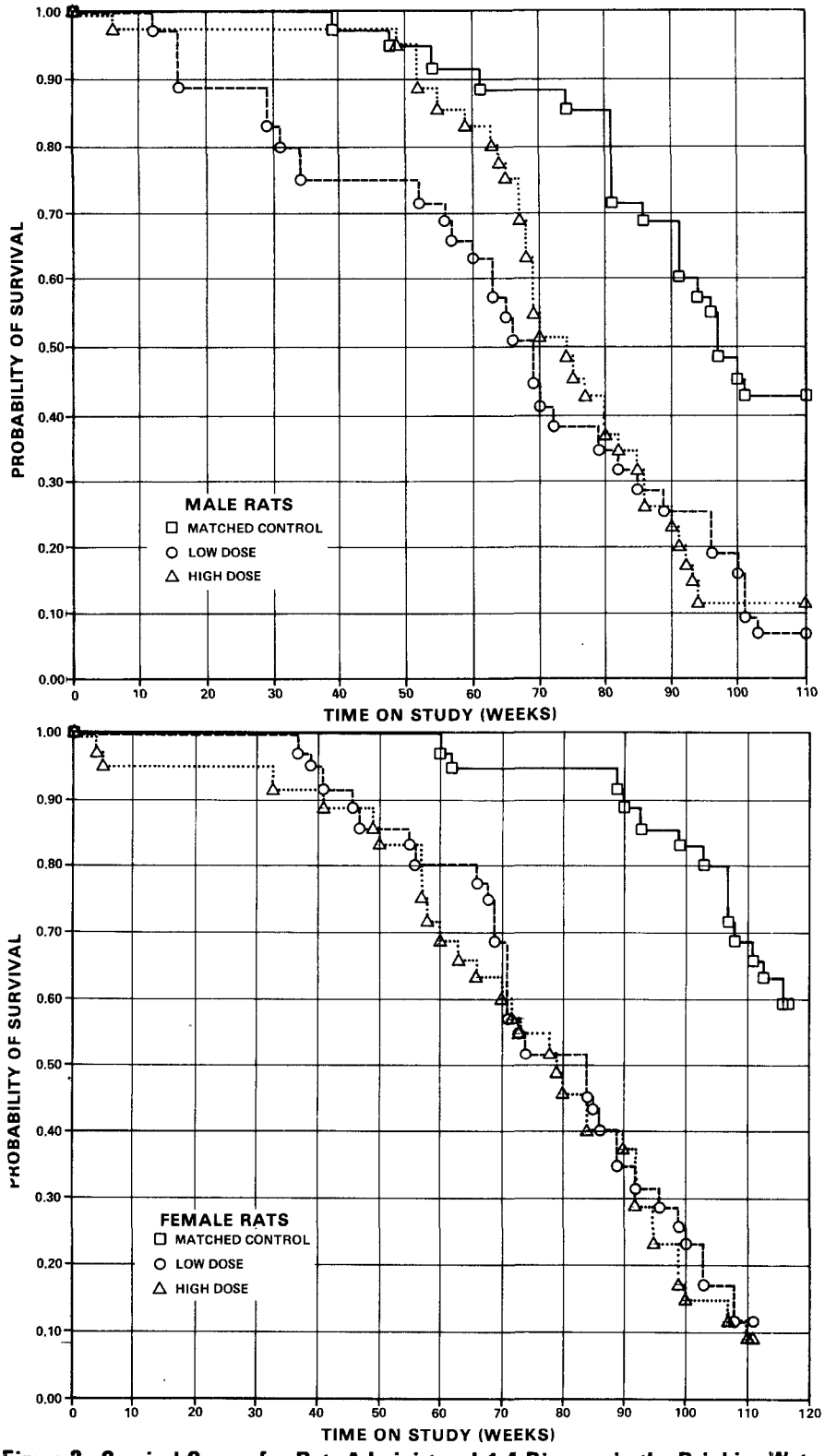


Figure 2. Survival Curves for Rats Administered 1,4-Dioxane in the Drinking Water

observed in the dosed groups. In male rats, 33/35 (94%) of the high-dose group, 26/35 (74%) of the low-dose group, and 33/35 (94%) of the matched controls lived at least as long as 52 weeks on study. In female rats, 29/35 (83%) of the high-dose group, 30/35 (86%) of the low-dose group, and all 35 of the matched controls lived beyond week 52. Sufficient numbers of rats of each sex were at risk for development of tumors appearing within this period.

C. Pathology (Rats)

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

Neoplasms associated with administration of dioxane occurred in the nasal cavity (squamous-cell carcinomas, adenocarcinomas, and rhabdomyomas) in each sex, liver (hepatocellular adenomas) in females, and testis/epididymis (mesotheliomas) in males.

The incidence of tumors of the nasal cavity was related to the dioxane to which the rats were exposed. Squamous-cell carcinomas occurred in 12/33 (36%) low-dose males, 16/34 (47%) high-dose males, 10/35 (29%) low-dose females, and 8/35 (23%) high-dose females. The first tumors were observed at week 52 in males and

at week 66 in females. None were found in the 33 male controls and 34 female controls.

Nasal squamous-cell carcinomas varied morphologically from minimal foci of locally invasive squamous-cell proliferation to advanced growth consisting of extensive columns of epithelial cells projecting either into free spaces of the nasal cavity and/or infiltrating the submucosa. Although reasonably well differentiated (formation of cell nests and cornification), local invasiveness was common and extended to the retrobulbar tissues of the eye in 1/16 high-dose males, and to the brain in 1/12 low-dose males. Distant metastasis to the lung occurred in 1/8 high-dose females. Adenocarcinomas (nonkeratinizing) arose from nasal mucosal epithelium in 3/34 (9%) high-dose males, 1/35 (3%) low-dose, and 1/35 (3%) high-dose females. They extended primarily into the free space of the nasal cavity. The neoplasms were reasonably well differentiated, with varying infiltrations into the submucosal tissue. Metastasis to the lung occurred in 1/3 high-dose males having these tumors. The single instance of a benign skeletal muscle tumor (rhabdomyoma) was observed in 1/33 (3%) low-dose males.

Although hepatocellular hyperplasia (cytomegaly) occurred in both dosed and control groups, hepatocellular adenomas were primarily seen in livers of female rats (0/31 [0%] controls, 10/33 [30%]

low-dose, 11/32 [34%] high-dose). These neoplastic foci consisted of proliferating hepatic cells oriented as concentric cords. The foci were sharply delineated from immediate normal parenchyma which yielded to compression. Hepatic cell size was variable; mitoses and necrosis were rare.

Mesotheliomas involving the vaginal tunics of the testis/epididymis were apparent in dosed animals more frequently than in the control group (2/33 [6%] high-dose controls, 4/33 [12%] low-dose, and 5/34 [15%] high-dose). Microscopically, these growths were characterized as rounded and papillary projections of mesothelial cells, each supported by a core of fibrous tissue.

Although other benign and malignant neoplasms occurred in various tissues, each type has been encountered previously as a spontaneous lesion in the rat. Moreover, the incidences of neoplasms are not related to administration of the test chemical by type, site, test group, or sex.

Nonneoplastic responses associated with exposure to dioxane were observed in the kidney (tubular degeneration), liver (cytomegaly), and stomach (ulceration). Renal changes were characterized within the proximal cortical tubular epithelium by marked vacuolar degeneration and/or focal tubular epithelial regeneration. Hyaline casts were seen on occasion. Gastric

ulceration of the stomach was observed in 5/28 (18%) low-dose, 5/30 (17%) high-dose, and no control males. Females were affected negligibly.

Dosed rats had higher incidences of pneumonia than the controls (8/30 [27%] controls, 15/31 [48%] low-dose, and 14/33 [42%] high-dose males; 6/30 [20%] control, 5/34 [15%] low-dose, and 25/32 [78%] high-dose females), and the development of nasal carcinomas may have been a contributing factor.

A variety of other nonneoplastic lesions were represented among both control and dosed animals. Such lesions have been encountered previously and are considered spontaneous events not unlike those commonly observed in aging rats.

Based on the histopathologic examination, dioxane was carcinogenic, producing squamous-cell carcinomas of the nasal cavity in male and female Osborne-Mendel rats exposed to the chemical in drinking water.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group. The statistical analyses in

the male rats consist only of Fisher exact tests, comparing incidences in the high-dose with those in the control groups. These groups were tested concurrently; the low-dose group, however, was started a year earlier without appropriate controls. Although the incidences of tumors in the low-dose group of male rats were not used for statistical analysis, they are shown in table E1.

Squamous-cell carcinomas of the nasal turbinate occurred in a significantly ($P < 0.001$) higher proportion in the high-dose group of male rats than in the control group. While no tests were made using the proportion of 12/33 (36%) seen in the low-dose group, this proportion approaches the 16/34 (47%) seen in the high-dose group. In females, the Cochran-Armitage test is significant ($P = 0.008$). An indicated departure from linear trend is observed ($P = 0.039$), because the proportion in the low-dose group is slightly greater than that in the high-dose group. The Fisher exact test shows that the incidences in both the dosed groups are significantly higher ($P \leq 0.003$) than that in the matched controls. The statistical conclusion is that this tumor in both sexes of rats is associated with the administration of the test chemical.

