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

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FOREWORD: This report presents the results of the bioassay of trimethylphosphate 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 trimethylphosphate was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., the prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were chosen by Drs. E. K. Weisburger[1], J. H. Weisburger[1,2], N. P. Page[1,3], and F. M. Garner[4]. Administration of test chemical and observation of animals were supervised by Dr. F. M. Garner, with the technical assistance of Mr. R. Cypher[4], Mr. H. D. Thornett[4], and Mr. D. J. Howard[4].

Histopathologic examinations of rats were performed by Drs. R. A. Montali[4], P. Hildebrandt[4], N. J. Wosu[4], and H. R. Seibold[4] and those of mice by Drs. Montali and Wosu. Histologic sections of all tumors and hyperplasias were reexamined by Dr. Montali, who also examined all diagnoses and prepared the interpretive pathology summary included in this report.

Animal pathology tables and survival tables were complied 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. E. Murrill[8], and the analytical results were reviewed by Dr. S. S. Olin[6]. The structural formula was supplied by NCI[1].

This report was prepared at Tracor Jitco[6] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller 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: 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, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cirpiano 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], and Dr. Jerrold M. Ward.

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SUMMARY

A bioassay of trimethylphosphate for possible carcinogenicity was conducted by administering the compound by gavage to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were administered trimethylphosphate in distilled water three times per week at one of two doses, either 50 or 100 mg/kg body weight for the rats and either 250 or 500 mg/kg body weight for the mice. Vehicle controls consisted of groups of 20 rats and 20 mice of each sex. The rats were dosed for 104 weeks and the mice for 103 weeks. All surviving rats were killed at week 105 and all surviving mice at week 103.

Mean body weights of dosed male and female rats and female mice were slightly lower than those of the corresponding vehicle controls throughout the study; mean body weights of the male mice were comparable to those of the vehicle controls. Survival rates of both rats and mice were high, and adequate numbers of animals were at risk for the development of late-appearing tumors.

In the male rats, the incidence of fibromas of the subcutanous tissue was higher (P = 0.036) in the high-dose group than in the vehicle controls (control 0/20, low-dose 2/50, high-dose 9/49), and there was a dose-related trend (P = 0.006) in the incidences of these fibromas. In the female rats, no tumors occurred in the dosed groups at significantly increased incidences, compared with corresponding controls.

In the male mice, no tumors occurred in the dosed groups at significantly increased incidences, compared with controls. In the female mice, the incidence of adenocarcinomas of the endometrium was higher (P = 0.004) in the high-dose group than in the vehicle controls (controls 0/16, low-dose 7/40, high-dose 13/37), and there was a significant dose-related trend (P = 0.003) in the incidences of these adenocarcinomas.

It is concluded that under the conditions of this bioassay, trimethylphosphate was carcinogenic in female B6C3F1 mice, inducing adenocarcinomas of the uterus/endometrium. Trimethylphosphate was associated with the induction of benign fibromas of the subcutaneous tissue in male Fischer 344 rats. No evidence of carcinogenicity of the compound was obtained in female rats or in male mice.

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I. INTRODUCTION

Trimethylphosphate (CAS 512-56-1; NCI C03781) is an alkylating agent which has been used as a gasoline additive (Hinkamp and Warren, 1958), a methylating agent (Billman et al., 1942), an intermediate for the production of polymethyl polyphosphates (Toy, 1949), and a flame retardant in polymers (Matsunaga et al., 1975). Although it is still available commercially, it is no longer manufactured in the U.S. (Chem Sources - U.S.A., 1977).

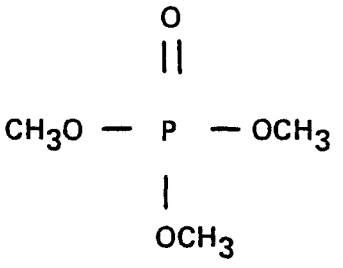
Trimethylphosphate is known to cause sterility in mice, rats, and rabbits (Harbison et al., 1976). It produces chromosomal abnormalities in the bone-marrow cells of rats (Adler et al., 1971) and dominant lethal effects in mice (Epstein, 1970).

Trimethylphosphate was selected for testing because its production and industrial applications could result in the exposure of workers in the chemical industry to the compound.

II. MATERIALS AND METHODS

A. Chemical

TRIMETHYLPHOSPHATE



Trimethylphosphate was obtained in two batches (Lot Nos. 062927 and 112737) from Aldrich Chemical Company, Milwaukee, Wisconsin. The identity and purity of both batches were checked by analysis. Elemental analyses (C, H, P) were consistent with $C_3H_9PO_4$, the molecular formula of trimethylphosphate. Infrared spectra were identical to those given in the literature (Aldrich Library of Infrared Spectra, 1976). Thin-layer chromatography using silica gel F-254(B) showed one major spot (with a slight trace at the origin in Lot No. 062927). Lot No. 112737 was estimated to be > 99% pure by vapor-phase chromatography (vpc). Lot No. 062927 contained approximately 2.4% methanol by vpc and nuclear magnetic resonance, and 1.8% water by Karl Fisher analysis.

The chemical was stored at 4°C in the original containers.

B. Dosage Preparation

Test solutions were prepared with distilled water on each day that a dose was to be administered. Excess solutions were not stored.

At 25°C, the half-life for the hydrolysis of the first methyl group in trimethylphosphate in water at neutral or slightly acid pH is calculated to be approximately 53 days (by extrapolation from Barnard et al., 1961). About 0.1% hydrolysis is expected in 24 hours. This is in essential agreement with the report of Newell et al. (1977) and Spanggord (1977) that no hydrolysis of aqueous solutions of trimethylphosphate was detected after 7 days at ambient temperature. Thus, no significant hydrolysis of trimethylphosphate in the test solutions for this bioassay should have occurred.

C. Animals

Fischer 344 rats and B6C3F1 mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, under a contract with the Division of Cancer Treatment, NCI. On arrival at the laboratory, the animals were approximately 4 weeks of age.

All animals were quarantined for 2 weeks and were then assigned to control or dosed groups.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature was maintained at 21-25°C and the relative humidity at 45-55%. There were 15 changes of room air per hour, and the incoming and exhaust air was passed through HEPA filters. The animal rooms were positively pressurized with respect to an exit hall and negatively pressurized with respect to an entrance hall. Cool white fluorescent lighting was provided 8 hours per day. Ground Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) was available *ad libitum* and was replenished three times per week. Tap water acidified with HCl to pH 2.5 also was available *ad libitum*.

Rats were housed four per cage and mice five per cage in solid polycarbonate cages. Each cage was covered with a wire mesh screen and a sheet of filter paper. Heat-treated hardwood chip bedding (Absorb-Dri®, Lab Products, Garfield, N. J.) was used in the cages. Cages and water bottles were sanitized twice per week and feed hoppers once per week at approximately 82°C; bedding was replaced twice per week.

Rats and mice were housed in separate rooms. Control animals

were housed in the same room as the respective dosed animals. Animals administered trimethylphosphate were housed in the same room as animals of the same species administered the following chemicals:

RATS

Feed Studies

1-H-1,2,4-triazole-3-amine (amitrole) (CAS 61-82-5)
2,4-dimethoxybenzenamine hydrochloride (NCI C02255)
N′-(chloroacetyl)-N-phenylacetamide (CAS 140-49-8)
N-ethyl-N′-(5-nitro-2-thiazolyl)urea (nithiazide) (CAS 139-94-6)

MICE

Feed Studies

2,4-dimethoxybenzenamine hydrochloride (NCI C02255)
N′-(chloroacetyl)-N-phenylacetamide (CAS 149-99-8)
N-ethyl-N′-(5-nitro-2-thiazolyl)urea (nithiazide) (CAS 139-94-6)
1,4-benzenediamine dihydrochloride (CAS 624-18-0)
4-nitro-o-phenylenediamine (CAS 99-56-9)
2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one (CAS 89-25-8)

Gavage Studies

3-(chloromethyl)pyridine hydrochloride (NCI C03838)
2-(chloromethyl)pyridine hydrochloride (CAS 4377-33-7)
3,3-dimethyl-2-oxethanone (pivalolactone) (CAS 1955-45-9)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated concentrations (hereinafter referred to as "low doses" and "high doses") of trimethylphosphate, on the basis of which low and high doses were determined for use in the chronic study.

Trimethylphosphate was administered by gavage in a vehicle of distilled water three times per week for 7 weeks. Doses for rats were 100, 147, 215, 316, 464, 681, 1,000, or 1,470 mg/kg body weight. Doses for mice were 147, 215, 316, 464, 681, 1,000, 1,470, or 2,150 mg/kg. Five animals were tested at each dose. Control groups consisted of five animals of each species and sex and were administered distilled water by gavage on the same schedule as dosed groups. All surviving animals were killed and necropsied 1 week after the end of the period of administration of the trimethylphosphate.

In the rats, all males and females died when administered doses of 681 mg/kg and greater, and one male died at a dose of 464 mg/kg. At the end of the period of administration, the gains in mean body weight in males and females at doses up to and including 316 mg/kg were approximately 80% of those of the controls. At 464 mg/kg, the gain in mean weight in males was 56% of that in controls; in females, it was 68% of that in controls. Rats dying before the end of the study had distended bladders and gastrointestinal hemorrhages. Low and high doses for the chronic studies using rats were set at 50 and 100 mg/kg.

