National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 16
1979

BIOASSAY OF PHOSPHAMIDON FOR POSSIBLE CARCINOGENICITY

CAS No. 13171-21-6

NCI-CG-TR-16

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

PHOSPHAMIDON

FOR POSSIBLE CARCINOGENICITY

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

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 79-816

BIOASSAY OF

PHOSPHAMIDON

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

FOREWORD: This report presents the results of the bioassay of phosphamidon 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 chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of phosphamidon was conducted by Gulf South Research Institute, New Iberia, Louisiana, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by Drs. J. H. Weisburger (1,2) and R. R. Bates (1,3); the doses were selected by Drs. T. E. Shellenberger (4,5,), J. H. Weisburger, and R. R. Bates. Chemical administration and observation of animals were supervised by Drs. T. E. Shellenberger and H. P. Burchfield (4),

with the technical assistance of Ms. D. H. Monceaux (4) and Mr. D. Broussard (4). Histopathology was performed by Drs. E. Bernal (4) and B. Buratto (4) at Gulf South Research Institute, and the diagnoses included in this report represent the interpretations of these pathologists.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (6). Statistical analyses were performed by Dr. J. R. Joiner (7) and Ms. P. L. Yong (7), using methods selected for the bioassay program by Dr. J. J. Gart (8). Chemicals used in this bioassay were analyzed under the direction of Dr. H. P. Burchfield, and the results of the analyses were reviewed by Dr. S. S. Olin (7).

This report was prepared at Tracor Jitco (7) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Ms. Y. E. Presley, technical writer; and Dr. E. W. Gunberg, technical editor, assisted by Ms. P. J. Graboske.

The following 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 (9), Dr. Richard A. Griesemer, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire (10), Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

---

(1) Carcinogenesis Testing Program, Divison of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

(2) Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammond House Road, Valhalla, New York.

(3) Now with the National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina.

(4) Gulf South Research Institute, Atchafalaya Basin Laboratories, P. O. Box 1177, New Iberia, Louisiana.

(5) Now with the National Center for Toxicological Research, Jefferson, Arkansas.

(6) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

(7) Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

(8) Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

(9) Now with Clement Associates, Inc., 1010 Wisconsin Avenue, N.W., Suite 660, Washington, D. C.

(10) Now with the Division of Camparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of technical-grade phosphamidon for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F1 mice. The test material was administered in feed to 50 rats and 50 mice of each sex at one of two doses, either 80 or 160 ppm. The rats were fed the test chemical for 80 weeks, then observed without compound administration for 30 or 31 weeks; the low-dose male mice were fed for 71 weeks, then observed for 19 weeks; the high-dose male mice were fed for 62 weeks, then observed for 28 weeks; and the low- and high-dose female mice were fed for 80 weeks, then observed for 10 or 11 weeks. Matched controls consisted of groups of 10 untreated rats or 10 untreated mice of each sex; pooled controls consisted of the matched controls combined with 85 male and 85 female untreated rats or 80 male and 80 female untreated mice from similar bioassays of eight other test chemicals. All surviving rats were killed at 110 or 111 weeks; all surviving mice were killed at 90 or 91 weeks.

Hyperexcitability and tremors, both indications of phosphamidon toxicity, were observed in dosed rats and mice. However, sufficient numbers of all groups of both species were at risk for the development of late-appearing tumors.

In male rats, the combined incidence of hemangiomas and hemangiosarcomas in the spleen showed a statistically significant ($P = 0.012$) dose-related trend. However, the comparison with matched controls was not significant, and the historical records of this laboratory on untreated males of this strain show a tumor incidence of 6/240 (3%) with incidences in individual control groups as high as 3/9 (33%) and 2/9(22%), compared with 5/49 (10%) seen in the high-dose group in this study. No hemangiomas or hemangiosarcomas were found in the females.

In female rats, the Cochran-Armitage test for dose-related trend was significant ($P = 0.003$) for C-cell adenomas and carcinomas of the thyroid when pooled controls were compared with the dosed groups. The incidences of these tumors were also significant when low-dose females ($P = 0.003$) and high-dose females ($P = 0.004$) were compared directly with pooled controls. However, the historical records of this laboratory show a tumor incidence of 16/235 (7%) in untreated female rats of this strain of female rats, with incidences in individual control groups as high as 3/9 (33%) and 3/10 (30%); these data are therefore considered marginal and insufficient to establish an association between the tumors and administration of the chemical. In males, the incidence of these tumors was not statistically significant.

In mice, no tumor occurred at a higher incidence in dosed animals than in controls.

It is concluded that under the conditions of this bioassay, technical- grade phosphamidon was not carcinogenic for B6C3F1 mice. The data obtained in this bioassay with Osborne-Mendel rats are insufficient to allow the interpretation that technical-grade phosphamidon is carcinogenic in this species.

TABLE OF CONTENTS

		Page
I.	Introduction	1
II.	Materials and Methods	3
	A. Chemical	3
	B. Dietary Preparation	3
	C. Animals	5
	D. Animal Maintenance	5
	E. Subchronic Studies	7
	F. Chronic Studies	8
	G. Clinical and Pathologic Examinations	11
	H. Data Recording and Statistical Analyses	12
III.	Results - Rats	19
	A. Body Weights and Clinical Signs (Rats)	19
	B. Survival (Rats)	21
	C. Pathology (Rats)	23
	D. Statistical Analyses of Results (Rats)	25
IV.	Results - Mice	29
	A. Body Weights and Clinical Signs (Mice)	29
	B. Survival (Mice)	31
	C. Pathology (Mice)	33
	D. Statistical Analyses of Results (Mice)	33
V.	Discussion	35
VI.	Bibliography	39

APPENDIXES

Appendix A	Summary of the Incidence of Neoplasms in Rats Fed Phosphamidon in the Diet	41
Table A1	Summary of the Incidence of Neoplasms in Male Rats Fed Phosphamidon in the Diet	43
Table A2	Summary of the Incidence of Neoplasms in Female Rats Fed Phosphamidon in the Diet	47