In female rats, the Cochran-Armitage test result for the incidence of hepatocellular adenomas is significant ($P = 0.001$),

and the Fisher exact test shows that the incidences in both the low- and high-dose groups are significantly higher ($P \leq 0.001$) than that in the matched controls. The statistical conclusion is that the incidence of this tumor in the female rats is associated with administration of the test chemical. The statistical test results on the incidences of this tumor in male rats are not significant.

Significant results in the negative direction are observed in the incidence of C-cell adenomas in female rats.

The statistical conclusion is that the incidence of squamous-cell carcinomas of the nasal turbinate in both sexes of rats and the incidence of hepatocellular adenomas in female rats are associated with the administration of dioxane.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of male mice at the low-dose were comparable to those of the matched controls, while at the high-dose, the mean body weights were slightly elevated (figure 3). Mean body weights of low-dose female mice were higher than those of the controls, and body weights of the high-dose animals were lower. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No clinical signs other than those of altered body weights were reported.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered dioxane in the drinking water at the doses of this bioassay, together with those of the matched controls, are shown in figure 4.

In male mice, the Tarone test result for positive dose-related trend in mortality is not significant, with at least 90% of the animals in each group (45/50 [90%] in the high-dose group, 46/50 [92%] in the low-dose group, and 48/50 [96%] in the control group) still alive at week 91. In females, the Tarone test

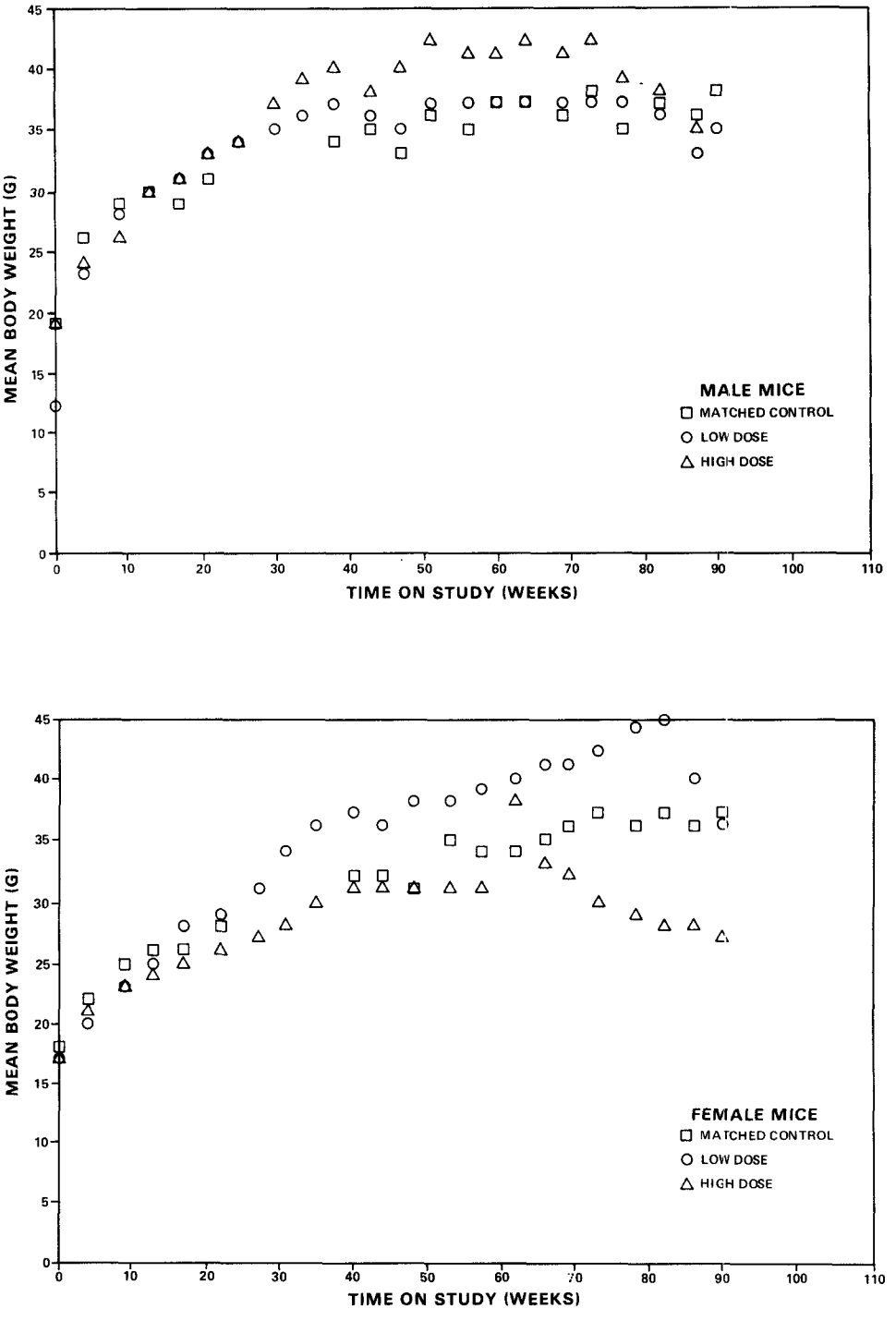


Figure 3. Growth Curves For Mice Administered 1,4-Dioxane in the Drinking Water

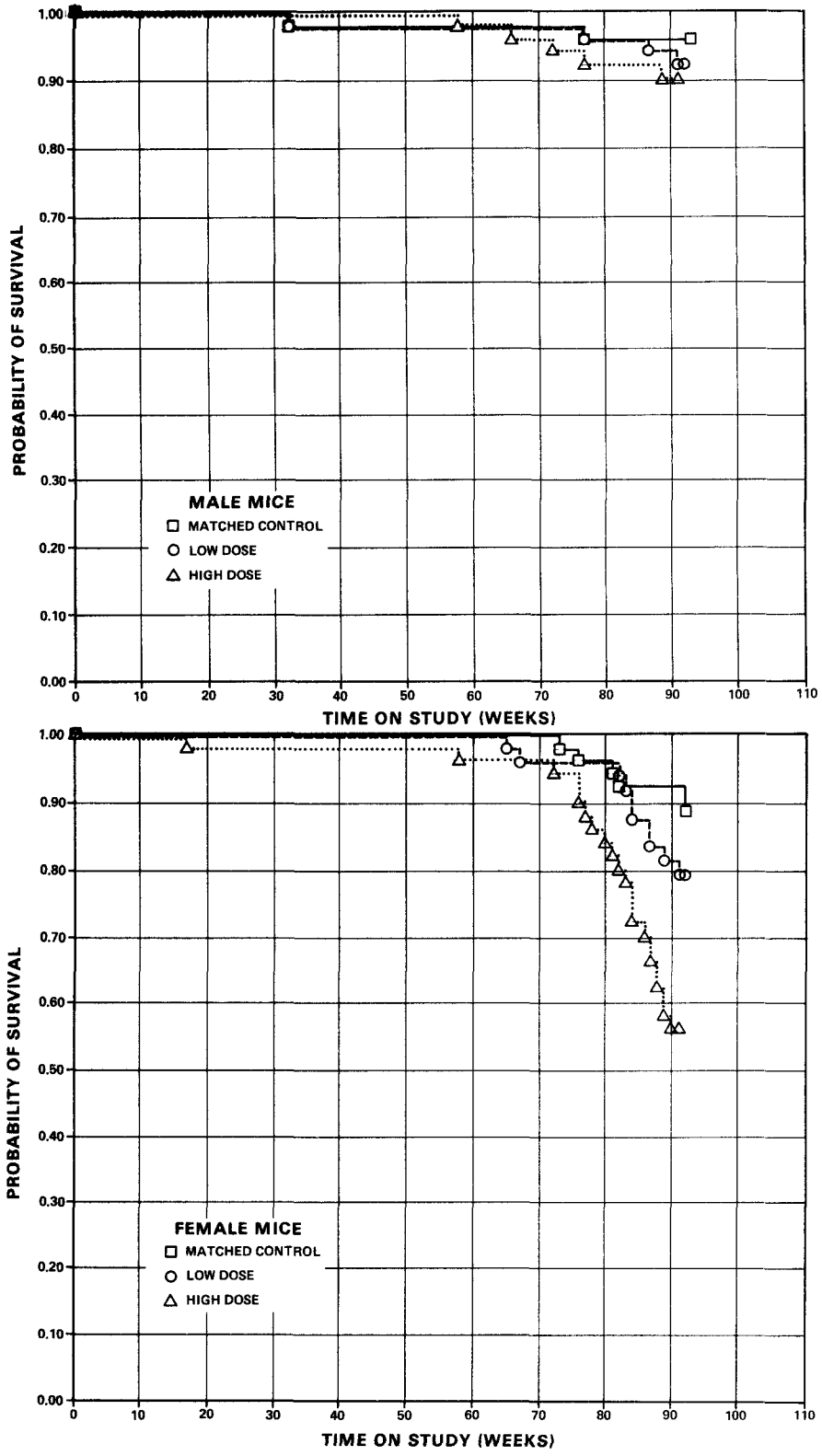


Figure 4. Survival Curves for Mice Administered 1,4-Dioxane in the Drinking Water

result is significant ($P < 0.001$), with 28/50 (56%) of the high-dose group, 39/50 (78%) of the low-dose group, and 45/50 (90%) of the matched controls still alive at week 91. Sufficient numbers of mice of each sex were at risk for development of late-appearing tumors.