In the mice, five males and one female died at a dose of 2,150 mg/kg, and two females died at 1,470 mg/kg. There were no other deaths. Gains in mean body weight were slightly depressed at

doses of 681 mg/kg and above in males, but were not greatly affected in any of the groups of females. Low and high doses for the chronic studies using mice were set at 250 and 500 mg/kg.

F. Designs of Chronic Studies

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

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied. Rats and mice were weighed individually at regular intervals. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of all major tissues, organs, or gross lesions taken from killed animals and from animals found dead. The following tissues and organs were routinely subjected to microscopic examination: skin, lymph nodes, mammary gland, salivary gland, bone marrow, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, large intestine, liver, gallbladder (mice), pancreas, spleen, kidney, adrenal, urinary bladder, prostate or uterus, testis or ovary, brain, and pituitary. Peripheral blood smears were prepared from each animal whenever possible. Occasionally, additional tissues

Table 1. Design of Chronic Studies of Trimethylphosphate in Rats

Sex and Test Group	Initial No. of Animals[a]	Trimethyl-phosphate Dose (mg/kg)[b]	Time on Study	
			Dosed (weeks)	Observed (weeks)
Males				
Vehicle-Control[c]	20	0		105
Low-Dose	50	50	104	1
High-Dose	50[d]	100	104	1
Females				
Vehicle-Control[c]	20	0		105
Low-Dose	50	50	104	1
High-Dose	50[d]	100	104	1

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

[b]Trimethylphosphate in distilled water was administered by gavage three times per week. Doses were based on the mean body weight of the group and were adjusted once per month.

[c]Vehicle controls received distilled water by gavage three times per week.

[d]One animal in the high-dose male group was found to be a female, and one in the high-dose female group was found to be a male.

Table 2. Design of Chronic Studies of Trimethylphosphate in Mice

Sex and Test Group	Initial No. of Animals[a]	Trimethyl-phosphate Dose (mg/kg)[b]	Time on Study	
			Dosed (weeks)	Observed (weeks)
Males				
Vehicle-Control[c]	20	0		103
Low-Dose	50	250	103	0
High-Dose	49	500	103	0
Females				
Vehicle-Control[c]	20	0		103
Low-Dose	50	250	103	0
High-Dose	50[d]	500	103	0

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

[b]Trimethylphosphate in distilled water was administered by gavage three times per week. Doses were based on the mean body weight of the group and were adjusted once per month.

[c]Vehicle controls received distilled water by gavage three times per week.

[d]One animal in the high-dose female group was found to be a male.

were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. An occasional section was subjected to special staining techniques for more definitive diagnosis.

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

H. Data Recording and Statistical Analyses

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

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

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

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling

(e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

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

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

ship. 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- and high-dose rats of each sex were slightly lower than those of the corresponding vehicle controls, and the depressions in weight were dose related (figure 1). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other chemical-related clinical signs were reported.

B. Survival (Rats)

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

The results of the Tarone test for positive dose-related trend in mortality are not significant in either sex. All of the rats lived beyond week 52 on study, and adequate numbers of animals of each sex were at risk for development of late-appearing tumors. In male rats, 17/49 (35%) of the high-dose group, 28/50 (56%) of the low-dose group, and 8/20 (40%) of the matched controls lived to the end of the study. In females, the percentages of survival