		Page
Appendix B	Analyses of the Incidence of Primary Tumors in Mice Fed Phosphamidon in the Diet.....	51
Table B1	Summary of the Incidence of Neoplasms in Male Mice Fed Phosphamidon in the Diet.....	53
Table B2	Summary of the Incidence of Neoplasms in Female Mice Fed Phosphamidon in the Diet.....	56
Appendix C	Summary of the Incidence of Nonneoplastic Lesions in Rats Fed Phosphamidon in the Diet...	59
Table C1	Summary of the Incidence of Nonneoplastic Lesions in Male Rats Fed Phosphamidon in the Diet.....	61
Table C2	Summary of the Incidence of Nonneoplastic Lesions in Female Rats Fed Phosphamidon in the Diet.....	64
Appendix D	Summary of the Incidence of Nonneoplastic Lesions in Mice Fed Phosphamidon in the Diet...	67
Table D1	Summary of the Incidence of Nonneoplastic Lesions in Male Mice Fed Phosphamidon in the Diet.....	69
Table D2	Summary of the Incidence of Nonneoplastic Lesions in Female Mice Fed Phosphamidon in the Diet.....	72
Appendix E	Analyses of the Incidence of Primary Tumors in Rats fed Phosphamidon in the Diet.....	75
Table E1	Analyses of the Incidence of Primary Tumors in Male Rats Fed Phosphamidon in the Diet.....	77
Table E2	Analyses of the Incidence of Primary Tumors in Female Rats Fed Phosphamidon in the Diet....	80
Appendix F	Analyses of the Incidence of Primary Tumors in Mice Fed Phosphamidon in the Diet.....	85
Table F1	Analyses of the Incidence of Primary Tumors in Male Mice Fed Phosphamidon in the Diet.....	87

Page

Table F2 Analyses of the Incidence of Primary Tumors in Female Mice Fed Phosphamidon in the Diet.... 89

Appendix G Analysis of Formulated Diets for Concentrations of Phosphamidon..... 93

TABLES

Table 1 Phosphamidon Chronic Feeding Studies in Rats..... 9

Table 2 Phosphamidon Chronic Feeding Studies in Mice..... 10

FIGURES

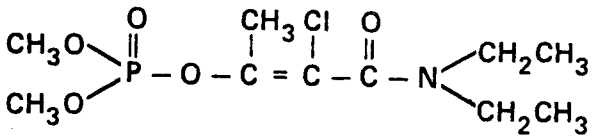
Figure 1 Growth Curves for Rats Fed Phosphamidon in the Diet..... 20

Figure 2 Survival Curves for Rats Fed Phosphamidon in the Diet..... 22

Figure 3 Growth Curves for Mice Fed Phosphamidon in the Diet..... 30

Figure 4 Survival Curves for Mice Fed Phosphamidon in the Diet..... 32

I. INTRODUCTION



Phosphamidon

Phosphamidon (CAS 13171-21-6; NCI C00588) is an organophosphorus compound used as a broad-spectrum insecticide in agriculture since 1956 (Spencer, 1973). It is toxic both systemically and by contact, and acts through the inhibition of cholinesterase (Hazleton and Weir, 1958). Phosphamidon is currently registered for use by both ground and aerial applications on vegetables, fruits, and field crops with tolerances for residues from 0.1 to 1.0 ppm (EPA Compendium, 1974).

Phosphamidon was one of a series of pesticides selected for screening for carcinogenic activity because of the potential for long-term human exposure to the chemical during agricultural application or to residues in food products.

II. MATERIALS AND METHODS

A. Chemical

Technical-grade phosphamidon (dimethyl 2-chloro-2-diethylcarbamoyl-1-methylvinyl phosphate) was obtained from Chevron Chemical Company, Ortho Division (San Francisco, Calif.), in one batch for the chronic study. The identity of the chemical was confirmed by analyses at Gulf South Research Institute. Elemental analysis (C, H, N, Cl, P) was correct for the molecular formula of phosphamidon, $C_{10}H_{19}ClNO_5P$. Infrared, nuclear magnetic resonance, and mass spectra were as expected for phosphamidon. No attempt was made to identify or quantitate any impurities that may have been present. The chemical was stored at 5°C in the original amber glass container until used.

B. Dietary Preparation

All diets containing phosphamidon were formulated once per week using Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) to which was added the required amount of phosphamidon for each dietary concentration. The test chemical was first

dissolved in a small amount of acetone (Mallinckrodt, Inc., St. Louis, Mo.), which was then added to the feed. Corn oil (LouAna®, Opelousas Refinery Co., Opelousas, La.) was also added to the feed, primarily as a dust suppressant, and the diets were mixed mechanically to assure homogeneity of the mixtures and evaporation of the acetone. Final diets, including those for the control groups of animals, contained corn oil equal to 2% of the final weight of feed. The diets were stored at approximately 17°C until used, but no longer than 1 week.

The stability of phosphamidon in feed was tested by determining the concentration of the chemical in formulated diets at intervals over a 7-day period. Diets containing either 80 or 160 ppm phosphamidon showed no change in concentration on standing at ambient temperature for this period.

As a quality control test on the accuracy of preparation of the diets, the concentration of phosphamidon was determined in different batches of formulated diets during the chronic study. The results are summarized in Appendix G. The means of the analytical concentrations for the checked samples were 81.7 $\pm$ 3.8 and 161.5 $\pm$ 6.3 ppm for target concentrations of 80 and 160 ppm, respectively.

C. Animals

Rats and mice of each sex, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were of the Osborne-Mendel strain obtained from Battelle Memorial Institute, Columbus, Ohio, and the mice were B6C3F1 hybrids obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. On arrival at the laboratory, all animals were quarantined (rats for 6 days, mice for 13 days) and then were assigned to either control or dosed groups.