C. Pathology (Mice)

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

The incidences of neoplasms observed in the liver are tabulated below:

	MICE					
	Male			Female		
	Untreated Control	Low Dose	High Dose	Untreated Control	Low Dose	High Dose
No. of tissues examined microscopically	(49)	(50)	(47)	(50)	(48)	(37)
Liver						
Hepatocellular carcinoma	2(4%)	18(36%)	24(51%)	0	12(25%)	29(78%)
Hepatocellular adenoma or carcinoma	8(16%)	19(38%)	28(60%)	0	21(44%)	35(95%)

The neoplastic hepatic parenchymal cells were irregular in size and arrangement. Cells were often hypertrophic with hyper-

chromatic nuclei. Despite extensive proliferation, the interlacing cords of hepatic cells seldom revealed mitoses. Although locally invasive within the liver, metastasis to the lung was rarely observed (1/50 [2%] low-dose males).

The few nasal adenocarcinomas (1/48 [2%] low-dose females and 1/49 [2%] high-dose males) that were observed arose from proliferating respiratory epithelium lining the nasal turbinates. The neoplasms extended into the nasal cavity, and local tissue infiltration was not extensive. Nasal mucosal polyps were rare (1/48 [2%] low-dose females and 1/49 [2%] high-dose males). The polyps were derived from mucus-secreting epithelium and were not otherwise remarkable.

A variety of other benign and malignant neoplasms occurred; however, each type has been encountered previously as a spontaneous lesion in the B6C3F1 mouse. It is apparent that the incidences of these neoplasms are unrelated by type, site, group, or sex of animal, and hence, are unattributable to exposure to the chemical.

Of the nonneoplastic lesions represented among both control and dosed animals, the increased incidence of pneumonia (inflammation) and rhinitis (acute inflammation, acute suppurative inflammation) was significant. Pneumonia occurred in

1/49 (2%) control, 9/50 (18%) low-dose, and 17/47 (36%) high-dose males; 2/50 (4%) control, 33/47 (70%) low-dose, and 32/36 (89%) high-dose females. Rhinitis was observed in 1/50 (2%) low-dose, 1/49 (2%) high-dose males; and in 7/48 (14%) low-dose and 8/39 (21%) high-dose females. Hepatic cytomegaly was commonly observed in dosed mice. A variety of other nonneoplastic lesions were observed; such lesions have been encountered previously, however, and are considered to be similar to those commonly observed in aging mice.

Based on the histopathologic examination, dioxane was carcinogenic, producing hepatocellular neoplasms in male and female B6C3F1 mice exposed to the chemical in drinking water.

D. Statistical Analyses of Results (Mice)

Tables F3 and F4 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In each sex, the result of the Cochran-Armitage test for positive dose-related trend in proportions for the incidence of the animals with either hepatocellular adenomas or carcinomas is significant ($P < 0.001$) and the Fisher exact test shows that the incidences in any of the dosed groups are significantly higher

($P \leq 0.014$) than those in the matched controls. The statistical conclusion is that the incidence of this tumor in male and female mice is associated with administration of the test chemical.

In male mice, the result of the Cochran-Armitage test on the incidence of lymphomas is not significant, and the Fisher exact test comparing the incidence in the low-dose group with that in the matched controls indicates a probability level of 0.030, which is above the 0.025 level required by the Bonferroni inequality criterion when a multiple comparison is considered. In females, the statistical test results have probability levels greater than 0.05.

The result of the Cochran-Armitage test on the combined incidences of hemangiomas and hemangiosarcomas in male mice is significant ($P = 0.047$). The Fisher exact test shows that the incidence in the low-dose group is significantly higher ($P = 0.014$) than that in the matched controls. Neither the Fisher exact test results using the high-dose males nor the results using the female groups are significant.

A significant trend in the negative direction is observed in the incidence of animals with alveolar/bronchiolar adenomas or carcinomas of the lung in male mice, where the incidence in the matched controls exceeds the incidences in the dosed groups. The

probable reason for this negative trend is that the dosed animals did not live as long as the control animals, thus suppressing the possibility of the development of tumors in the dosed groups.

The statistical conclusion is that the incidence of hepatocellular carcinomas in both sexes of mice is associated with the administration of dioxane.

V. DISCUSSION

In this bioassay, the total doses received by the "low-" and "high-dose" groups in both rats and mice do not reflect the twofold difference in concentration of chemical in the drinking water, because of variations in the intake of the dosed water — presumably due in part to decreased palatability. In addition, there were wide fluctuations in intake at different time periods within the groups. The mean body weights of the rats and mice were not consistently affected by the administration of dioxane. Rates of survival of the dosed groups of male and female rats were lower than those of the corresponding controls, but sufficient numbers of rats were at risk beyond week 52 on study for development of tumors appearing within this period. There was a positive dose-related trend in mortality in the female but not in the male mice. Although only 56% of the high-dose female mice survived until the end of the bioassay, sufficient numbers of both male and female mice were at risk for development of late-appearing tumors.

In rats, the incidence of squamous-cell carcinomas of the nasal turbinates was statistically significant in tests both for dose-related trend in females ($P = 0.008$) and for direct comparison of high-dose with control males ($P < 0.001$) and direct comparison of dosed with control females ($P \leq 0.003$) (males: controls 0/33,

low-dose 12/33, high-dose 16/34; females: controls 0/34, low-dose 10/35, high-dose 8/35). These carcinomas commonly invaded local tissues and extended to the retrobulbar tissues of the eye in one male and to the brain in another male. In addition, adenocarcinomas (nonkeratinizing) arose from the nasal mucosal epithelium in three high-dose males and in one low-dose and one high-dose female. In the female, but not in the male rats, the incidence of hepatocellular adenomas also was significant ($P \leq 0.001$) in tests for dose-related trend and for direct comparison of both low- and high-dose groups with controls (controls 0/31, low-dose 10/33, high-dose 11/32).

In both male and female mice, the incidence of hepatocellular carcinomas was statistically significant ($P \leq 0.001$) in tests for both dose-related trend and direct comparison of both low- and high-dose groups with controls (males: controls 2/49, low-dose 18/50, high-dose 24/47; female: controls 0/50, low-dose 12/48, high-dose 29/37). The incidences remained significant when hepatocellular adenomas were combined with hepatocellular carcinomas. Hemangiomas or hemangiosarcomas occurred in six low-dose and three high-dose male mice but in no controls. The incidence in the low-dose group was significantly higher than in controls. Since neither the dose-related trend nor the incidence

in the high-dose group is significant, the tumors are not considered to be related to administration of the chemical.

Several investigators have reported induction of carcinomas in animals by dioxane. Argus et al. (1965) reported that dioxane given to male Wistar rats in drinking water at a concentration of 1% was a hepatocarcinogen; 7/26 rats developed liver tumors at days 448-455. Hoch-Ligeti et al. (1970) and Argus et al. (1973) reported that administration of the compound to 120 male rats (Charles River random bred, Sprague-Dawley descendant, 1950) at concentrations of 0.75% to 1.8% in the drinking water for 13 months led to the development of both hepatocellular carcinomas and carcinomas of the nasal cavity. Kociba et al. (1974) maintained Sherman strain male and female rats on drinking water containing 0, 1.0, 0.1, or 0.01% dioxane for up to 716 days; hepatocellular carcinomas developed in 10/66 rats at the 1% level, 1/100 rats at the 0.1% level, 0/110 rats at the 0.01% level, and 1/106 control rats. Nasal carcinomas occurred in 3/66 rats at the 1% level and in none at any other level. The high dose used in the present bioassay would be comparable to the 1% level used in Kociba's experiment, and nasal carcinomas and hepatocellular carcinomas were found in both tests. A relatively high concentration of peroxide (0.109%) was found several months after completion of the bioassay in one of the lots of dioxane

used for the present study. It is not known whether peroxide was present in the dioxane during the study. However, dioxane containing no detectable peroxide has produced similar lesions to those seen in this study in rats (Argus et al., 1973), so it is unlikely that the lesions in the current study were due to peroxide. Torkelson et al. (1974) conducted a 2-year inhalation study in rats with dioxane, using 111 ppm 5 days per week for 7 hours per day. Under these conditions, no lesions related to administration of the dioxane were observed. Thus, carcinomas of the nasal cavity of rats were observed in both the present study and in previously reported studies. The hepatocellular carcinomas previously reported in rats were not found in the present study in rats, but they did occur in both sexes of mice, and hepatocellular adenomas were found in the female rats.