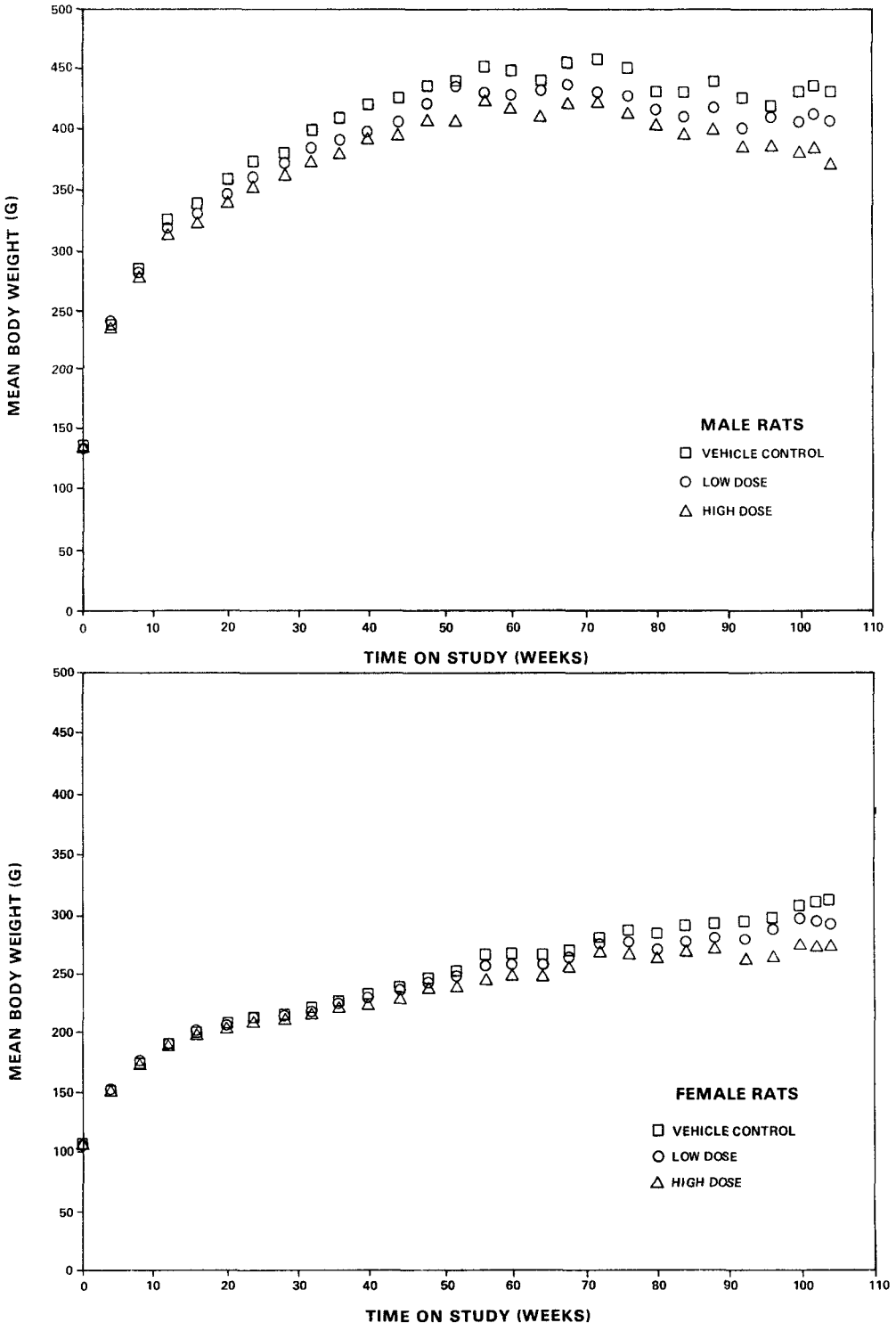


Figure 1. Growth Curves for Rats Administered Trimethylphosphate by Gavage

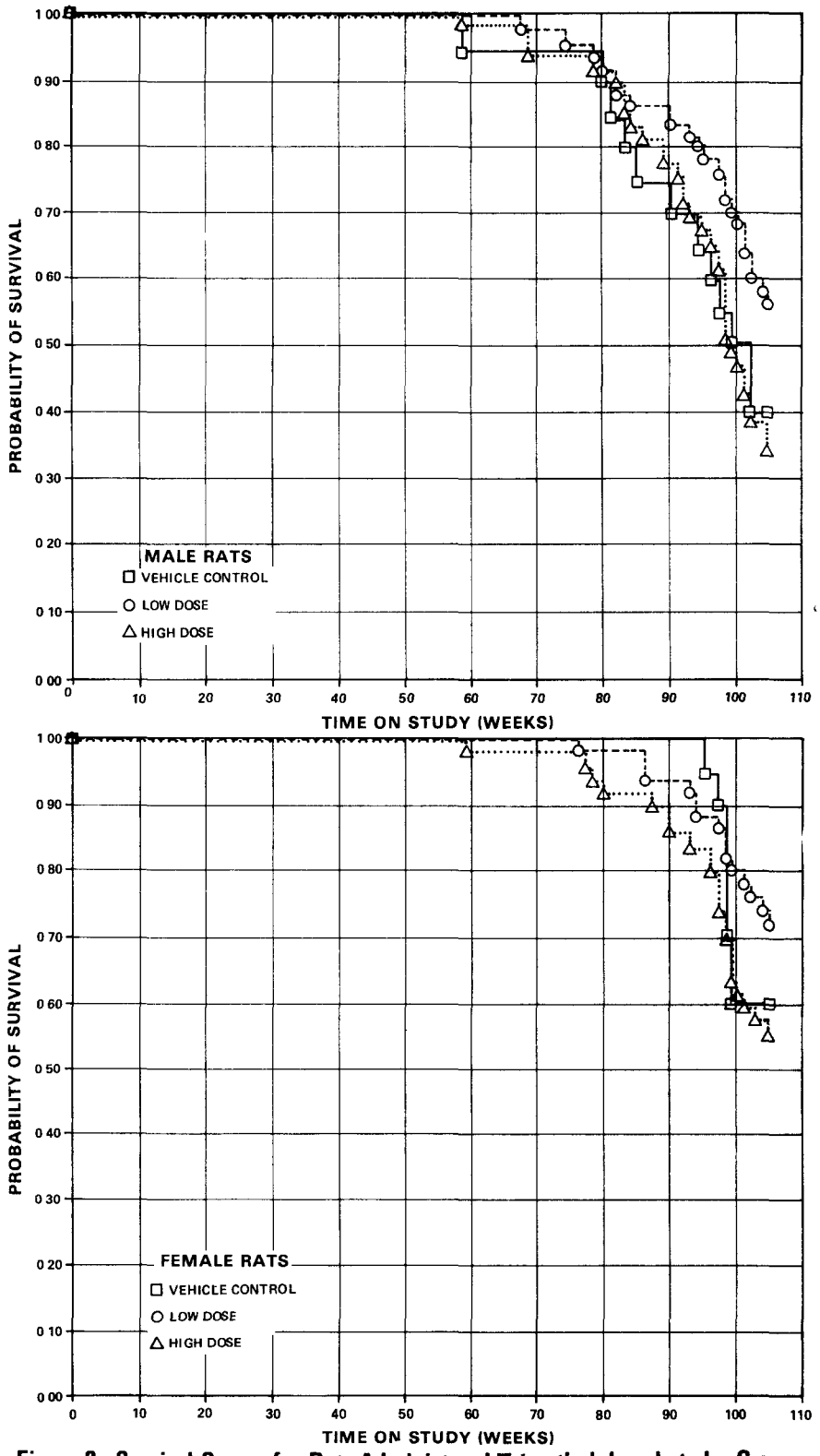


Figure 2. Survival Curves for Rats Administered Trimethylphosphate by Gavage

to termination of the study are 27/49 (55%) in high-dose group, 36/50 (72%) in low-dose group, and 12/20 (60%) in the matched controls.

C. Pathology (Rats)

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

There was a relatively high incidence of subcutaneous fibromas occurring only in the dosed male rats while none occurred in the dosed females or in the controls. The incidence is as follows:

	Male Rats		
	Matched Control	Low Dose	High Dose
Number of animals necropsied	(20)	(50)	(49)
Integumentary System			
Fibromas	0	3 (6%)	9 (18%)

Grossly, the fibromas ranged from 5 cm tc 9 cm in diameter and were located at points along the axillary, thoracic, abdominal, and inguinal regions. Microscopically, they consisted of layers of well-differentiated fibroblastic cells separated by dense bands of mature collagen. A fibroadenoma of the mammary gland also occurred in a high-dose male. Fibromas are occasionally

encountered spontaneously in aging rats, but this incidence is considered unusual. In addition, several other unusual tumors were observed in this study. These included three tumors of the brain and two of subcutaneous tissue: astrocytoma in 1/48 (2%) high-dose males; glioblastoma multiforme in 1/48 (2%) high-dose females; malignant reticulosis in 1/50 (2%) low-dose females; and myxosarcoma in 2/49 (4%) high-dose females.

As shown in the summary tables, there were malignant tumors in the dosed rats that did not occur in the controls. These tumors appeared to be randomly distributed, and the isolated or low incidence minimizes any possible significance. The remaining tumors that occurred in the dosed and control rats are commonly observed as spontaneous tumors in aging rats. In some cases the incidences of these spontaneous tumors exceeded those of the matched controls, but the overall incidence did not exceed the expected figures for aging rats.

A variety of degenerative and inflammatory conditions of the type usually encountered in aged rats were observed, but none was attributed to the test compound.

In the judgment of the pathologist, there was an increased incidence of subcutaneous fibromas in dosed male rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

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

In male rats, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of fibromas of the subcutaneous tissue is significant (P = 0.006). The Fisher exact comparison of the incidences of these tumors in the high-dose group and its vehicle-control indicates a probability level of 0.036, which is above the 0.025 level required by the Bonferroni inequality when multiple comparison is considered.

In female rats, a significant dose-related trend in the negative direction (P = 0.043) is observed in the incidence of endometrial stromal polyps of the uterus, where the incidence in the control group exceeds those in the dosed groups; this significant negative trend cannot, however, be accounted for by differential survival.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the dosed groups of male mice were comparable to those of the vehicle controls throughout the bioassay, while those of the dosed groups of female mice were lower, with a dose-related depression (figure 3). 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 other clinical signs related to administration of the trimethylphosphate were recorded.

B. Survival (Mice)

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

The results of the Tarone test for positive dose-related trend in mortality are not significant in either sex. At least 70% of the male mice (39/49 [80%] in the high-dose group, 44/50 [88%] in the low-dose group, and 14/20 [70%] in the matched controls) and at least 59% of the female mice (29/49 [59%] in the high-dose group, 31/50 [62%] in the low-dose group, and 18/20 [90%] in the matched