D. Animal Maintenance

All animals were housed by species in rooms maintained at 22 to 24°C, and 40 to 70% relative humidity. The air in each room was filtered (Air Maze Incom International, Cleveland, Ohio), and room air was changed 10 to 12 times per hour. Fluorescent lighting provided illumination 10 hours per day. Food and water were provided ad libitum. Fresh feed was provided daily, and excess remaining feed was discarded.

The rats were housed individually in hanging galvanized steel

mesh cages (Hoeltge, Inc., Cincinnati, Ohio), and the mice were housed in polypropylene cages (Lab Products, Inc., Garfield, N.J.), five females per cage or two or three males per cage. Mouse cages were covered with polyester filter bonnets (Lab Products, Inc.). The rat racks and cages were sanitized every 2 weeks. The mouse cages were sanitized each week. These cages and racks were washed in an industrial washer at 82°C with Acclaim® detergent (Economics Laboratory, Inc., St. Paul, Minn.) and then rinsed. Absorbent Kimpak cage liners (Kimberly Clark Corp., Neenah, Wis.) were placed under the rat cages and were changed three times per week. Absorb-dri® hardwood chip bedding (Lab Products, Inc.), used in the mouse cages, was provided two times per week for males and three times per week for females. Filter bonnets were sanitized each week. Feed jars and water bottles were changed and sanitized three times per week. Sipper tubes and stoppers were sanitized two times per week.

The filter bonnets, feed jars, water bottles, sipper tubes, and stoppers were washed in a Vulcan Autosan washer (Louisville, Ky.).

Cage racks for each species were rotated to a new position in the room once per week; at the same time, each cage was moved to a different row within the same column of a rack. Rats receiving

phosphamidon, along with their matched controls, were housed in a room by themselves. Mice were maintained in a room housing mice from the following studies:

Feed Studies

(CAS 56-38-2) parathion
(CAS 60-51-5) dimethoate

E. Subchronic Studies

Subchronic feeding studies were conducted with rats and mice to estimate the maximum tolerated doses of phosphamidon, on the basis of which two concentrations (referred to in this report as "low" and "high" doses) were selected for administration in the chronic studies. In the subchronic studies, phosphamidon was added to the animal feed in twofold increasing concentrations, ranging from 10 to 1,280 ppm for both rats and mice. The chemical was provided in feed to dosed groups of five male and five female animals of each species for 6 weeks, followed by observation for 2 weeks. Untreated-control groups consisted of five animals of each species and sex.

At 320 ppm and higher, all rats and all male mice died by week 2. No animals died at 160 ppm, although weight gains in dosed

rats were lower than those of their controls during the second week of the study; for the remainder of the study, the behavior of these animals was generally similar to that of the controls. Male mice fed 160 ppm lost weight during the first week, but generally gained weight throughout the remainder of the study. The weight gains in the female mice at 160 ppm were similar to those of their controls throughout the 8-week study. The low and high doses for the chronic studies were set at 80 and 160 ppm for both rats and mice.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in tables 1 and 2.

Since the numbers of animals in the matched-control groups were small, pooled-control groups also were used for statistical comparisons. For rats, matched controls from the current bioassay of phosphamidon were combined with matched controls from the bioassays of tetrachlorvinphos (CAS 961-11-5), toxaphene (CAS 8001-35-2), endrin (CAS 72-20-8), lindane (CAS 58-89-9), chlorothalonil (CAS 1897-45-6), chloramben (CAS 133-90-4), picloram (CAS 1918-02-1), and malathion (CAS 121-75-5). The

Table 1. Phosphamidon Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	Phosphamidon Doses (ppm)	Time on Study	
			Dosed (weeks)	Observed (b) (weeks)
Male				
Matched-Control	10	0		110
Low-Dose	50	80	80	
		0		30
High-Dose	50	160	80	
		0		30
Female				
Matched-Control	10	0		111
Low-Dose	50	80	80	
		0		31
High-Dose	50	160	80	
		0		31

(a) All animals were 5 weeks of age when placed on study.

(b) When diets containing phosphamidon were discontinued, all rats received control diets (2% corn oil added) until termination.

Table 2. Phosphamidon Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals (a)	Phosphamidon Doses (ppm)	Time on Study	
			Dosed (weeks)	Observed (b) (weeks)
Male				
Matched-Control	10	0		90-91
Low-Dose	50	80	71	
		0		19
High-Dose	50	160	62	
		0		28
Female				
Matched-Control	10	0		90-91
Low-Dose	50	80	80	
		0		11
High-Dose	50	160	80	
		0		11

(a) All animals were 5 weeks of age when placed on study.

(b) When diets containing phosphamidon were discontinued, high-dose males received control diets without corn oil added for 8.5 weeks, then control diets (2% corn oil added) for 19.5 weeks. Low-dose male and all female mice were fed control diets until termination.

pooled controls for statistical tests consisted of 95 rats and 90 mice of each sex. These studies overlapped the bioassay of phosphamidon by at least 1 year. The pooled-control animals were of the same strain as the test animals (Osborne-Mendel rats or B6C3F1 mice) and from the same supplier, and their tissues were diagnosed by the same pathologists.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for deaths. Observations to identify sick, tumor-bearing, and moribund animals were made daily. Animals were weighed every 2 weeks for the first 12 weeks and monthly thereafter, and palpated for masses at each weighing. Moribund animals and animals that survived to the end of the bioassay were killed using ether and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions. The tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen,

lymph nodes, heart, salivary gland, liver, gallbladder (mice), pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain. Occasionally, additional tissues were also examined microscopically.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

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

tables were generated for verification of data transcription and for statistical review.

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), is also used. Assuming 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, and Tarone's extension to testing for linear trend is used for three groups. All 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 less than 0.05, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a 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 left endpoint of the confidence interval is greater than zero, the occurrence of a statistically significant result (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

The mean body weights of the high-dose male and high-dose female rats were lower than those of the matched controls for most of the study (figure 1). The mean body weights of the low-dose males and low-dose females were essentially unaffected by the administration of phosphamidon. 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 wide variation.