It is concluded that under the conditions of this bioassay, 1,4-dioxane induced hepatocellular adenomas in female Osborne-Mendel rats. 1,4-Dioxane was carcinogenic in both sexes of rats, producing squamous-cell carcinomas of the nasal turbinates, and to both sexes of B6C3F1 mice, producing hepatocellular carcinomas.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	33	33	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	33	32	33
INTEGUMENTARY SYSTEM			
*SKIN	(33)	(33)	(34)
SQUAMOUS CELL CARCINOMA, INVASIV			1 (3%)
SQUAMOUS CELL CARCINOMA, METASTA		2 (6%)	
FIBROMA		1 (3%)	
*SUBCUT TISSUE	(33)	(33)	(34)
FIBROMA	3 (9%)	1 (3%)	1 (3%)
FIBROSARCOMA			1 (3%)
LIPOMA	1 (3%)		
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(33)	(33)	(34)
SQUAMOUS CELL CARCINOMA		12 (36%)	16 (47%)
ADENOCARCINOMA, NOS			3 (9%)
RHABDOMYOMA		1 (3%)	
#LUNG	(30)	(31)	(33)
SQUAMOUS CELL CARCINOMA, METASTA			1 (3%)
TRANSITIONAL-CELL CARCINOMA, MET		1 (3%)	
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (3%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (3%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(33)	(33)	(34)
MALIGNANT LYMPHOMA, NOS			1 (3%)
#SPLEEN	(31)	(32)	(30)
SARCOMA, NOS	1 (3%)		
HEMANGIOMA			1 (3%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MANDIBULAR L. NODE	(22)		(15)
SQUAMOUS CELL CARCINOMA, METASTA			1 (7%)
ADENOCARCINOMA, NOS, METASTATIC			1 (7%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(31)	(32)	(33)
HEPATOCELLULAR ADENOMA	2 (6%)	1 (3%)	1 (3%)
HEPATOCELLULAR CARCINOMA		1 (3%)	
HEMANGIOSARCOMA	1 (3%)		
URINARY SYSTEM			
#KIDNEY	(31)	(31)	(33)
LIPOSARCOMA	1 (3%)	1 (3%)	1 (3%)
HAMARTOMA	1 (3%)		
#KIDNEY/CORTEX	(31)	(31)	(33)
ADENOMA, NOS		1 (3%)	
#URINARY BLADDER	(28)	(2)	(27)
TRANSITIONAL-CELL CARCINOMA		1 (50%)	
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(1)	(15)
ADENOMA, NOS	2 (13%)		1 (7%)
CHROMOPHOBE ADENOMA	1 (6%)		
#ADRENAL	(30)	(24)	(33)
CORTICAL ADENOMA			1 (3%)
PHEOCHROMOCYTOMA	6 (20%)		2 (6%)
#ADRENAL CORTEX	(30)	(24)	(33)
ADENOCARCINOMA, NOS		1 (4%)	
#THYROID	(29)	(17)	(31)
FOLLICULAR-CELL ADENOMA	2 (7%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FOLLICULAR-CELL CARCINOMA	1 (3%)		
C-CELL ADENOMA	3 (10%)	1 (6%)	
CYSTADENOMA, NOS		1 (6%)	1 (3%)
#THYROID FOLLICLE	(29)	(17)	(31)
CYSTADENOMA, NOS		1 (6%)	
#PARATHYROID	(25)	(4)	(24)
ADENOMA, NOS	2 (8%)		
#PANCREATIC ISLETS	(24)	(12)	(24)
ISLET-CELL ADENOMA	1 (4%)		1 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(33)	(33)	(34)
ADENOCARCINOMA, NOS		1 (3%)	
FIBROADENOMA		2 (6%)	
#PROSTATE	(29)	(2)	(31)
ADENOCARCINOMA, NOS			1 (3%)
#TESTIS	(32)	(23)	(31)
INTERSTITIAL-CELL TUMOR		1 (4%)	
#TUNICA ALBUGINEA	(32)	(23)	(31)
MESOTHELIOMA, NOS		3 (13%)	2 (6%)
NERVOUS SYSTEM			
#BRAIN	(31)	(29)	(32)
SQUAMOUS CELL CARCINOMA, METASTA		1 (3%)	
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)
GLIOMA, NOS			2 (6%)
SPECIAL SENSE ORGANS			
*EYE	(33)	(33)	(34)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*TUNICA VAGINALIS	(33)	(33)	(34)
MESOTHELIOMA, NOS	2 (6%)	4 (12%)	5 (15%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
LIPOMA	1	1	1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	35	35	35
NATURAL DEATH@	20	31	26
MORIBUND SACRIFICE			5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		2	
TERMINAL SACRIFICE	15	2	4
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	18	27
TOTAL PRIMARY TUMORS	32	36	43
TOTAL ANIMALS WITH BENIGN TUMORS	17	8	7
TOTAL BENIGN TUMORS	25	12	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	15	23
TOTAL MALIGNANT TUMORS	5	17	25
TOTAL ANIMALS WITH SECONDARY TUMORS#		3	5
TOTAL SECONDARY TUMORS		4	7
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2	4	5
TOTAL UNCERTAIN TUMORS	2	7	7
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	34	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	31	34	32
INTEGUMENTARY SYSTEM			
*SKIN	(34)	(35)	(35)
FIBROMA		1 (3%)	1 (3%)
*SUBCUT TISSUE	(34)	(35)	(35)
FIBROMA	1 (3%)	2 (6%)	2 (6%)
FIBROSARCOMA	1 (3%)		
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(34)	(35)	(35)
SQUAMOUS CELL CARCINOMA		10 (29%)	8 (23%)
ADENOCARCINOMA, NOS		1 (3%)	1 (3%)
#LUNG	(30)	(34)	(32)
SQUAMOUS CELL CARCINOMA, METASTA			1 (3%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(30)	(34)	(32)
HEMANGIOMA		2 (6%)	
#MESENTERIC L. NODE	(25)	(5)	(5)
MALIGNANT LYMPHOMA, NOS		1 (20%)	
CIRCULATORY SYSTEM			
*MESENTERIC ARTERY	(34)	(35)	(35)
HEMANGIOMA		1 (3%)	
DIGESTIVE SYSTEM			
#LIVER	(31)	(33)	(32)
ADENOCARCINOMA, NOS			1 (3%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR ADENOMA		10 (30%)	11 (34%)
HEMANGIOSARCOMA			1 (3%)
*BILE DUCT	(34)	(35)	(35)
BILE DUCT ADENOMA			1 (3%)
URINARY SYSTEM			
#KIDNEY	(31)	(34)	(32)
FIBROSARCOMA, METASTATIC	1 (3%)		
FIBROADENOMA			1 (3%)
HAMARTOMA		1 (3%)	1 (3%)
#KIDNEY/CORTEX	(31)	(34)	(32)
ADENOMA, NOS			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(3)	(2)
ADENOMA, NOS		1 (33%)	
CHROMOPHOBE ADENOMA	4 (22%)		
#ADRENAL	(30)	(32)	(29)
CORTICAL ADENOMA	1 (3%)	1 (3%)	
PHEOCHROMOCYTOMA			1 (3%)
#THYROID	(28)	(20)	(18)
C-CELL ADENOMA	4 (14%)		
CYSTADENOMA, NOS			1 (6%)
#THYROID FOLLICLE	(28)	(20)	(18)
CYSTADENOMA, NOS	2 (7%)	1 (5%)	
#PANCREATIC ISLETS	(29)	(15)	(16)
ISLET-CELL ADENOMA	1 (3%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(34)	(35)	(35)
ADENOMA, NOS	3 (9%)	3 (9%)	1 (3%)
ADENOCARCINOMA, NOS	1 (3%)		
CYSTADENOMA, NOS		1 (3%)	
FIBROMA	1 (3%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROADENOMA	13 (38%)	16 (46%)	10 (29%)
#UTERUS	(30)	(34)	(28)
ADENOCARCINOMA, NOS, INVASIVE	1 (3%)		
PAPILLARY CYSTADENOMA, NOS	1 (3%)		
PAPILLARY CYSTADENOCARCINOMA,NOS			1 (4%)
FIBROMA		1 (3%)	
#OVARY	(26)	(23)	(22)
CYSTADENOMA, NOS			1 (5%)
THECOMA		1 (4%)	
HEMANGIOMA			2 (9%)
NERVOUS SYSTEM			
#FRONTAL LOBE	(31)	(31)	(28)
ADENOCARCINOMA, NOS, METASTATIC			1 (4%)
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(34)	(35)	(35)
ADENOCARCINOMA, NOS, INVASIVE	1 (3%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL WALL	(34)	(35)	(35)
FIBROSARCOMA	1 (3%)		
ALL OTHER SYSTEMS			
SITE UNKNOWN			
SQUAMOUS CELL CARCINOMA			1