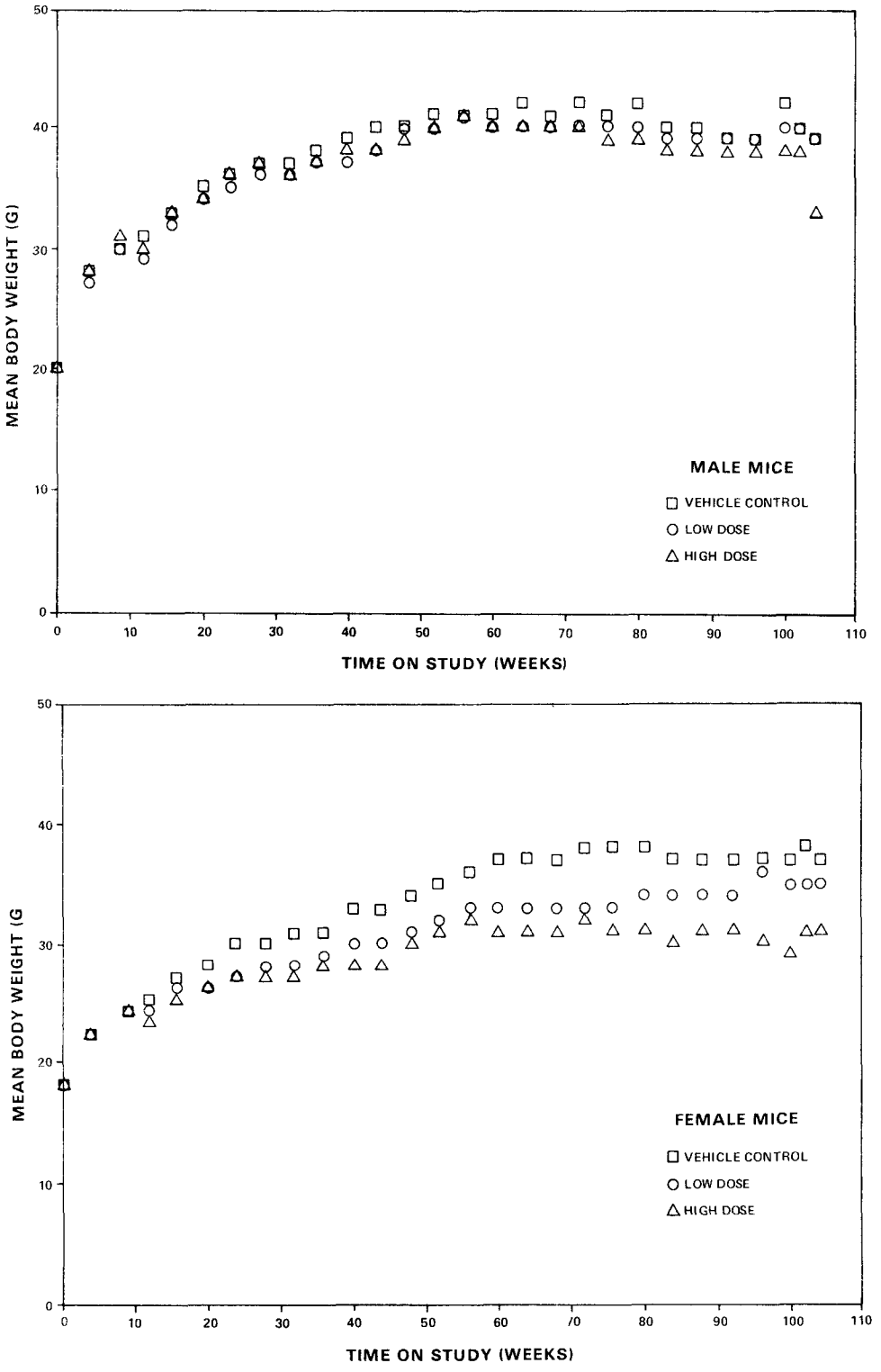


Figure 3. Growth Curves for Mice Administered Trimethylphosphate by Gavage

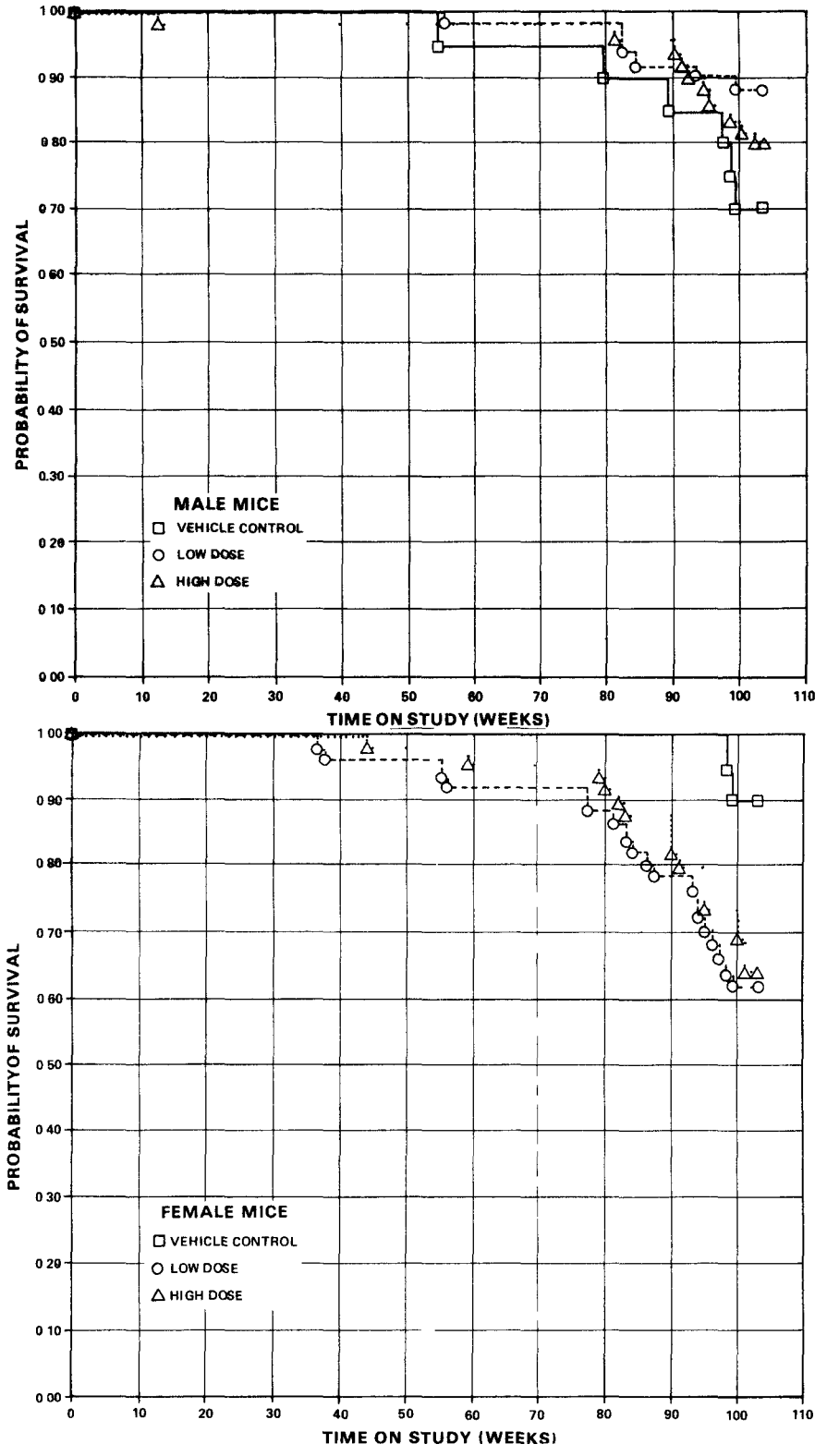


Figure 4. Survival Curves for Mice Administered Trimethylphosphate by Gavage

controls) lived to the end of the study, providing adequate numbers of animals of each sex 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.

A high incidence of endometrial adenocarcinomas and a few other types of malignant uterine tumors occurred only in the female mice administered trimethylphosphate, as shown in the following tabulation:

	Female Mice		
	Matched Control	Low Dose	High Dose
Number of animals with tissues examined microscopically	(16)	(40)	(37)
Uterus/Endometrium			
Adenocarcinoma, NOS*	0	7(18%)	13(35%)
Squamous-cell carcinoma			1(3%)
Uterus			
Leiomyosarcoma	0	1(3%)	0

*Not otherwise specified

Grossly, the uterine tumors were masses 1 cm to 2 cm in diameter, which were usually limited to one horn. Microscopically, the

majority of the tumors appeared to arise from the endometrium as irregular acinar structures with slit-like lumens that were composed of flat to low cuboidal hyperchromatic epithelial cells. The neoplastic glandular structures widely invaded the myometrium and often extended to the serosa. There was a scirrhous reaction in some areas in which tumors were involved. The remainder of the tumors appeared to arise from endometrial polypoid structures that contained columnar shaped cells with high nuclear/cytoplasmic ratios and numerous mitoses. A few of these formed papillary structures and had cystic areas. Overall, the uterine adenocarcinomas appeared to be highly malignant. There was vascular involvement and pulmonary metastases in one low-dose and four high-dose mice. The tumors appeared to be more aggressive in the high-dose animals, since metastases frequently occurred in those groups.

Spontaneous adenocarcinomas of the uterus are uncommon in mice. Their high incidence in this study is attributed to the administration of trimethylphosphate.

There were interstitial-cell tumors of the testes in two low-dose male mice, which consisted of sheets of basophilic, round to polygonal cells that separated and displaced seminiferous tubules. These tumors also do not occur commonly in mice.

There were a few malignant or unusual tumors that occurred in the dosed mice, which were not seen in the controls. In the male mice, these included a rhabdomyosarcoma (one low-dose), a gastric squamous-cell carcinoma (one high-dose), and an adenocarcinoma of the lacrimal gland (one high-dose). In the female mice, there was an osteosarcoma that metastasized to the lung and kidney (one low-dose), an oligodendroglioma of the brain (one low-dose), an ameloblastoma of the mandible (one high-dose), and an arrhenoblastoma (Sertoli-cell tumor) of the ovary (one high-dose). Although these represent an unusual array of tumors, little significance was attributed to them because of their apparent sporadic and low incidence. Other tumors observed included those of hepatocellular and hematopoietic origin that had equivalent frequencies in the control and dosed mice.

There were two nonneoplastic changes that appeared to be associated with the uterine tumors described above. One was hydronephrosis; four of the six mice with this lesion had endometrial adenocarcinomas, and one had a uterine leiomyosarcoma. Although involvement of the urinary tract was microscopically evident in only two cases, it is likely that obstruction by the tumor occurred in the other cases at some point along the urinary tract. The other change was extensive thrombosis of the pulmo-

nary arteries that occurred in three high-dose mice with pulmonary metastases of the endometrial adenocarcinomas.

There were several other nonneoplastic changes considered to be either spontaneous or intercurrent disease processes that are common in mice on long-term studies.

In the judgment of the pathologist, it was concluded that because of the high incidence and apparent dose response of endometrial adenocarcinomas in the dosed female mice, trimethylphosphate was clearly carcinogenic in female B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that 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 male mice, neither the result of the Cochran-Armitage test for positive dose-related trend in incidences of tumors nor the result of the Fisher exact test for direct comparison of incidences of tumors in dosed groups with those in the controls is significant.

In females, the result of the Cochran-Armitage test for the

incidence of adenocarcinoma of the uterus/endometrium is significant (P = 0.003) and the Fisher exact test shows that the incidence of the tumors in the high-dose group is significantly higher (P = 0.004) than that in the controls. No occurrence of such tumors has been reported in the laboratory historical controls, which consist of 100 female B6C3F1 mice. The statistical conclusion suggests that this incidence of tumors in female mice is related to the administration of the test chemical.

The incidence of lung tumors in the low-dose group of female mice is significantly lower (P = 0.023) than that in the controls (controls 3/20 [15%], low-dose 0/48). This significance in the negative direction cannot be accounted for by the fact that the survival in the low-dose group (62%) is lower than that in the controls (90%), since the high-dose group has a survival (59%) comparable to that of the low-dose group yet has an incidence of tumors (6/45 [13%]) that is not substantially different from that of the controls.

V. DISCUSSION

Under the conditions of this bioassay, mean body weights of the dosed male and female rats and of the dosed female mice were slightly lower than those of corresponding vehicle controls, with dose-related depression in the weights, while those of the dosed male mice were essentially unaffected. Survival of all groups of animals was high, and adequate numbers of both rats and mice were at risk for development of late-developing tumors.

In the male rats, the incidence of fibromas of the subcutanous tissue was significantly higher (P = 0.036) in the high-dose group than in the vehicle controls (control 0/20, low-dose 2/50, high-dose 9/49), and there was a significant dose-related trend (P = 0.006) in the incidences of these fibromas. In the female rats, no tumors occurred in the dosed groups at significantly increased incidences.

In the male mice, no tumors were observed in the dosed groups at significant incidences, compared with the vehicle controls. In the female mice, the incidence of adenocarcinomas of the endometrium was significantly higher (P = 0.004) in the high-dose group than in the vehicle controls (controls 0/16, low-dose 7/40, high-dose 13/37), and there was a significant dose-related trend (P = 0.003) in the incidences of these adenocarcinomas. None of

these tumors occurred among the 100 historical-control female B6C3F1 mice at this laboratory. In addition, a squamous-cell carcinoma occurred in the uterus of a high-dose female mouse and a leimyosarcoma in the uterus of a low-dose female mouse.

No significant gonadal effects were found in either rats or mice.

No long-term toxicity studies with trimethylphosphate have been reported. In subacute experiments with rabbits, Deichmann and Witherup (1946) observed tremors and a flaccid paralysis after 6 days of oral gavage at 300 mg/kg body weight.