During the first year of the study, the high-dose males and the low-dose females were generally comparable to the controls in appearance and behavior. The high-dose females appeared to be hyperexcitable from weeks 6 to 10. At week 16, one low-dose male and one high-dose male showed generalized body tremors. Convulsions were observed in one low-dose male at week 19. Beginning at week 28, a majority of the low-dose males had generalized alopecia.

During the second year of the study, clinical signs were noted with increasing frequency in both matched controls and dosed

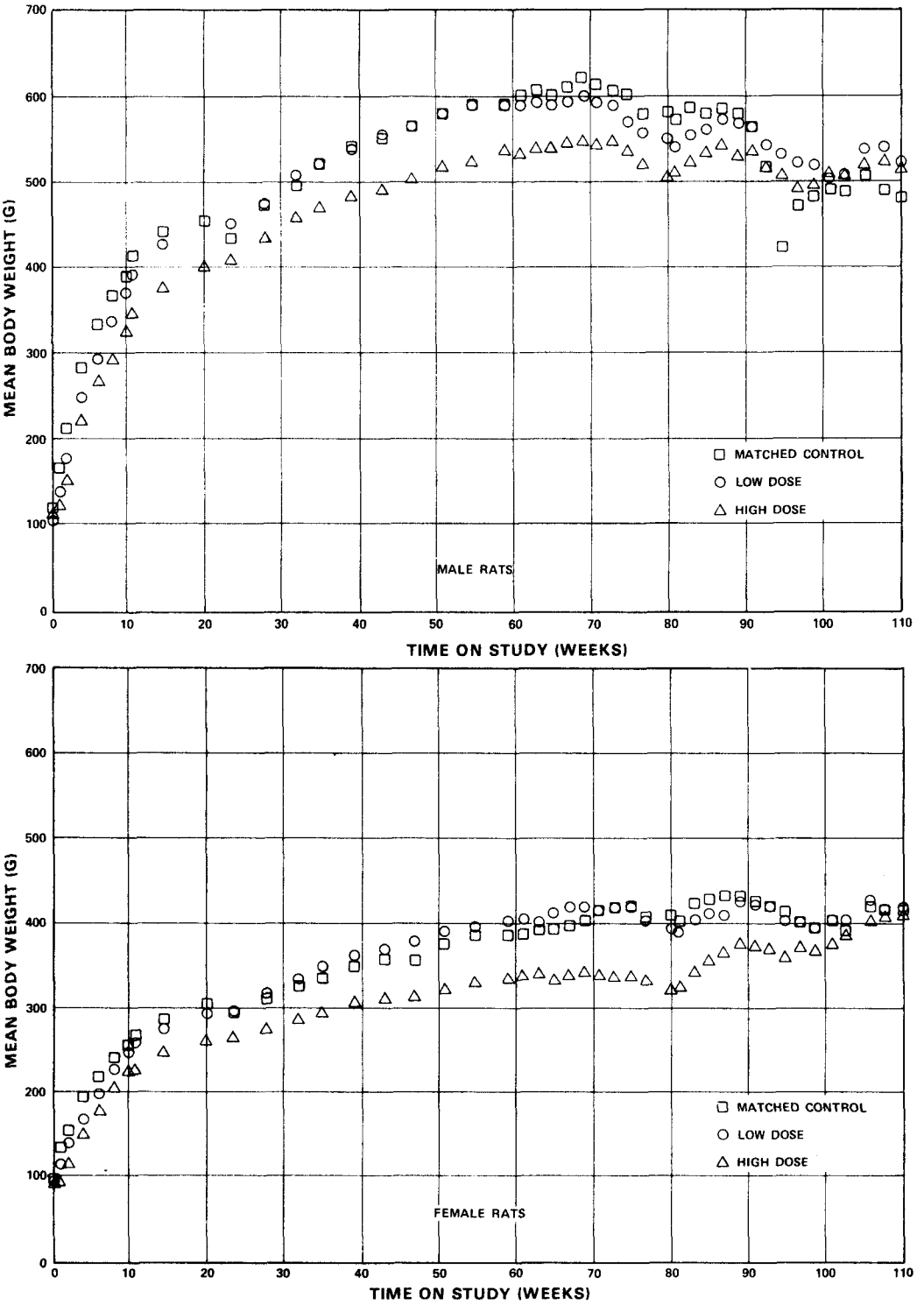


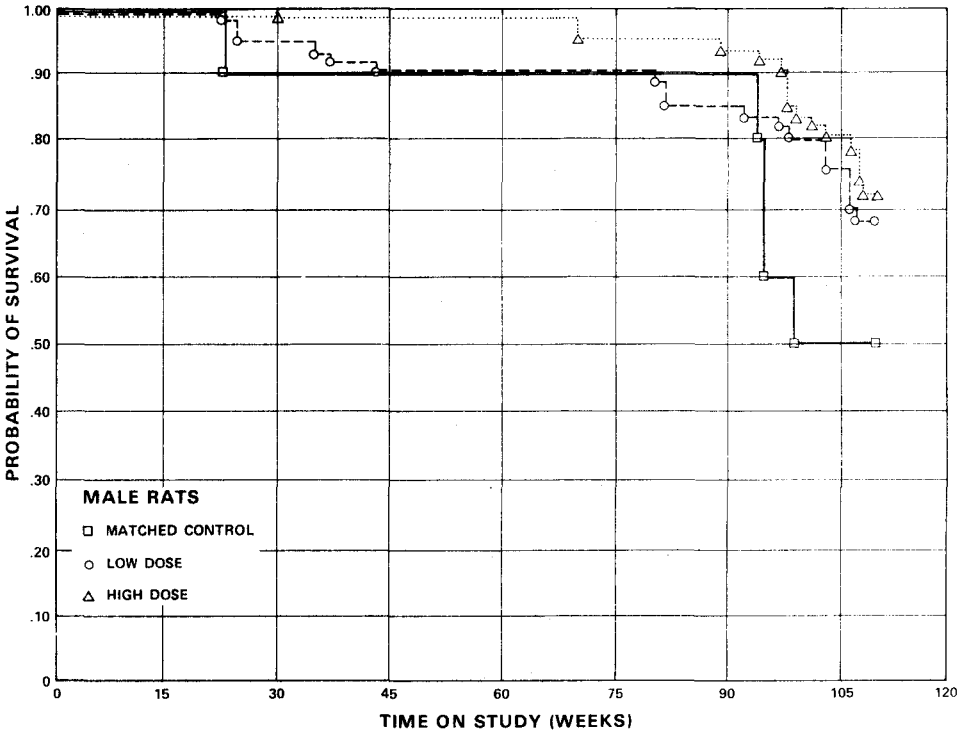
Figure 1. Growth Curves for Rats Fed Phosphamidon in the Diet