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	35	35	35
NATURAL DEATH@	14	29	31
MORIBUND SACRIFICE		2	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	21	4	3
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	22	28	21
TOTAL PRIMARY TUMORS	34	54	47
TOTAL ANIMALS WITH BENIGN TUMORS	20	22	18
TOTAL BENIGN TUMORS	31	42	34
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	12	12
TOTAL MALIGNANT TUMORS	3	12	13
TOTAL ANIMALS WITH SECONDARY TUMORS#	3		2
TOTAL SECONDARY TUMORS	3		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(49)
PAPILLOMA, NOS		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
*SUBCUT TISSUE	(49)	(50)	(49)
SEBACEOUS ADENOMA	1 (2%)		
FIBROSARCOMA		4 (8%)	
LEIOMYOSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(49)	(50)	(49)
ADENOCARCINOMA, NOS			1 (2%)
#LUNG	(49)	(50)	(47)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	8 (16%)	3 (6%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		2 (4%)	1 (2%)
#SPLEEN	(48)	(49)	(43)
HEMANGIOMA		2 (4%)	2 (5%)
HEMANGIOSARCOMA		2 (4%)	
HEMANGIOSARCOMA, METASTATIC			1 (2%)
MALIGNANT LYMPHOMA, NOS		3 (6%)	1 (2%)
MAST-CELL SARCOMA, METASTATIC		1 (2%)	
#PANCREATIC L.NODE	(1)	(2)	(1)
HEMANGIOSARCOMA, METASTATIC		1 (50%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(49)	(50)	(47)
MAST-CELL SARCOMA, METASTATIC		1 (2%)	
#STOMACH	(49)	(49)	(47)
MAST-CELL SARCOMA		1 (2%)	
#KIDNEY	(49)	(50)	(48)
MAST-CELL SARCOMA, METASTATIC		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(47)
HEPATOCELLULAR ADENOMA	6 (12%)	1 (2%)	4 (9%)
HEPATOCELLULAR CARCINOMA	2 (4%)	18 (36%)	24 (51%)
*BILE DUCT	(49)	(50)	(49)
BILE DUCT CARCINOMA	1 (2%)		
#PANCREAS	(42)	(38)	(31)
HEMANGIOMA		2 (5%)	
#STOMACH	(49)	(49)	(47)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
SQUAMOUS CELL CARCINOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(39)	(38)	(38)
PAPILLARY CYSTADENOMA, NOS	1 (3%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(49)	(50)	(49)
SEBACEOUS ADENOMA		1 (2%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	4	5
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	48	46	45
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	16	28	33
TOTAL PRIMARY TUMORS	21	40	38
TOTAL ANIMALS WITH BENIGN TUMORS	14	7	8
TOTAL BENIGN TUMORS	17	10	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	24	27
TOTAL MALIGNANT TUMORS	4	30	30
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	1
TOTAL SECONDARY TUMORS		5	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 ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	48	39
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	48	39
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(48)	(39)
FIBROSARCOMA	1 (2%)	2 (4%)	
RHABDOMYOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(50)	(48)	(39)
PAPILLARY ADENOCARCINOMA		1 (2%)	
#LUNG	(50)	(47)	(36)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)		2 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (3%)
FIBROSARCOMA, METASTATIC		1 (2%)	
HEMANGIOSARCOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(48)	(39)
MALIGNANT LYMPHOMA, NOS	4 (8%)	3 (6%)	4 (10%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)	1 (2%)	
GRANULOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(50)	(46)	(37)
HEMANGIOMA		2 (4%)	
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
MALIGNANT LYMPHOMA, NOS		1 (2%)	4 (11%)
#LYMPH NODE	(5)	(1)	(4)
HEMANGIOSARCOMA, METASTATIC	1 (20%)		
*ADIPOSE TISSUE	(50)	(48)	(39)
MALIGNANT LYMPHOMA, NOS		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LUNG	(50)	(47)	(36)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#LIVER	(50)	(48)	(37)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(37)
HEPATOCELLULAR ADENOMA		9 (19%)	6 (16%)
HEPATOCELLULAR CARCINOMA		12 (25%)	29 (78%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(39)	(35)	(19)
FOLLICULAR-CELL ADENOMA		1 (3%)	
#PANCREATIC ISLETS	(26)	(30)	(19)
ISLET-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
*VAGINA	(50)	(48)	(39)
HEMANGIOSARCOMA	1 (2%)		
#UTERUS	(49)	(46)	(34)
HEMANGIOSARCOMA		1 (2%)	
#OVARY	(20)	(24)	(20)
TERATOMA, BENIGN		1 (4%)	
TERATOMA, NOS			1 (5%)
NERVOUS SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE	(50)	(48)	(39)
SQUAMOUS CELL CARCINOMA			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(50)	(48)	(39)
LYMPHANGIOMA	1 (2%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	10	22
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	45	39	28
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	31	35
TOTAL PRIMARY TUMORS	12	41	48
TOTAL ANIMALS WITH BENIGN TUMORS	4	14	6
TOTAL BENIGN TUMORS	4	14	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	21	30
TOTAL MALIGNANT TUMORS	8	27	39
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	2	
TOTAL SECONDARY TUMORS	1	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	33	33	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	33	32	33
INTEGUMENTARY SYSTEM			
*SKIN	(33)	(33)	(34)
EPIDERMAL INCLUSION CYST		1 (3%)	1 (3%)
*SUBCUT TISSUE	(33)	(33)	(34)
GRANULOMA, NOS	1 (3%)		
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(33)	(33)	(34)
INFLAMMATION, HEMORRHAGIC		2 (6%)	
INFLAMMATION, ACUTE	5 (15%)	2 (6%)	
INFLAMMATION, ACUTE SUPPURATIVE	6 (18%)	16 (48%)	16 (47%)
INFLAMMATION, CHRONIC	2 (6%)		1 (3%)
#TRACHEA	(30)	(23)	(33)
INFLAMMATION, ACUTE SUPPURATIVE	1 (3%)	2 (9%)	4 (12%)
INFLAMMATION, CHRONIC	7 (23%)		1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV	2 (7%)		
ABSCESS, CHRONIC		1 (4%)	
#LUNG	(30)	(31)	(33)
CONGESTION, NOS	1 (3%)	5 (16%)	
EDEMA, NOS	1 (3%)		
PNEUMONIA, ASPIRATION			1 (3%)
PNEUMONIA, CHRONIC MURINE	8 (27%)	15 (48%)	14 (42%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(31)	(15)	(32)
HEMATOPOIETIC TISSUE DISORDER	1 (3%)		
HYPERPLASIA, HEMATOPOIETIC	3 (10%)	3 (20%)	9 (28%)
#SPLEEN	(31)	(32)	(30)
INFLAMMATION, CHRONIC		6 (19%)	3 (10%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMOSIDEROSIS	3 (10%)	5 (16%)	11 (37%)
ATROPHY, NOS			8 (27%)
LYMPHOID DEPLETION			1 (3%)
HEMATOPOIESIS	3 (10%)	8 (25%)	4 (13%)
#SPLENIC FOLLICLES	(31)	(32)	(30)
ATROPHY, NOS	1 (3%)		
#MANDIBULAR L. NODE	(22)		(15)
INFLAMMATION, CHRONIC			1 (7%)
HYPERPLASIA, LYMPHOID	5 (23%)		1 (7%)
#BRONCHIAL LYMPH NODE	(22)		(15)
HEMORRHAGE	1 (5%)		
#THYMUS	(3)		(2)
ATROPHY, NOS	3 (100%)		2 (100%)
CIRCULATORY SYSTEM			
#HEART	(30)	(32)	(33)
CALCIFICATION, DYSTROPHIC		2 (6%)	1 (3%)
#MYOCARDIUM	(30)	(32)	(33)
INFLAMMATION, NOS			1 (3%)
INFLAMMATION, CHRONIC	4 (13%)	2 (6%)	1 (3%)
DEGENERATION, NOS		1 (3%)	
#ENDOCARDIUM	(30)	(32)	(33)
FIBROSIS			1 (3%)
*AORTA	(33)	(33)	(34)
METAPLASIA, OSSEOUS			1 (3%)
*PULMONARY ARTERY	(33)	(33)	(34)
CALCIFICATION, DYSTROPHIC	1 (3%)		
DIGESTIVE SYSTEM			
#LIVER	(31)	(32)	(33)
CYST, NOS	1 (3%)		
DEGENERATION, NOS		3 (9%)	
NECROSIS, FOCAL		1 (3%)	
METAMORPHOSIS FATTY	2 (6%)	6 (19%)	7 (21%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, NOS			2 (6%)
HYPERPLASIA, NOS	5 (16%)	3 (9%)	11 (33%)
ANGIECTASIS	1 (3%)	2 (6%)	2 (6%)
#LIVER/CENTRILOBULAR	(31)	(32)	(33)
NECROSIS, NOS			1 (3%)
*BILE DUCT	(33)	(33)	(34)
INFLAMMATION, CHRONIC		1 (3%)	
HYPERPLASIA, NOS	8 (24%)	3 (9%)	2 (6%)
#PANCREAS	(24)	(12)	(24)
PERIARTERITIS	1 (4%)		
#STOMACH	(31)	(28)	(30)
ULCER, NOS		1 (4%)	
ULCER, ACUTE		3 (11%)	5 (17%)
ULCER, CHRONIC		1 (4%)	
URINARY SYSTEM			
#KIDNEY	(31)	(31)	(33)
MINERALIZATION			5 (15%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (3%)	
ABSCESS, NOS			1 (3%)
INFLAMMATION, CHRONIC	23 (74%)		
PYELONEPHRITIS, CHRONIC	1 (3%)		
CALCIFICATION, DYSTROPHIC		2 (6%)	2 (6%)
#KIDNEY/CORTEX	(31)	(31)	(33)
CALCIFICATION, DYSTROPHIC			1 (3%)
#PERIRENAL TISSUE	(31)	(31)	(33)
HEMORRHAGE		1 (3%)	
#KIDNEY/TUBULE	(31)	(31)	(33)
CAST, NOS			1 (3%)
DEGENERATION, NOS		20 (65%)	27 (82%)
ATROPHY, NOS			1 (3%)
REGENERATION, NOS			1 (3%)
#URINARY BLADDER	(28)	(2)	(27)
EDEMA, NOS			2 (7%)
INFLAMMATION, CHRONIC	2 (7%)		
HYPERPLASIA, PAPILLARY			1 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(1)	(15)
CYST, NOS	2 (13%)		1 (7%)
#ADRENAL	(30)	(24)	(33)
HEMORRHAGE			1 (3%)
ANGIECTASIS	1 (3%)	3 (13%)	2 (6%)
#ADRENAL CORTEX	(30)	(24)	(33)
LIPOIDOSIS	11 (37%)	4 (17%)	1 (3%)
ATROPHY, NOS			1 (3%)
#PARATHYROID	(25)	(4)	(24)
CYST, NOS			1 (4%)
HYPERPLASIA, NOS	4 (16%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(29)	(2)	(31)
INFLAMMATION, ACUTE	2 (7%)		
INFLAMMATION, CHRONIC	4 (14%)		3 (10%)
*SEMINAL VESICLE	(33)	(33)	(34)
DILATATION, NOS	1 (3%)		
INFLAMMATION, CHRONIC	1 (3%)		
ABSCESS, CHRONIC		1 (3%)	
#TESTIS	(32)	(23)	(31)
ABSCESS, NOS	1 (3%)		
PERIARTERITIS	2 (6%)		
CALCIFICATION, DYSTROPHIC		1 (4%)	
ATROPHY, NOS	9 (28%)	12 (52%)	10 (32%)
ASPERMATOGENESIS	1 (3%)		1 (3%)
#TESTIS/TUBULE	(32)	(23)	(31)
ATROPHY, FOCAL		1 (4%)	
NERVOUS SYSTEM			
#BRAIN	(31)	(29)	(32)
ABSCESS, NOS			1 (3%)
ABSCESS, CHRONIC		1 (3%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE	(33)	(33)	(34)
INFLAMMATICN, ACUTE		2 (6%)	
*EYE/RETINA	(33)	(33)	(34)
INFLAMMATICN, NOS		2 (6%)	1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(33)	(33)	(34)
PERIARTERITIS	1 (3%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHCIOGY SUMMARY			
NO LESION REPORTED		1	
ACCIDENTAL DEATH		2	
AUTO/NECROPSY/HISTO PERF	1		
AUTO/NECROPSY/NO HISTO		1	1
AUTOLYSIS/NO NECROPSY	2		1