It is concluded that under the conditions of this bioassay, trimethylphosphate was carcinogenic in female B6C3F1 mice, inducing adenocarcinomas of the uterus/endometrium. Trimethylphosphate was associated with the induction of benign fibromas of the subcutaneous tissue in male Fischer 344 rats. No evidence of carcinogenicity of the compound was obtained in female rats or in male mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED TRIMETHYLPHOSPHATE BY GAVAGE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED TRIMETHYLPHOSPHATE BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	@50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
SEBACEOUS ADENOMA		1 (2%)	
FIBROMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(49)
NEOPLASM, NOS			1 (2%)
FIBROMA		2 (4%)	9 (18%)
LIPOMA		1 (2%)	
LEIOMYOSARCOMA		1 (2%)	
HEMANGIOSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(46)
ALVEOLAR/BRONCHIOLAR ADENOMA			4 (9%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	1 (2%)
LEIOMYOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
LEUKEMIA,NOS	3 (15%)	4 (8%)	8 (16%)
UNDIFFERENTIATED LEUKEMIA	5 (25%)	15 (30%)	15 (31%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
#LYMPH NODE	(20)	(46)	(46)
OSTEOSARCOMA, METASTATIC		1 (2%)	
#LUNG	(19)	(49)	(46)
LEUKEMIA,NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A FEMALE IN A MALE GROUP.

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(48)
NEOPLASTIC NODULE		1 (2%)	1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
#COLON	(12)	(41)	(43)
ADENOCARCINOMA, NOS		1 (2%)	
*RECTUM	(20)	(50)	(49)
ADENOCARCINOMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
ADENOMA, NOS		1 (2%)	
TUBULAR-CELL ADENOCARCINOMA			1 (2%)
LIPOSARCOMA			1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
#URINARY BLADDER	(11)	(36)	(35)
TRANSITIONAL-CELL CARCINOMA		1 (3%)	
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(44)	(38)
CHROMOPHOBE ADENOMA	4 (25%)	13 (30%)	8 (21%)
#ADRENAL	(20)	(48)	(47)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA	1 (5%)	4 (8%)	7 (15%)
#THYROID	(19)	(45)	(46)
FOLLICULAR-CELL CARCINOMA			2 (4%)
C-CELL ADENOMA	1 (5%)	3 (7%)	
C-CELL CARCINOMA			2 (4%)
#PANCREATIC ISLETS	(18)	(44)	(45)
ISLET-CELL ADENOMA	1 (6%)	1 (2%)	1 (2%)
ISLET-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
FIBROADENOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*PREPUTIAL GLAND	(20)	(50)	(49)
ADENOMA, NOS	1 (5%)		
#TESTIS	(16)	(46)	(46)
INTERSTITIAL-CELL TUMOR	11 (69%)	33 (72%)	25 (54%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(49)
OSTEOSARCOMA, UNC PRIM OR META		1 (2%)	
*TUNICA VAGINALIS	(20)	(50)	(49)
MESOTHELIOMA, NOS		2 (4%)	1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	11	9
MORIBUND SACRIFICE	8	11	23
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	28	17
ANIMAL MISSING			
ANIMAL DELETED(WRONG SEX)			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	48	43
TOTAL PRIMARY TUMORS	28	91	92
TOTAL ANIMALS WITH BENIGN TUMORS	15	41	33
TOTAL BENIGN TUMORS	19	61	55
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	24	29
TOTAL MALIGNANT TUMORS	9	26	34
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		4	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		3	3
TOTAL UNCERTAIN TUMORS		3	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC		1	
TOTAL UNCERTAIN TUMORS		1	

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED TRIMETHYLPHOSPHATE BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	@50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
KERATOACANTHOMA			1 (2%)
MYXOSARCOMA			2 (4%)
LIPOMA	1 (5%)		
OSTEOSARCOMA			1 (2%)
NEUROFIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(45)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
FIBROSARCOMA, INVASIVE			1 (2%)
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
#BRAIN	(20)	(50)	(48)
MALIGNANT RETICULOSIS		1 (2%)	
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
LEUKEMIA,NOS	2 (10%)	4 (8%)	1 (2%)
UNDIFFERENTIATED LEUKEMIA	1 (5%)	9 (18%)	11 (22%)
#SPLEEN	(20)	(49)	(46)
SARCOMA, NOS	1 (5%)		
CIRCULATORY SYSTEM			
NONE			

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
#URINARY BLADDER	(17)	(32)	(31)
TRANSITIONAL-CELL CARCINOMA	1 (6%)		
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(48)	(41)
CHROMOPHOBE ADENOMA	9 (45%)	21 (44%)	18 (44%)
#ADRENAL	(19)	(48)	(48)
COPTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA		2 (4%)	1 (2%)
#THYROID	(19)	(47)	(43)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA		6 (13%)	3 (7%)
C-CELL CARCINOMA	2 (11%)		2 (5%)
#PARATHYROID	(10)	(28)	(23)
CHIEF-CELL ADENOMA		1 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
ADENOCARCINOMA, NOS	1 (5%)		
FIBROADENOMA	2 (10%)	3 (6%)	5 (10%)
#UTERUS	(20)	(45)	(44)
ENDOMETRIAL STROMAL POLYP	2 (10%)	1 (2%)	
#CERVIX UTERI	(20)	(45)	(44)
LEIOMYOSARCOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
GLIOBLASTOMA MULTIFORME			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(50)	(49)
SEBACEOUS ADENOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
*STERNUM	(20)	(50)	(49)
FIBROSARCOMA			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	9	10
MORIBUND SACRIFICE	5	5	12
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	36	27
ANIMAL MISSING			
ANIMAL DELETED(WRONG SEX)			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	40	42
TOTAL PRIMARY TUMORS	22	51	54
TOTAL ANIMALS WITH BENIGN TUMORS	12	28	29
TOTAL BENIGN TUMORS	14	34	33
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	17	19
TOTAL MALIGNANT TUMORS	8	17	21
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	2
TOTAL SECONDARY TUMORS		1	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 TRIMETHYLPHOSPHATE BY GAVAGE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED TRIMETHYLPHOSPHATE BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	49
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
RHABDOMYOSARCOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(49)
HEMANGIOSARCOMA			1 (2%)
ANGIOSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
HEPATOCELLULAR CARCINOMA, METAST		3 (6%)	2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	3 (6%)	6 (12%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (10%)	8 (16%)	3 (6%)
SARCOMA, NOS, METASTATIC			1 (2%)
ANGIOSARCOMA, METASTATIC	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	2 (4%)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	1 (2%)
#SPLEEN	(18)	(45)	(44)
HEMANGIOMA	1 (6%)		
#MEDIASTINAL L.NODE	(18)	(45)	(41)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
#MESENTERIC L. NODE	(18)	(45)	(41)
MALIGNANT LYMPHOMA, NOS	1 (6%)		