animals. These signs included loss of weight, pale mucous membranes, diarrhea, dermatitis, vaginal bleeding, rough hair coats, epistaxis, and tachypnea. A majority of the dosed animals had wet and urine-stained hair coats during the second year.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered phosphamidon in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. In neither sex is the Tarone test result significant (P greater than 0.05) for positive dose-related trend in mortality over the bioassay.

In male rats, 36/50 (72%) of the high-dose group, 34/50 (68%) of the low-dose group, and 5/10 (50%) of the matched-control group survived to the end of the bioassay. In females, 39/50 (78%) of the high-dose group, 41/50 (82%) of the low-dose group, and 9/10 (90%) of the matched-control group lived to termination of the study. Thus, sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.



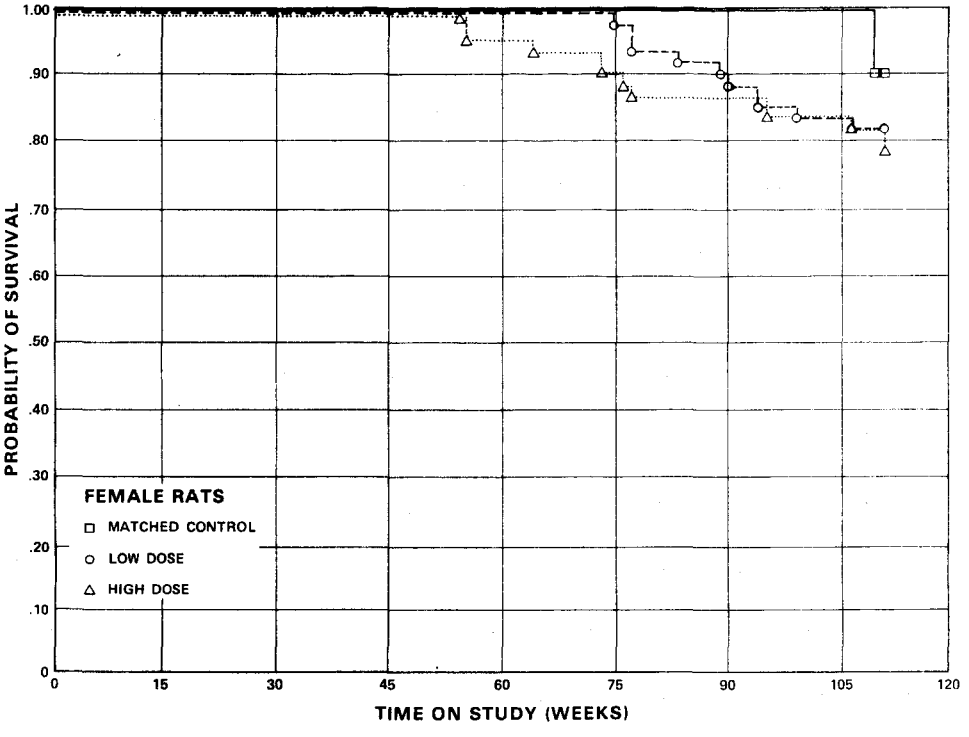


Figure 2. Survival Curves for Rats Fed Phosphamidon in the Diet

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.

A variety of neoplasms are represented among both the dosed and the control rats. In general, these lesions are common in this strain of rat, independent of any treatment. However, hemangiosarcomas of the spleen occurred in one control male, three low-dose males, and five high-dose males; a hemangioma occurred in one other low-dose male. No hemangiomas or hemangiosarcomas of the spleen occurred in any of the matched-control or dosed females. The hemangiosarcomas in the spleen of the male rats varied from very cellular areas in the red pulp with minimal vascularity, to lesions with prominent vascular spaces, hemorrhage, and necrosis. The tumor cells in the stroma and lining vesicular spaces were large, hyperchromatic oval to spindle cells, with prominent nuclei and nucleoli, and several mitotic figures. The boundaries of the tumors were irregular and indistinct, and some lesions involved the splenic capsule. Although these tumors have been encountered in control animals in the past, their occurrence at the incidences noted in this study is infrequent.

C-cell adenomas of the thyroid also occurred with relatively high incidences in low- and high-dose rats of each sex but not in any of the matched controls. C-cell carcinomas were found in one matched-control male, one low-dose female, and one high-dose female. C-cell hyperplasias of the thyroid were also observed. Proliferative lesions of the C-cells occurred as follows:

	Males			Females		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Animals with Tissue Examined Microscopically	(8)	(48)	(47)	(10)	(50)	(46)
Thyroid						
C-cell Hyperplasia	0	7	3	0	4	0
C-cell Adenoma	0	7	5	0	8	7
C-cell Carcinoma	1	0	0	0	1	1

Although these thyroid lesions have occurred in control rats of this strain used in other similar studies, the incidence with which they occurred in this experiment may indicate an association with the administration of phosphamidon.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals of the control and dosed groups. For the most part, these nonneoplastic lesions were similar to those commonly encountered in aged rats.

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 of one group and at an incidence of at least 5% in one or more than one group.