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	35	35	35
ANIMALS NECROPSIED	34	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	31	34	32
INTEGUMENTARY SYSTEM			
*SKIN	(34)	(35)	(35)
EPIDERMAL INCLUSION CYST		1 (3%)	
*SUBCUT TISSUE	(34)	(35)	(35)
GRANULOMA, FOREIGN BODY	1 (3%)		
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(34)	(35)	(35)
INFLAMMATION, HEMORRHAGIC		1 (3%)	
INFLAMMATION, ACUTE	1 (3%)	7 (20%)	2 (6%)
INFLAMMATION, ACUTE SUPPURATIVE	1 (3%)	16 (46%)	16 (46%)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
INFLAMMATION, CHRONIC			1 (3%)
#TRACHEA	(29)	(31)	(24)
INFLAMMATION, NOS	5 (17%)		
INFLAMMATION, ACUTE		2 (6%)	
INFLAMMATION, ACUTE SUPPURATIVE	1 (3%)	5 (16%)	4 (17%)
INFLAMMATION, CHRONIC			1 (4%)
#LUNG/BRONCHUS	(30)	(34)	(32)
INFLAMMATION, CHRONIC		1 (3%)	
#LUNG	(30)	(34)	(32)
CONGESTION, NOS	2 (7%)		
INFLAMMATION, ACUTE SUPPURATIVE	1 (3%)		
BRONCHOPNEUMONIA ACUTE SUPPURATI		4 (12%)	
PNEUMONIA, CHRONIC MURINE	6 (20%)	5 (15%)	25 (78%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
BRONCHOPNEUMONIA CHRONIC SUPPURA		2 (6%)	1 (3%)
GRANULOMA, NOS	1 (3%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(31)	(24)	(20)
HYPERPLASIA, HEMATOPOIETIC	4 (13%)	3 (13%)	1 (5%)
#SPLEEN	(30)	(34)	(32)
HEMORRHAGE			1 (3%)
INFLAMMATION, ACUTE	4 (13%)	1 (3%)	
INFLAMMATION, CHRONIC	1 (3%)		
HEMOSIDEROSIS	2 (7%)	6 (18%)	7 (22%)
ATROPHY, NOS	1 (3%)	1 (3%)	
HEMATOPOIESIS	6 (20%)	7 (21%)	8 (25%)
#MANDIBULAR L. NODE	(25)	(5)	(5)
HEMORRHAGIC CYST	1 (4%)		
INFLAMMATION, ACUTE	1 (4%)		
PLASMA-CELL INFILTRATE	3 (12%)		
HYPERPLASIA, LYMPHOID	5 (20%)	3 (60%)	
#MESENTERIC L. NODE	(25)	(5)	(5)
HYPERPLASIA, LYMPHOID	1 (4%)		
#THYMUS	(9)	(3)	(1)
CYST, NOS	2 (22%)		
ATROPHY, NOS	9 (100%)	3 (100%)	1 (100%)
CIRCULATORY SYSTEM			
#HEART	(31)	(34)	(32)
FIBROSIS			1 (3%)
CALCIFICATION, DYSTROPHIC	1 (3%)		
#MYOCARDIUM	(31)	(34)	(32)
INFLAMMATION, CHRONIC			1 (3%)
*MESENTERIC ARTERY	(34)	(35)	(35)
THROMBOSIS, NOS	1 (3%)		
INFLAMMATION, CHRONIC	1 (3%)		
DIGESTIVE SYSTEM			
#LIVER	(31)	(33)	(32)
CONGESTION, NOS	1 (3%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, NOS	1 (3%)	3 (9%)	3 (9%)
NECROSIS, FOCAL	1 (3%)	1 (3%)	
NECROSIS, DIFFUSE			1 (3%)
METAMORPHOSIS FATTY		6 (18%)	2 (6%)
LIPOIDOSIS	2 (6%)	1 (3%)	
HYPERTROPHY, NOS		2 (6%)	2 (6%)
HYPERPLASIA, NOS	7 (23%)	11 (33%)	17 (53%)
ANGIECTASIS		1 (3%)	1 (3%)
HEMATOPOIESIS	1 (3%)		
#LIVER/CENTRILOBULAR	(31)	(33)	(32)
METAMORPHOSIS FATTY	1 (3%)		
*BILE DUCT	(34)	(35)	(35)
DILATATION, NOS	1 (3%)		
INFLAMMATION, CHRONIC	1 (3%)		1 (3%)
HYPERPLASIA, NOS	13 (38%)	3 (9%)	5 (14%)
#PANCREAS	(29)	(15)	(16)
INFLAMMATION WITH FIBROSIS	1 (3%)		
#PANCREATIC DUCT	(29)	(15)	(16)
HYPERPLASIA, NOS	3 (10%)		1 (6%)
#PANCREATIC ACINUS	(29)	(15)	(16)
ATROPHY, NOS			1 (6%)
#STOMACH	(31)	(33)	(30)
EDEMA, NOS			1 (3%)
ULCER, ACUTE		1 (3%)	1 (3%)
CALCIFICATION, DYSTROPHIC	1 (3%)		
#GASTRIC MUCOSA	(31)	(33)	(30)
EROSION		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(31)	(34)	(32)
MINERALIZATION	17 (55%)	12 (35%)	15 (47%)
HEMATOMA, ORGANIZED			1 (3%)
PYELONEPHRITIS, NOS	1 (3%)		
PYELONEPHRITIS, ACUTE	1 (3%)		
INFLAMMATION, CHRONIC	5 (16%)	2 (6%)	1 (3%)
DEGENERATION, NOS		1 (3%)	
NEPHROSIS, NOS		1 (3%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#KIDNEY/MEDULLA	(31)	(34)	(32)
MINERALIZATION	1 (3%)	4 (12%)	1 (3%)
#KIDNEY/TUBULE	(31)	(34)	(32)
DILATATION, NOS			2 (6%)
CYST, NOS	4 (13%)		
DEGENERATION, NOS			10 (31%)
#URINARY BLADDER	(25)	(8)	(4)
EDEMA, NOS			1 (25%)
INFLAMMATION, NOS	1 (4%)		
INFLAMMATION, ACUTE	1 (4%)		
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(3)	(2)
CYST, NOS	3 (17%)		
#ADRENAL	(30)	(32)	(29)
HEMORRHAGE			1 (3%)
ANGIECTASIS	15 (50%)	9 (28%)	7 (24%)
#ADRENAL CORTEX	(30)	(32)	(29)
LIPOIDOSIS	9 (30%)	3 (9%)	1 (3%)
HYPERPLASIA, NOS	2 (7%)		
#THYROID	(28)	(20)	(18)
CYSTIC FOLLICLES	1 (4%)		
FOLLICULAR CYST, NOS	1 (4%)		
HYPERPLASIA, C-CELL	3 (11%)		
REPRODUCTIVE SYSTEM			
*VAGINA	(34)	(35)	(35)
INFLAMMATION, ACUTE		1 (3%)	
#UTERUS	(30)	(34)	(28)
INFLAMMATION, ACUTE	2 (7%)		1 (4%)
#UTERUS/ENDOMETRIUM	(30)	(34)	(28)
CYST, NOS	2 (7%)	11 (32%)	4 (14%)
INFLAMMATION, ACUTE	2 (7%)	3 (9%)	1 (4%)
INFLAMMATION, ACUTE SUPPURATIVE		2 (6%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS		1 (3%)	
#OVARY/OVIDUCT	(30)	(34)	(28)
INFLAMMATION, ACUTE		1 (3%)	
#OVARY	(26)	(23)	(22)
CYSTIC FOLLICLES	1 (4%)		
FOLLICULAR CYST, NOS	1 (4%)	2 (9%)	
NERVOUS SYSTEM			
#BRAIN	(31)	(31)	(28)
HEMORRHAGE			1 (4%)
NECROSIS, NOS			1 (4%)
SPECIAL SENSE ORGANS			
*EYE	(34)	(35)	(35)
INFLAMMATION, ACUTE	3 (9%)		
CATARACT	1 (3%)		
*EYE/RETINA	(34)	(35)	(35)
INFLAMMATION, NOS	21 (62%)	4 (11%)	3 (9%)
*EYE/LACRIMAL GLAND	(34)	(35)	(35)
INFLAMMATION, ACUTE SUPPURATIVE	1 (3%)		
*HARDERIAN GLAND	(34)	(35)	(35)
ABSCESS, NOS	1 (3%)		
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(34)	(35)	(35)
GRANULOMA, FOREIGN BODY	1 (3%)		
BODY CAVITIES			
*ABDOMINAL WALL	(34)	(35)	(35)
INFLAMMATION, CHRONIC	1 (3%)		
ALL OTHER SYSTEMS			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECRCPSY/NO HISTO	3	1	3
AUTOLYSIS/NO NECROPSY	1		