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

TABLE B1. MALE MICE : NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	1 (2%)
#SMALL INTESTINE	(20)	(49)	(45)
MALIGNANT LYMPHOMA, NOS			2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (5%)		
#COLON	(20)	(48)	(47)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(49)
ADENOCARCINOMA, NOS			1 (2%)
HEPATOCELLULAR ADENOMA		1 (2%)	
HEPATOCELLULAR CARCINOMA	4 (20%)	9 (19%)	8 (16%)
#STOMACH	(18)	(50)	(46)
SQUAMOUS CELL CARCINOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(48)	(43)
PHEOCHROMOCYTOMA	1 (6%)		
#THYROID	(9)	(35)	(39)
FOLLICULAR-CELL ADENOMA	1 (11%)	1 (3%)	
#PANCREATIC ISLETS	(20)	(46)	(42)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(20)	(45)	(47)
INTERSTITIAL-CELL TUMOR		2 (4%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(50)	(49)
ADENOCARCINOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(49)
SARCOMA, NOS			1 (2%)
LIPOMA			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	49
NATURAL DEATH@	5	3	9
MORIBUND SACRIFICE	1	3	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	44	39
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE : NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	26	26
TOTAL PRIMARY TUMORS	14	31	32
TOTAL ANIMALS WITH BENIGN TUMORS	4	8	7
TOTAL BENIGN TUMORS	4	8	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	20	22
TOTAL MALIGNANT TUMORS	10	23	25
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	3	3
TOTAL SECONDARY TUMORS	1	4	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED TRIMETHYLPHOSPHATE BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	@50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(47)
SARCOMA, NOS	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(45)
NEOPLASM, NOS, UNC PRIM OR META		1 (2%)	
CARCINOMA, NOS, UNC PRIM OR META	1 (5%)		
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
TRANSITIONAL-CELL CARCINOMA, MET	1 (5%)		
ADENOCARCINOMA, NOS, METASTATIC			4 (9%)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)		5 (11%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		1 (2%)
SARCOMA, NOS, METASTATIC	1 (5%)		
LEIOMYOSARCOMA, METASTATIC		1 (2%)	
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(47)
MALIGNANT LYMPHOMA, NOS	1 (5%)	3 (6%)	2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (5%)	4 (8%)	2 (4%)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	2 (4%)
LYMPHOCYTIC LEUKEMIA	1 (5%)	1 (2%)	2 (4%)
GRANULOCYTIC SARCOMA		1 (2%)	
#SPLEEN	(17)	(48)	(44)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#MANDIBULAR L. NODE	(17)	(46)	(43)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#LYMPH NODE OF THORAX	(17)	(46)	(43)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
#MEDIASTINAL L.NODE	(17)	(46)	(43)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#MESENTERIC L. NODE	(17)	(46)	(43)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	1 (2%)
#RENAL LYMPH NODE	(17)	(46)	(43)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
#LIVER	(20)	(50)	(44)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (5%)		
#KIDNEY	(19)	(50)	(44)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
#THYMUS	(2)	(1)	(1)
MALIGNANT LYMPHOMA, NOS			1 (100%)
CIRCULATORY SYSTEM			
*LYMPHATICS OF UTERUS	(20)	(50)	(47)
SQUAMOUS CELL CARCINOMA, INVASIV			1 (2%)
ADENOCARCINOMA, NOS, INVASIVE			1 (2%)
*PULMONARY ARTERY	(20)	(50)	(47)
ADENOCARCINOMA, NOS, INVASIVE			2 (4%)
*UTERINE VEIN	(20)	(50)	(47)
SQUAMOUS CELL CARCINOMA, INVASIV			1 (2%)
ADENOCARCINOMA, NOS, INVASIVE		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(44)
HEPATOCELLULAR ADENOMA	1 (5%)	2 (4%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA	1 (5%)	2 (4%)	
HEMANGIOSARCOMA			1 (2%)
#PANCREAS	(20)	(48)	(42)
ADENOCARCINOMA, NOS, INVASIVE			2 (5%)
#STOMACH	(17)	(46)	(47)
ADENOMATOUS POLYP, NOS	1 (6%)		
URINARY SYSTEM			
#KIDNEY	(19)	(50)	(44)
TRANSITIONAL-CELL CARCINOMA	1 (5%)		
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
#URINARY BLADDER	(13)	(27)	(25)
ADENOCARCINOMA, NOS, INVASIVE			1 (4%)
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(46)	(39)
ADENOCARCINOMA, NOS, INVASIVE			1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(16)	(40)	(37)
TRANSITIONAL-CELL CARCINOMA, MET	1 (6%)		
LEIOMYOSARCOMA		1 (3%)	
ENDOMETRIAL STROMAL POLYP		2 (5%)	1 (3%)
#UTERUS/ENDOMETRIUM	(16)	(40)	(37)
SQUAMOUS CELL CARCINOMA			1 (3%)
ADENOCARCINOMA, NOS		7 (18%)	13 (35%)
#OVARY	(10)	(26)	(28)
SERTOLI-CELL TUMOR			1 (4%)
HEMANGIOMA			1 (4%)
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(46)
OLIGODENDROGLIOMA		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(20)	(50)	(47)
OSTEOSARCOMA		1 (2%)	
*MANDIBLE	(20)	(50)	(47)
AMELOBLASTOMA			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(47)
LIPOMA		1 (2%)	
*PERITONEUM	(20)	(50)	(47)
ADENOCARCINOMA, NOS, INVASIVE			1 (2%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
SQUAMOUS CELL CARCINOMA, METASTA			1
ADENOCARCINOMA, NOS, INVASIVE			2
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	14	15
MORIBUND SACRIFICE		5	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	18	31	29
ANIMAL MISSING			2
ANIMAL DELETED(WRONG SEX)			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	28	30
TOTAL PRIMARY TUMORS	14	33	36
TOTAL ANIMALS WITH BENIGN TUMORS	4	5	9
TOTAL BENIGN TUMORS	4	5	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	25	26
TOTAL MALIGNANT TUMORS	9	27	27
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	4	11
TOTAL SECONDARY TUMORS	3	5	22
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC	1	1	
TOTAL UNCERTAIN TUMORS	1	1	

* 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 TRIMETHYLPHOSPHATE BY GAVAGE

TABLE C1.

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

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	@50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(49)
DERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, GRANULOMATOUS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(46)
COLLAPSE			1 (2%)
CONGESTION, NOS		1 (2%)	1 (2%)
EDEMA, NOS			1 (2%)
HEMORRHAGE	1 (5%)	1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	8 (42%)	21 (43%)	13 (28%)
HYPERPLASIA, ADENOMATOUS		1 (2%)	4 (9%)
METAPLASIA, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(42)	(43)
HYPOPLASIA, NOS			1 (2%)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	3 (7%)
#SPLEEN	(20)	(47)	(47)
CONGESTION, NOS		1 (2%)	1 (2%)
HEMORRHAGE		1 (2%)	
FIBROSIS			2 (4%)
SCLEROSIS			1 (2%)
INFARCT, HEALED			1 (2%)
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HEMATOPOIESIS			1 (2%)
#MANDIBULAR L. NODE	(20)	(46)	(46)
INFLAMMATION, ACUTE		1 (2%)	

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A FEMALE IN A MALE GROUP.

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, NOS			1 (2%)
#MESENTERIC L. NODE	(20)	(46)	(46)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, PLASMA CELL		1 (2%)	
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(20)	(49)	(46)
THROMBOSIS, NOS		3 (6%)	2 (4%)
ATHEROSCLEROSIS			1 (2%)
#MYOCARDIUM	(20)	(49)	(46)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
FIBROSIS	11 (55%)	29 (59%)	27 (59%)
FIBROSIS, DIFFUSE		2 (4%)	1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(43)	(46)
HEMORRHAGIC CYST			1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
GRANULATION, TISSUE			1 (2%)
ATROPHY, DIFFUSE		1 (2%)	
#SALIVARY MUCOUS GLAN	(20)	(43)	(46)
NUCLEAR-SHAPE ALTERATION			1 (2%)
#LIVER	(20)	(48)	(48)
DEGENERATION, NOS	1 (5%)		1 (2%)
DEGENERATION, HYDROPIC		1 (2%)	
METAMORPHOSIS FATTY	2 (10%)	4 (8%)	2 (4%)
BASOPHILIC CYTO CHANGE	3 (15%)	2 (4%)	
CLEAR-CELL CHANGE	1 (5%)		
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, FOCAL	2 (10%)	3 (6%)	3 (6%)
#LIVER/CENTRILOBULAR	(20)	(48)	(48)
NECROSIS, NOS			1 (2%)
*BILE DUCT	(20)	(50)	(49)
HYPERPLASIA, NOS	7 (35%)	20 (40%)	21 (43%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#PANCREAS	(18)	(44)	(45)
FIBROSIS, DIFFUSE	1 (6%)		
ATROPHY, FOCAL		3 (7%)	3 (7%)
#PANCREATIC ACINUS	(18)	(44)	(45)
ATROPHY, NOS		1 (2%)	
#STOMACH	(15)	(45)	(47)
ULCER, NOS			1 (2%)
#LARGE INTESTINE	(12)	(41)	(43)
NEMATODIASIS	3 (25%)		
#COLON	(12)	(41)	(43)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
RUPTURE, INFLAMMATORY			1 (2%)
PARASITISM		6 (15%)	11 (26%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
GLOMERULONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	13 (65%)	33 (67%)	38 (79%)
FIBROSIS, DIFFUSE		1 (2%)	
NEPHROPATHY, TOXIC	1 (5%)		2 (4%)
#KIDNEY/GLOMERULUS	(20)	(49)	(48)
DEGENERATION, HYALINE		1 (2%)	
#KIDNEY/TUBULE	(20)	(49)	(48)
DILATATION, NOS		1 (2%)	
NECROSIS, NOS		1 (2%)	1 (2%)
#URINARY BLADDER	(11)	(36)	(35)
DILATATION, NOS			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(44)	(38)
CYST, NOS	3 (19%)		
HEMORRHAGIC CYST		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANGIECTASIS			1 (3%)
#ADRENAL	(20)	(48)	(47)
CYST, NOS			1 (2%)
THROMBOSIS, NOS			1 (2%)
GRANULOMA, NOS		1 (2%)	
LIPOIDOSIS			1 (2%)
CALCIFICATION, NOS		1 (2%)	
#ADRENAL CORTEX	(20)	(48)	(47)
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(19)	(45)	(46)
HYPERPLASIA, C-CELL		5 (11%)	1 (2%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
DILATATION/DUCTS	1 (5%)		
*SEMINAL VESICLE	(20)	(50)	(49)
CALCULUS, NOS		1 (2%)	
INFLAMMATION, ACUTE	1 (5%)		
INFLAMMATION, ACUTE FOCAL	1 (5%)		
#TESTIS	(16)	(46)	(46)
ATROPHY, NOS		1 (2%)	2 (4%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
MINERALIZATION	1 (5%)		
HEMORRHAGE		1 (2%)	
#BRAIN STEM	(20)	(50)	(48)
HEMORRHAGE		1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(49)
NECROSIS, FAT	1 (5%)		3 (6%)
*MESENTERY	(20)	(50)	(49)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, GRANULOMATOUS		1	
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	1
AUTO/NECROPSY/HISTO PERF			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 TRIMETHYLPHOSPHATE BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	@50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(45)
COLLAPSE			1 (2%)
ATELECTASIS			1 (2%)
CONGESTION, NOS	1 (5%)	5 (10%)	3 (7%)
HEMORRHAGE		4 (8%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	10 (50%)	19 (38%)	16 (36%)
PERIVASCULAR CUFFING		1 (2%)	
FOAM-CELL	1 (5%)	1 (2%)	1 (2%)
HYPERPLASIA, ADENOMATOUS	2 (10%)	1 (2%)	4 (9%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(18)	(42)	(37)
HYPERPLASIA, HEMATOPOIETIC			2 (5%)
#SPLEEN	(20)	(49)	(46)
CONGESTION, NOS		1 (2%)	
CONGESTION, CHRONIC		1 (2%)	
HEMOSIDEROSIS		3 (6%)	3 (7%)
HEMATOPOIESIS			3 (7%)
#MESENTERIC L. NODE	(19)	(46)	(46)
MASTOCYTOSIS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(48)	(47)
HYPERPLASIA, FOCAL			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(20)	(48)	(47)
INFLAMMATION, FOCAL			1 (2%)
FIBROSIS	11 (55%)	23 (48%)	22 (47%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(46)	(43)
DILATATION/DUCTS			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
ATROPHY, FOCAL			1 (2%)
#LIVER	(20)	(48)	(46)
GRANULOMA, NOS			1 (2%)
DEGENERATION, NOS		1 (2%)	
BASOPHILIC CYTO CHANGE	9 (45%)	11 (23%)	11 (24%)
EOSINOPHILIC CYTO CHANGE		1 (2%)	
CLEAR-CELL CHANGE		1 (2%)	
CYTOLOGIC DEGENERATION		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL	3 (15%)	7 (15%)	5 (11%)
*BILE DUCT	(20)	(50)	(49)
HYPERPLASIA, NOS	4 (20%)	8 (16%)	8 (16%)
#PANCREAS	(20)	(48)	(46)
FIBROSIS, DIFFUSE			2 (4%)
ATROPHY, NOS		1 (2%)	1 (2%)
ATROPHY, FOCAL	2 (10%)	2 (4%)	2 (4%)
#PANCREATIC ACINUS	(20)	(48)	(46)
ATROPHY, NOS			1 (2%)
ATROPHY, DIFFUSE			1 (2%)
#ILEUM	(17)	(44)	(43)
HYPERPLASIA, LYMPHOID		1 (2%)	
#LARGE INTESTINE	(16)	(44)	(43)
NEMATODIASIS		4 (9%)	
#COLON	(16)	(44)	(43)
PARASITISM	2 (13%)		6 (14%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(48)
HEMORRHAGIC CYST		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
PYELONEPHRITIS, NOS		1 (2%)	
INFLAMMATICN, CHRONIC	14 (70%)	28 (56%)	28 (58%)
NEPHROSIS, CHOLEMIC		1 (2%)	
#KIDNEY/TUBULE	(20)	(50)	(48)
NECROSIS, NOS	1 (5%)		1 (2%)
NECROSIS, FOCAL		1 (2%)	
#URINARY BLADDER	(17)	(32)	(31)
INFLAMMATICN, NOS	1 (6%)		
METAPLASIA, SQUAMOUS	1 (6%)		
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(48)	(41)
CYST, NOS	1 (5%)	4 (8%)	5 (12%)
HEMORRHAGIC CYST		3 (6%)	
ANGIECTASIS	1 (5%)		
#ADRENAL	(19)	(48)	(48)
LIPOIDOSIS			3 (6%)
ANGIECTASIS		1 (2%)	2 (4%)
#ADRENAL CORTEX	(19)	(48)	(48)
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(19)	(47)	(43)
HYPERPLASIA, C-CELL		2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
DILATATICN/DUCTS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
#UTERUS	(20)	(45)	(44)
HEMORRHAGE		1 (2%)	1 (2%)
HYPERPLASIA, STROMAL	1 (5%)		
#UTERUS/ENDOMETRIUM	(20)	(45)	(44)
INFLAMMATICN, ACUTE			1 (2%)
HYPERPLASIA, NOS	2 (10%)	2 (4%)	2 (5%)
HYPERPLASIA, CYSTIC			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#OVARY	(20)	(45)	(43)
FOLLICULAR CYST, NOS	1 (5%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
MINERALIZATION			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(49)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	1
AUTO/NECROPSY/NO HISTO			1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED TRIMETHYLPHOSPHATE BY GAVAGE