In male rats, the Cochran-Armitage test for positive dose-related trend in proportions for combined hemangioma and hemangiosarcoma is significant (P = 0.012) using the pooled controls. The result of the Fisher exact test shows a significantly higher incidence of this tumor in the high-dose group (P = 0.020) than in the pooled controls. The historical records of this laboratory show an incidence of these tumors of 6/240 (3%) in untreated male rats of this strain of male rats, with incidences in individual control groups as high as 3/9 (33%) and 2/9 (22%), compared with 5/49 (10%) seen in the high-dose groups in this study. This tumor was not observed in female rats.

The results of the Cochran-Armitage test for positive dose-related trend in the incidence of C-cell adenoma of the thyroid in male rats are not significant. The Fisher exact comparison of incidence between the low-dose and pooled-control groups shows a P value of 0.031, which is above the 0.025 level required for

significance when the Bonferroni inequality criterion is used for multiple comparison.

In females, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of animals with either C-cell adenoma or carcinoma of the thyroid is significant (P = 0.003) using the pooled controls. The result of the Fisher exact test shows significantly higher incidences of this tumor both in the low-dose group (P = 0.003) and the high-dose group (P = 0.004) than in the pooled controls. The historical records of this laboratory show an incidence of these tumors of 16/235 (7%) in untreated female rats of this strain, with incidences in individual control groups as high as 3/9 (33%) and 3/10 (30%), compared with 8/46 (17%) seen in the high-dose group in this study.

In male rats, the incidence of cortical adenoma of the adrenal in the low-dose group is significantly higher (P = 0.023) than that in the pooled-control group, but not higher than that in the matched-control group. The incidence in the high-dose group is not significant, nor are the results of the Cochran-Armitage test using either control group.

Significant trends in the negative direction are observed in the incidence of carcinoma of the pituitary in female rats.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights of the dosed male mice were lower than those of the matched controls, and there was a dose-related effect (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 wide variation. The mean body weights of the dosed females were essentially the same as the control values.

During the first year of the study, the dosed animals were generally comparable to the controls in appearance and behavior. At week 29, a majority of the dosed and control males were observed fighting, which resulted in alopecia and in wounds.

During the second half of the study, clinical signs including alopecia, rough hair coats, and abdominal distention were noted with increasing frequency in both control and dosed animals. A few low-dose males and a majority of high-dose males showed generalized tremors at week 60. Tremors persisted in the high-dose males until week 80. At week 69, a majority of

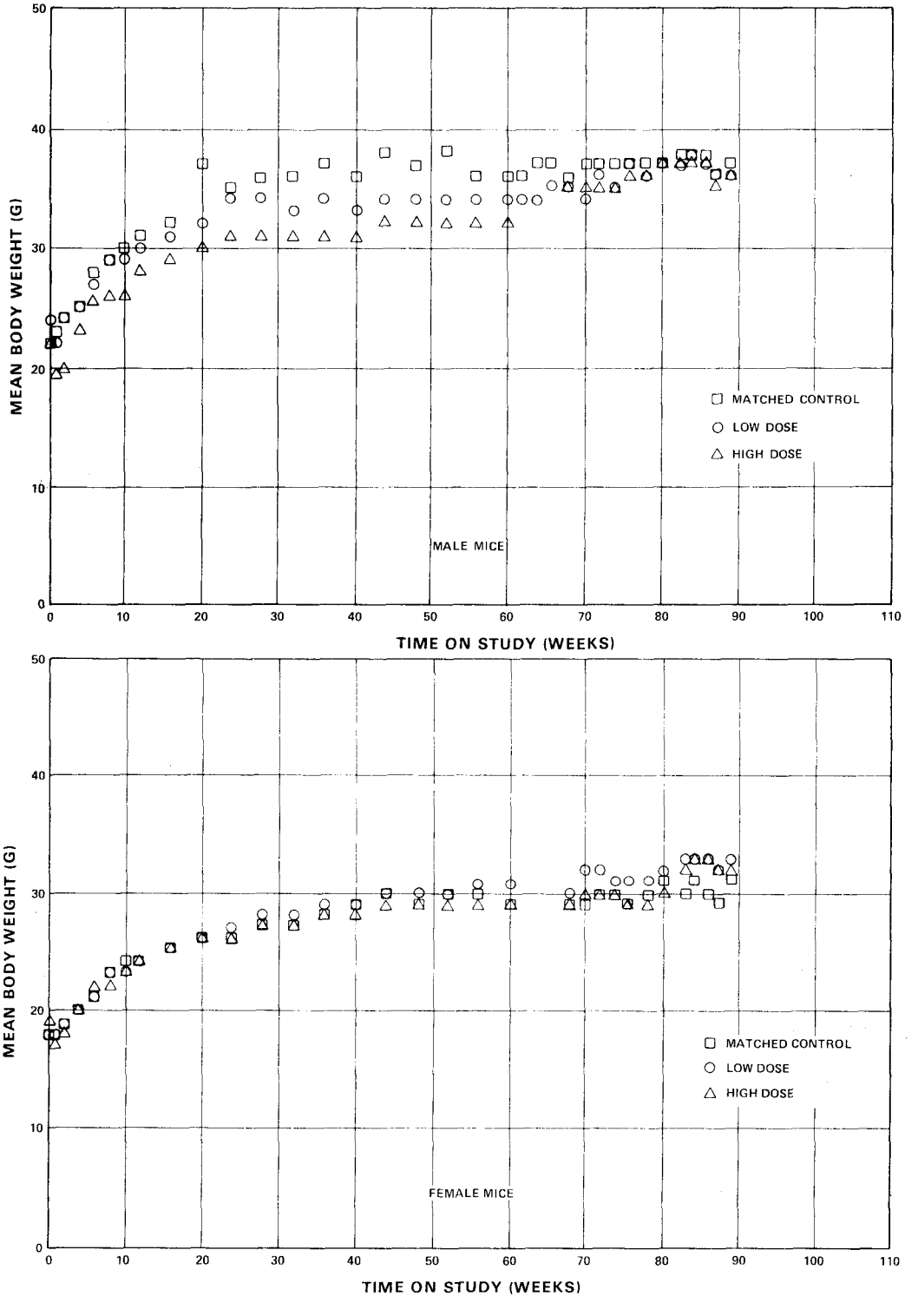


Figure 3. Growth Curves for Mice Fed Phosphamidon in the Diet

low-dose and high-dose males had rough hair coats and generally appeared to be in poor physical condition.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered phosphamidon in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. In neither sex is the result of the Tarone test significant (P greater than 0.05) for positive dose-related trend in mortality.