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(49)
ULCER, CHRONIC		1 (2%)	1 (2%)
ACARIASIS			2 (4%)
CALCIFICATION, DYSTROPHIC		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(49)	(50)	(49)
INFLAMMATION, ACUTE		1 (2%)	1 (2%)
POLYP			1 (2%)
#LUNG	(49)	(50)	(47)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS	1 (2%)	9 (18%)	17 (36%)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(48)	(49)	(43)
HEMORRHAGE			1 (2%)
HEMATOPOIESIS		1 (2%)	
#LYMPH NODE	(1)	(2)	(1)
HYPERPLASIA, RETICULUM CELL			1 (100%)
HYPERPLASIA, LYMPHOID		1 (50%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(49)	(50)	(48)
INFLAMMATION, CHRONIC			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(47)
NECROSIS, NOS		2 (4%)	5 (11%)
HYPERPLASIA, NOS		2 (4%)	1 (2%)
HYPERPLASIA, CYSTIC		1 (2%)	
ANGIECTASIS		2 (4%)	1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(49)	(50)	(49)
DILATATION, NOS	1 (2%)	1 (2%)	3 (6%)
CYST, NOS		1 (2%)	2 (4%)
INFLAMMATION, NOS		1 (2%)	
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)	3 (6%)	1 (2%)
#TESTIS	(49)	(34)	(35)
GRANULOMA, SPERMATIC	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	29	10	7
AUTO/NECROPSY/HISTO PERF		1	
AUTOLYSIS/NO NECROPSY	1		1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	48	39
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	48	39
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(50)	(48)	(39)
INFLAMMATION, ACUTE		3 (6%)	5 (13%)
INFLAMMATION, ACUTE SUPPURATIVE		4 (8%)	3 (8%)
POLYP		1 (2%)	
#TRACHEA	(45)	(41)	(25)
POLYP		1 (2%)	
#LUNG	(50)	(47)	(36)
INFLAMMATION, NOS	2 (4%)	33 (70%)	32 (89%)
INFLAMMATION, ACUTE			2 (6%)
ABSCESS, NOS			1 (3%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(46)	(37)
INFLAMMATION, ACUTE			1 (3%)
INFLAMMATION, CHRONIC			1 (3%)
ATROPHY, NOS			1 (3%)
HYPERPLASIA, LYMPHOID	6 (12%)	2 (4%)	2 (5%)
HEMATOPOIESIS		1 (2%)	
#LYMPH NODE	(5)	(1)	(4)
HYPERPLASIA, LYMPHOID	1 (20%)		1 (25%)
#MESENTERIC L. NODE	(5)	(1)	(4)
INFLAMMATION, CHRONIC			1 (25%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(37)
ABSCESS, NOS		1 (2%)	
NECROSIS, NOS		2 (4%)	
METAMORPHOSIS FATTY		1 (2%)	
LIPOIDOSIS		1 (2%)	
HYPERPLASIA, NOS	1 (2%)	7 (15%)	
ANGIECTASIS		4 (8%)	2 (5%)
#LIVER/HEPATOCYTES	(50)	(48)	(37)
NECROSIS, NOS	1 (2%)		
#PANCREAS	(26)	(30)	(19)
DILATATION/DUCTS	1 (4%)		1 (5%)
ABSCESS, CHRONIC			1 (5%)
LIPOGRANULOMA		1 (3%)	
#PANCREATIC ACINUS	(26)	(30)	(19)
ATROPHY, NOS			1 (5%)
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(36)
LYMPHOCYTIC INFLAMMATORY INFILTR	2 (4%)	2 (4%)	1 (3%)
PLASMA-CELL INFILTRATE		1 (2%)	
#KIDNEY/GLOMERULUS	(50)	(48)	(36)
AMYLOIDOSIS	1 (2%)		
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(49)	(46)	(34)
HYDROMETRA	4 (8%)	1 (2%)	2 (6%)
HEMORRHAGIC CYST		1 (2%)	
ABSCESS, CHRONIC		1 (2%)	2 (6%)
#UTERUS/ENDOMETRIUM	(49)	(46)	(34)
CYST, NOS		7 (15%)	1 (3%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE		3 (7%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
HYPERPLASIA, DIFFUSE	1 (2%)		
HYPERPLASIA, CYSTIC	48 (98%)	26 (57%)	23 (68%)
#OVARY/PAROVARIAN	(49)	(46)	(34)
ABSCESS, CHRONIC		1 (2%)	
#OVARY	(20)	(24)	(20)
CYST, NOS	5 (25%)	8 (33%)	1 (5%)
FOLLICULAR CYST, NOS	5 (25%)	2 (8%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (5%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
LIPOGRANULOMA	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	2
ANIMAL MISSING/NO NECROPSY		1	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTO/NECROPSY/HISTO PERF			1
AUTOLYSIS/NO NECROPSY		1	11

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 1,4-Dioxane in the Drinking Water[a]

Topography: Morphology	High Dose Control	Low Dose	High Dose
Integumentary System: Fibroma[b]	3/33 (9)	1/33 (3)	1/34 (3)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			0.324
Lower Limit			0.006
Upper Limit			3.787
Weeks to First Observed Tumor	96	101	110
Nasal Turbinate: Squamous-cell Carcinoma[b]	0/33 (0)	12/33 (36)	16/34 (47)
P Values[c,d]			P < 0.001
Relative Risk (High Dose Control)[f]			Infinite
Lower Limit			5.028
Upper Limit			Infinite
Weeks to First Observed Tumor	--	60	52