TABLE D1.

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

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	49
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
HEMORRHAGIC CYST			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, ACUTE			1 (2%)
PNEUMONIA, CHRONIC MURINE			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU	1 (5%)	1 (2%)	1 (2%)
PERIVASCULAR CUFFING			1 (2%)
CRYSTALS, NOS	1 (5%)		
HYPERPLASIA, ADENOMATOUS		3 (6%)	
METAPLASIA, OSSEOUS			1 (2%)
HISTIOCYTOSIS			2 (4%)
#LUNG/ALVEOLI	(20)	(50)	(49)
HISTIOCYTOSIS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(45)	(44)
HYPERPLASIA, LYMPHOID	1 (6%)		
HEMATOPOIESIS	1 (6%)	1 (2%)	
#LYMPH NODE	(18)	(45)	(41)
HEMORRHAGE		1 (2%)	
#MANDIBULAR L. NODE	(18)	(45)	(41)
PLASMACYTOSIS			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(18)	(45)	(41)
HEMORRHAGE		2 (4%)	2 (5%)
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS	1 (6%)	1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(48)	(47)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
FIBROSIS	1 (5%)		
FIBROSIS, FOCAL			2 (4%)
CALCIFICATION, FOCAL			1 (2%)
*ARTERY	(20)	(50)	(49)
INFLAMMATION, CHRONIC			1 (2%)
MEDIAL CALCIFICATION		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(49)
CYST, NOS	1 (5%)		1 (2%)
INFLAMMATION, CHRONIC FOCAL		3 (6%)	1 (2%)
DEGENERATION, HYDROPIC			1 (2%)
NECROSIS, FOCAL	1 (5%)		1 (2%)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
HYPERPLASIA, FOCAL	1 (5%)		
ANGIECTASIS	1 (5%)		
*BILE DUCT	(20)	(50)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
#PANCREAS	(20)	(46)	(42)
DILATATION/DUCTS		1 (2%)	
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
#PANCREATIC ACINUS	(20)	(46)	(42)
ATROPHY, NOS		2 (4%)	
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(18)	(50)	(46)
INFLAMMATION, ACUTE			1 (2%)
#PEYERS PATCH	(20)	(49)	(45)
HYPERPLASIA, NOS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#COLON	(20)	(48)	(47)
NEMATODIASIS		1 (2%)	
PARASITISM	3 (15%)	12 (25%)	21 (45%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
INFLAMMATION, CHRONIC	1 (5%)		
NEPHROPATHY, TOXIC			1 (2%)
LYMPHOCYTOSIS			1 (2%)
#KIDNEY/GLOMERULUS	(20)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
#KIDNEY/TUBULE	(20)	(49)	(49)
ATROPHY, FOCAL			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(9)	(35)	(39)
FOLLICULAR CYST, NOS		1 (3%)	
HYPERPLASIA, ADENOMATOUS			1 (3%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(48)
CALCIFICATION, FOCAL	9 (45%)	15 (31%)	15 (31%)
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)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC	1	1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	9	7

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

TABLE D2.

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

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	@50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(48)	(45)
CRYSTALS, NOS	1 (5%)		1 (2%)
#LUNG	(20)	(48)	(45)
THROMBOSIS, NOS			1 (2%)
PNEUMONIA, CHRONIC MURINE	6 (30%)	13 (27%)	6 (13%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	1 (2%)
INFARCT, NOS			1 (2%)
HYPERPLASIA, ADENOMATOUS			2 (4%)
HISTIOCYTOSIS			2 (4%)
LEUKEMOID REACTION			2 (4%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(17)	(48)	(44)
HYPERPLASIA, RETICULUM CELL		2 (4%)	1 (2%)
HYPERPLASIA, LYMPHOID	1 (6%)	1 (2%)	1 (2%)
HEMATOPOIESIS		5 (10%)	3 (7%)
#MESENTERIC L. NODE	(17)	(46)	(43)
HEMORRHAGE		1 (2%)	
INFLAMMATION, GRANULOMATOUS			1 (2%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
CIRCULATORY SYSTEM			
*BLOOD VESSEL	(20)	(50)	(47)
INFLAMMATION, CHRONIC		1 (2%)	