In male mice, 42/50 (84%) of the high-dose group, 45/50 (90%) of the low-dose group, and 7/10 (70%) of the control animals lived to the end of the study. In females, 45/50 (90%) of the high-dose group, 46/50 (92%) of the low-dose group, and all 10 of the control animals survived to the end of the study. Thus, sufficient numbers of mice of each sex were at risk for the development of late-appearing tumors.

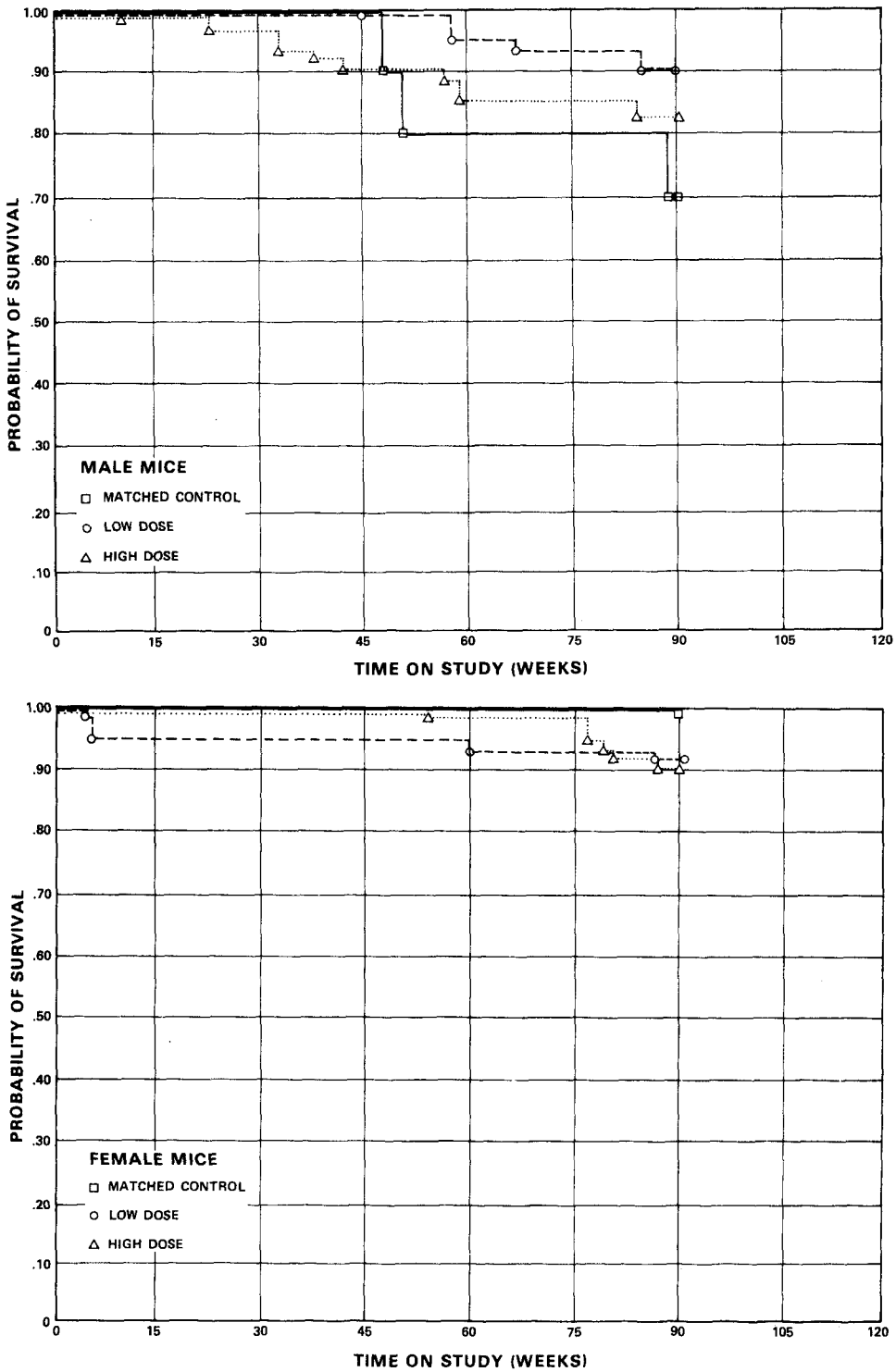


Figure 4. Survival Curves for Mice Fed Phosphamidon in the Diet

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.

Neoplasms occurred at approximately equal incidences in control and dosed mice. Hepatocellular adenomas and carcinomas, which occurred in both control and dosed mice, were of the type usually seen in aging mice of this strain. Under the conditions of this study, there was no evidence that phosphamidon was carcinogenic to B6C3F1 mice.

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 of one group and at an incidence of at least 5% in one or more than one group.

The results of the Cochran-Armitage test are not significant (P greater than 0.05) in either sex for positive dose-related trends in the proportions of tumors at any site, and none of the results

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

V. DISCUSSION

Phosphamidon is a member of the organophosphorus class of pesticides whose predominant mode of toxicity is inhibition of cholinesterase. Toxic effects characteristic of these chemicals were evident in the animals in this bioassay. During the first year of the study, both male and female rats were hyperexcitable. Several males were observed to have convulsions. In mice, the hyperexcitability and generalized tremors were more pronounced in males than in females and developed at a later time than in rats. Other clinical signs, such as rough hair coats, dermatitis, epistaxis, and vaginal bleeding, increased in frequency with age and could not definitely be correlated with the administration of phosphamidon. Administration of phosphamidon resulted in low mean body weights in high-dose male and female rats and in high-dose male mice, but had no adverse effect on the survival of either rats or mice. Sufficient numbers of all groups of both species were at risk for the development of late-appearing tumors.