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	High Dose Control	Low Dose	High Dose
Nasal Turbinate:			
. Adenocarcinoma, NOS[b]	0/33 (0)	0/33 (0)	3/34 (9)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			Infinite
Lower Limit			0.593
Upper Limit			Infinite
Weeks to First Observed Tumor	--	--	74
Liver: Hepatocellular			
Adenoma or Carcinoma[b]	2/31 (6)	2/32 (6)	1/33 (3)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			0.470
Lower Limit			0.008
Upper Limit			8.568
Weeks to First Observed Tumor	100	101	110

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	High Dose Control	Low Dose	High Dose
Adrenal: Pheochromocytoma[b]	6/30 (20)	0/24 (0)	2/33 (6)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			0.303
Lower Limit			0.032
Upper Limit			1.545
Weeks to First Observed Tumor	86	--	110
Pituitary: Chromophobe Adenoma or Adenoma, NOS[b]	3/16 (19)	0/1 (0)	1/15 (7)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			0.356
Lower Limit			0.007
Upper Limit			3.840
Weeks to First Observed Tumor	110	--	110

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	High Dose Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	3/29 (10)	1/17 (6)	0/31 (0)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			0.000
Lower Limit			0.000
Upper Limit			1.525
Weeks to First Observed Tumor	110	96	--
Thyroid or Thyroid Follicle:			
Follicular-cell Adenoma, Cystadenoma, NOS, or Carcinoma[b]	3/29 (10)	1/17 (6)	1/31 (3)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			0.312
Lower Limit			0.006
Upper Limit			3.626
Weeks to First Observed Tumor	97	96	85

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	High Dose Control	Low Dose	High Dose
Parathyroid: Adenoma, NOS[b]	2/25 (8)	0/4 (0)	0/24 (0)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			0.000
Lower Limit			0.000
Upper Limit			3.421
Weeks to First Observed Tumor	110	--	--
Mammary Gland: Fibroadenoma[b]	0/33 (0)	2/33 (6)	0/34 (0)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			--
Lower Limit			--
Upper Limit			--
Weeks to First Observed Tumor	--	89	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	High Dose Control	Low Dose	High Dose
Tunica Albuginea or Vaginalis: Mesothelioma, NOS[b]	2/33 (6)	4/33 (12)	5/34 (15)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			2.426
Lower Limit			0.432
Upper Limit			24.040
Weeks to First Observed Tumor	81	89	69
Brain: Glioma, NOS[b]	0/31 (0)	0/29 (0)	2/32 (6)
P Values[c,d]			N.S.
Relative Risk (High Dose Control)[f]			Infinite
Lower Limit			0.291
Upper Limit			Infinite
Weeks to First Observed Tumor	--	--	92

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 1,4-Dioxane in Drinking Water[a]

(continued)

[a]Dosed groups received average doses of 240 or 530 mg/kg per day in drinking water.

[b]Number of tumor-bearing animals/number of animals examined at site (percent). Controls were matched to the high-dose only and no statistics are provided for the low-dose group.

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

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

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

[f]The 95% confidence interval of the relative risk between the high-dose group and its control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 1,4-Dioxane in the Drinking Water[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Fibroma[b]	1/34 (3)	2/35 (6)	2/35 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.943	1.943
Lower Limit		0.106	0.106
Upper Limit		111.290	111.290
Weeks to First Observed Tumor	115	86	84
Nasal Turbinate: Squamous-cell Carcinoma[b]	0/34 (0)	10/35 (29)	8/35 (23)
P Values[c,d]	P = 0.008	P = 0.001	P = 0.003
Departure from Linear Trend[e]	P = 0.039		
Relative Risk (Matched Control)[b]		Infinite	Infinite
Lower Limit		2.942	2.258
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	69	66

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangioma or Hemangiosarcoma[b]	0/34 (0)	2/35 (6)	3/35 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.291	0.593
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	86	66
Liver: Hepatocellular Adenoma[b]	0/31 (0)	10/33 (30)	11/32 (34)
P Values[c,d]	P = 0.001	P = 0.001	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		2.860	3.296
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	73	70

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Adenoma, NOS[b]	4/18 (22)	1/3 (33)	0/2 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.500	0.000
Lower Limit		0.033	0.000
Upper Limit		6.475	4.985
Weeks to First Observed Tumor	116	110	--
Thyroid: C-cell Adenoma[b]	4/28 (14)	0/20 (0)	0/18 (0)
P Values[c,d]	P = 0.033(N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.444	1.593
Weeks to First Observed Tumor	115	--	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid or Thyroid Follicle:			
Cystadenoma, NOS[b]	2/28 (7)	1/20 (5)	1/18 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.700	0.778
Lower Limit		0.012	0.014
Upper Limit		12.385	13.643
Weeks to First Observed Tumor	116	111	92
Mammary Gland: Adenoma or			
Cystadenoma, NOS[b]	3/34 (9)	4/35 (11)	1/35 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.295	0.324
Lower Limit		0.237	0.006
Upper Limit		8.246	3.798
Weeks to First Observed Tumor	113	73	84

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	13/34 (38)	16/35 (46)	10/35 (29)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.196	0.747
Lower Limit		0.645	0.344
Upper Limit		2.249	1.583
Weeks to First Observed Tumor	107	46	92

[a]Dosed groups received average doses of 350 or 640 mg/kg per day in drinking water.

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

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

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

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED 1,4-DIOXANE IN THE DRINKING WATER

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 1,4-Dioxane in the Drinking Water[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Fibrosarcoma[b]	0/49 (0)	4/50 (8)	0/49 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.009		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.909	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	77	--
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	8/49 (16)	3/50 (6)	3/47 (6)
P Values[c,d]	P = 0.048(N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.368	0.391
Lower Limit		0.066	0.070
Upper Limit		1.430	1.516
Weeks to First Observed Tumor	92	91	89

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	0/49 (0)	5/50 (10)	2/49 (4)
P Values[c,d]	N.S.	P = 0.030	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		1.237	0.296
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	77	91
All Sites: Hemangioma or Hemangiosarcoma[b]	0/49 (0)	6/50 (12)	3/49 (6)
P Values[c,d]	P = 0.047	P = 0.014	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		1.569	0.602
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	91	66

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	2/49 (4)	18/50 (36)	24/47 (51)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Matched Control)[f]		8.820	12.511
Lower Limit		2.287	3.406
Upper Limit		74.477	101.955
Weeks to First Observed Tumor	93	91	58
Liver: Hepatocellular Carcinoma or Adenoma[b]	8/49 (16)	19/50 (38)	28/47 (60)
P Values[c,d]	P < 0.001	P = 0.014	P < 0.001
Relative Risk (Matched Control)[f]		2.328	3.649
Lower Limit		1.086	1.852
Upper Limit		5.517	7.934
Weeks to First Observed Tumor	92	91	58

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

[a]Dosed groups received average doses of 720 or 830 mg/kg per day in drinking water.

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

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

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

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

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 1,4-Dioxane in the Drinking Water[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	3/50 (6)	0/47 (0)	3/36 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	1.389
Lower Limit		0.000	0.196
Upper Limit		1.766	9.764
Weeks to First Observed Tumor	91	--	81
Hematopoietic System: Lymphoma[b]	6/50 (12)	8/48 (17)	8/39 (21)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.389	1.709
Lower Limit		0.457	0.566
Upper Limit		4.501	5.457
Weeks to First Observed Tumor	76	67	86

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangioma or Hemangiosarcoma[b]	2/50 (4)	4/48 (8)	0/39 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		2.083	0.000
Lower Limit		0.314	0.000
Upper Limit		22.174	4.305
Weeks to First Observed Tumor	73	87	--
Liver: Hepatocellular Carcinoma[b]	0/50 (0)	12/48 (25)	29/37 (78)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.822	13.395
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	82	83

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 1,4-Dioxane in the Drinking Water[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Adenoma[b]	0/50 (0)	21/48 (44)	35/37 (95)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		7.102	17.510
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	82	81

[a]Dosed groups received average doses of 380 or 860 mg/kg per day in drinking water.

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

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

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

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

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

Review of the Bioassay of 1,4-Dioxane* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

March 7, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 1,4-Dioxane for carcinogenicity.

The primary reviewer said that 1,4-Dioxane induced squamous-cell carcinomas of the nasal turbinates in treated rats and hepatocellular carcinomas in treated mice. He briefly described the experimental design and conditions under which 1,4-Dioxane was tested. In his critique, the primary reviewer noted the poor survival among the rats and the decreased water intake among the high dose treated male mice. He said, however, that these shortcomings did not effect the conclusion regarding the carcinogenicity of 1,4-Dioxane.

The secondary reviewer questioned the significance of the decreased water intake among the high dose treated male mice. A Program staff member commented that the mice may have increased their water retention as they decreased their water intake. As a result, 1,4-Dioxane may have concentrated in the animal urinary bladder.

It was pointed out that epidemiological studies have shown an increased incidence of cancer of the nose and related passages among furniture makers. A Subgroup member noted that other studies have shown experimentally the carcinogenicity of 1,4-Dioxane.

A motion was made that the report on the bioassay of 1,4-Dioxane be accepted as written. The motion was seconded and approved unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold Brown, Mayo Clinic
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Michael Shimkin, University of California at San Diego

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

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