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*PULMONARY ARTERY	(20)	(50)	(47)
THROMBOSIS, NOS			2 (4%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(44)
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)	1 (2%)	1 (2%)
INFLAMMATION, GRANULOMATOUS	1 (5%)		
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
PERIVASCULAR CUFFING			1 (2%)
DEGENERATION, HYDROPIC		1 (2%)	
NECROSIS, FOCAL	1 (5%)	1 (2%)	1 (2%)
METAMORPHOSIS FATTY	1 (5%)		
CYTOPLASMIC VACUOLIZATION			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
HEMATOPOIESIS		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(50)	(44)
NECROSIS, NOS		1 (2%)	
#LIVER/PERIPORTAL	(20)	(50)	(44)
INFLAMMATION, CHRONIC	1 (5%)		
*BILE DUCT	(20)	(50)	(47)
DILATATION, NOS		1 (2%)	
INFLAMMATION, CHRONIC	1 (5%)	2 (4%)	
#PANCREAS	(20)	(48)	(42)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#PANCREATIC ACINUS	(20)	(48)	(42)
ATROPHY, NOS		2 (4%)	2 (5%)
ATROPHY, FOCAL			1 (2%)
#STOMACH	(17)	(46)	(47)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
MASTOCYTOSIS		1 (2%)	
#SMALL INTESTINE	(19)	(48)	(43)
INFLAMMATION, GRANULOMATOUS	1 (5%)		
AMYLOIDOSIS			1 (2%)
#PEYERS PATCH	(19)	(48)	(43)
HYPERPLASIA, NOS		1 (2%)	1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#COLON	(19)	(49)	(47)
PARASITISM	2 (11%)	11 (22%)	9 (19%)
URINARY SYSTEM			
#KIDNEY	(19)	(50)	(44)
HYDRONEPHROSIS		3 (6%)	4 (9%)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
PERIVASCULAR CUFFING	1 (5%)		2 (5%)
NEPHROPATHY, TOXIC		1 (2%)	
#KIDNEY/TUBULE	(19)	(50)	(44)
CALCIFICATION, NOS		1 (2%)	
HEMOSIDEROSIS			1 (2%)
#URINARY BLADDER	(13)	(27)	(25)
HEMORRHAGIC CYST		1 (4%)	
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(46)	(39)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(16)	(40)	(37)
HYDROMETRA		1 (3%)	
INFLAMMATION, SUPPURATIVE	1 (6%)		1 (3%)
PYOMETRA		1 (3%)	
ABSCESS, NOS		1 (3%)	
#UTERUS/ENDOMETRIUM	(16)	(40)	(37)
CYST, NOS		4 (10%)	
MULTIPLE CYSTS			1 (3%)
HYPERPLASIA, CYSTIC		2 (5%)	
#UTERUS/MYOMETRIUM	(16)	(40)	(37)
INFLAMMATION, CHRONIC FOCAL		1 (3%)	
#OVARY	(10)	(26)	(28)
CYST, NOS		4 (15%)	3 (11%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(46)
HEMORRHAGE		1 (2%)	
CALCIFICATION, FOCAL	6 (32%)	8 (16%)	8 (17%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(20)	(50)	(47)
FIBROUS OSTEODYSTROPHY	11 (55%)	19 (38%)	11 (23%)
OSTEOSCLEROSIS	1 (5%)	1 (2%)	1 (2%)
*SKELETAL MUSCLE	(20)	(50)	(47)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC FOCAL	2 (10%)		
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(47)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
*MESENTERY	(20)	(50)	(47)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	3
ANIMAL MISSING/NO NECROPSY			2

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED TRIMETHYLPHOSPHATE BY GAVAGE

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

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b]	0/20 (0)	2/50 (4)	9/49 (18)
P Values[c,d]	P = 0.006	N.S.	P = 0.036
Relative Risk (Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.123	1.119
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	67	91
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/19 (0)	2/49 (4)	5/46 (11)
P Values[c,d]	N.S	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.119	0.545
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	84

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Leukemia or Lymphoma[b]	8/20 (40)	20/50 (40)	25/49 (51)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.000	1.276
Lower Limit		0.529	0.703
Upper Limit		2.242	2.746
Weeks to First Observed Tumor	81	79	82
Pituitary: Chromophobe Adenoma[b]	4/16 (25)	13/44 (30)	8/38 (21)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.182	0.842
Lower Limit		0.450	0.276
Upper Limit		4.430	3.406
Weeks to First Observed Tumor	83	79	92

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Adrenal: Pheochromocytoma[b]	1/20 (5)	4/48 (8)	7/47 (15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.667	2.979
Lower Limit		0.182	0.429
Upper Limit		80.315	131.059
Weeks to First Observed Tumor	105	93	97
Thyroid: C-cell Adenoma or Carcinoma[b]	1/19 (5)	3/45 (7)	2/46 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.267	0.826
Lower Limit		0.112	0.047
Upper Limit		64.997	47.694
Weeks to First Observed Tumor	105	67	97

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	11/16 (69)	33/46 (72)	25/46 (54)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.043	0.791
Lower Limit		0.742	0.544
Upper Limit		1.722	1.393
Weeks to First Observed Tumor	97	74	78

[a]Dosed groups received 50 or 100 mg/kg.

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

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

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

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

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

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

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/20 (0)	0/50 (0)	3/45 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		--	Infinite
Lower Limit		--	0.278
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	105
Hematopoietic System: Lymphoma or Leukemia[b]	3/20 (15)	14/50 (28)	12/49 (24)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.867	1.633
Lower Limit		0.609	0.513
Upper Limit		9.359	8.342
Weeks to First Observed Tumor	98	86	59

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	9/20 (45)	21/48 (44)	18/41 (44)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.972	0.976
Lower Limit		0.544	0.532
Upper Limit		2.025	2.049
Weeks to First Observed Tumor	98	86	93
Thyroid: C-cell Carcinoma[b]	2/19 (11)	0/47 (0)	2/43 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.044		
Relative Risk (Vehicle Control)[f]		0.000	0.442
Lower Limit		0.000	0.035
Upper Limit		1.357	5.796
Weeks to First Observed Tumor	97	--	105

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	2/19 (11)	6/47 (13)	5/43 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.213	1.105
Lower Limit		0.247	0.205
Upper Limit		11.660	10.982
Weeks to First Observed Tumor	97	99	105
Mammary Gland: Fibroadenoma[b]	2/20 (10)	3/50 (6)	5/49 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.600	1.020
Lower Limit		0.076	0.188
Upper Limit		6.860	10.204
Weeks to First Observed Tumor	97	101	96

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	2/20 (10)	1/45 (2)	0/44 (0)
P Values[c,d]	P = 0.043 (N)	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.222	0.000
Lower Limit		0.004	0.000
Upper Limit		4.077	1.524
Weeks to First Observed Tumor	105	105	--

[a]Dosed groups received 50 or 100 mg/kg.

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

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

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

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED TRIMETHYLPHOSPHATE BY GAVAGE

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

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	2/20 (10)	8/50 (16)	3/49 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.600	0.612
Lower Limit		0.364	0.078
Upper Limit		14.699	6.996
Weeks to First Observed Tumor	103	103	81
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	3/20 (15)	11/50 (22)	9/49 (18)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.467	1.224
Lower Limit		0.450	0.354
Upper Limit		7.594	6.533
Weeks to First Observed Tumor	103	103	81

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	3/20 (15)	5/50 (10)	9/49 (18)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.667	1.224
Lower Limit		0.147	0.354
Upper Limit		4.014	6.533
Weeks to First Observed Tumor	97	82	90
Liver: Hepatocellular Carcinoma[b]	4/20 (20)	9/48 (19)	8/49 (16)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.938	0.816
Lower Limit		0.307	0.255
Upper Limit		3.804	3.392
Weeks to First Observed Tumor	89	103	98

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	4/20 (20)	10/48 (21)	8/49 (16)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.402	0.816
Lower Limit		0.353	0.255
Upper Limit		4.148	3.392
Weeks to First Observed Tumor	89	103	98

[a]Dosed groups received 250 or 500 mg/kg.

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

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

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

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

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

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

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	3/20 (15)	0/48 (0)	6/45 (13)
P Values[c,d]	N.S.	P = 0.023 (N)	N.S.
Departure from Linear Trend[e]	P = 0.008		
Relative Risk (Vehicle Control)[f]		0.000	0.889
Lower Limit		0.000	0.218
Upper Limit		0.686	5.104
Weeks to First Observed Tumor	99	--	80
Hematopoietic System: Lymphoma or Leukemia[b]	5/20 (25)	14/50 (28)	11/47 (23)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		1.120	0.936
Lower Limit		0.457	0.358
Upper Limit		3.556	3.080
Weeks to First Observed Tumor	99	36	59

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	2/20 (10)	4/50 (8)	0/44 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.800	0.000
Lower Limit		0.128	0.000
Upper Limit		8.436	1.524
Weeks to First Observed Tumor	103	86	--
Uterus/Endometrium: Adenocarcinoma, NOS[b]	0/16 (0)	7/40 (18)	13/37 (35)
P Values[c,d]	P = 0.003	N.S.	P = 0.004
Relative Risk (Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.825	1.850
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	94	83

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

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	0/16 (0)	2/40 (5)	1/37 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		Infinite	Infinite
Lower Limit		0.125	0.024
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	93	103

[a]Dosed groups received 250 or 500 mg/kg.

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

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

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

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

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

March 6, 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 Trimethylphosphate for carcinogenicity.

The primary reviewer noted that Trimethylphosphate is used as a gas additive and in plastics as a flame retardant. After a brief description of the conditions of test, he said that the compound was associated with a higher incidence of fibromas and other tumors randomly distributed in this treatment group. In the treated female mice, Trimethylphosphate induced a statistically significant incidence of adenocarcinomas of the uterus/endometrium. Testicular interstitial-cell tumors were found in two low dose treated mice. The primary reviewer opined that the effects on the reproductive organs may be associated with the sterilent property of the chemical. In summary, he said that he agreed with the conclusion in the report that Trimethylphosphate was carcinogenic in at least the female mice.

The secondary reviewer said that there was a clear dose-response in the incidence of uterine/endometrium adenocarcinomas in treated female mice. After some discussion regarding the language of the conclusion in the report, it

was recommended that it be reworded to reflect the uncertainty regarding the carcinogenicity of Trimethylphosphate in the other treatment groups.

It was moved that the report be accepted as modified. The motion was seconded and approved unanimously.

Members present were

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold Brown, Mayo Clinic
Lawrence Garfinkel, American Cancer Society
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Sheldon Samuels, Industrial Union Department, AFL-CIO
Michael Shimkin, University of California at San Diego
John Weisburger, American Health Foundation
Sidney Wolfe, Health Research Group

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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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