The incidence of the combination of hemangioma and hemangiosarcoma in both high- and low-dose male rats was statistically significant ($P = 0.012$) when compared with the

pooled controls (pooled controls 1/91, matched controls 1/7, low-dose 3/50, high-dose 5/49). The comparison with matched controls was not significant. All of these tumors occurred in the spleen in the animals in the present study. However, the historical records of this laboratory on untreated males of this strain show a tumor incidence of 6/240 (3%) with incidences in individual control groups as high as 3/9 (33%) and 2/9 (22%). The data from this bioassay with male rats are considered marginal and insufficient to establish an association between the tumors and administration of the chemical. These lesions did not occur in female rats.

In male rats, the incidence of cortical adenoma of the adrenal in the low-dose group was significantly higher ($P = 0.023$) than that in the pooled control group (pooled controls 0/8, matched controls 2/90, low-dose 6/49, high-dose 2/49). However, the incidence in the high-dose group was not significant, and the occurrence of the tumor was not considered to be related to administration of the test chemical.

In female rats, the Cochran-Armitage test for dose-related trend was significant ($P = 0.003$) for C-cell adenomas and carcinomas of the thyroid when pooled controls were compared with the dosed groups (pooled controls 2/82, matched controls 0/10, low-dose

9/50, high-dose 8/46). The incidence of these tumors was also significant when low-dose females (P = 0.003) and high-dose females (P = 0.004) were compared with corresponding pooled controls. However, the historical records of this laboratory show a tumor incidence of 16/235 (7%) in untreated female rats of this strain, with incidences in individual control groups as high as 3/9 (33%) and 3/10 (30%). The data from this bioassay with female rats are considered marginal and insufficient to establish an association between these tumors and administration of the chemical. In males, the incidence of these tumors was not statistically significant.

In mice, no tumor occurred at a statistically significant incidence.

Sachsse and Voss (1971) reported that phosphamidon was not carcinogenic in rats. In their study, rats were fed diets containing 0.1 to 5 mg/kg of phosphamidon daily for 2 years, and various organs, including the thyroid glands and spleens of the high-dose animals, were examined. A different strain of rat from the one used in the present bioassay (Carworth CFN vs. Osborne-Mendel) and a lower maximum dose (5 mg/kg/day vs. approximately 10 mg/kg/day) were used.

It is concluded that under the conditions of this bioassay, technical-grade phosphamidon was not carcinogenic for B6C3F1 mice. The data obtained in this bioassay with Osborne-Mendel rats are insufficient to allow the interpretation that technical-grade phosphamidon is carcinogenic in this species.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Berenblum, I., ed., Carcinogenicity Testing, UICC Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B 34:187-220, 1972.

Cox, D. R., Analysis of Binary Data, Methuen and Co., Ltd., London, 1970, pp. 48-52.

Environmental Protection Agency, EPA Compendium of Registered Pesticides, U. S. Government Printing Office, Washington, D. C., 1974, III-P-12.1-12.5.

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratifiation. Rev. Int. Statist. Inst. 39(2):148-169, 1971.

Hazleton, L. W. and Weir, R. J., Organic Phosphate. In: Industrial Hygiene and Toxicology, Patty, F. A., ed., Interscience Publishers, New York, 1958, pp. 1935-1952.

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observation. J. Amer. Statist. Assn. 53:457-481, 1958.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. J. Comp. Biomed. Res. 7:230-248, 1974.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill, New York, 1966.

Sachsse, K. R. and Voss, G., Toxicology of phosphamidon. Residue Reviews 37:61-88,
1971.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F. and Kaufman, D. G., Respiratory tract carcinogenesis in hamster induced by different numbers of administration of benzo(a) pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Spencer, E. Y., Guide to Chemicals Used in Crop Protection, Research Institute, University of Western Ontario, London, Ontario, 1973, pp. 410-411.

Tarone, R. E., Tests for trend in life-table analysis. Biometrika 62(3):679-682, 1975.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED PHOSPHAMIDON IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED PHOSPHAMIDON IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(9)	(50)	(49)
FIBROUS HISTIOCYTOMA		1 (2%)	
HEMANGIOPERICYTOMA, MALIGNANT	1 (11%)		
RESPIRATORY SYSTEM			
#LUNG	(8)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(9)	(50)	(49)
UNDIFFERENTIATED LEUKEMIA			1 (2%)
#SPLEEN	(7)	(50)	(49)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA	1 (14%)	2 (4%)	5 (10%)
HAMARTOMA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(9)	(50)	(47)
ADENOMA, NOS			1 (2%)
#LIVER	(9)	(49)	(46)
NEOPLASTIC NODULE			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#STOMACH	(7)	(50)	(45)
SQUAMOUS CELL CARCINOMA			1 (2%)
#COLON		(38)	(40)
ADENOCARCINOMA, NOS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(9)	(49)	(49)
† HAMARTOMA		1 (2%)	
#URINARY BLADDER	(6)	(46)	(47)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(8)	(49)	(48)
CARCINOMA,NOS		1 (2%)	1 (2%)
ADENOMA, NOS			1 (2%)
CHROMOPHOBE ADENOMA	2 (25%)	8 (16%)	3 (6%)
#ADRENAL	(8)	(49)	(49)
CORTICAL ADENOMA		6 (12%)	2 (4%)
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(8)	(48)	(47)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA		7 (15%)	5 (11%)
C-CELL CARCINOMA	1 (13%)		
#PANCREATIC ISLETS	(7)	(47)	(49)
ISLET-CELL ADENOMA		2 (4%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(9)	(50)	(49)
FIBROMA	1 (11%)		
NERVOUS SYSTEM			
#BRAIN	(9)	(50)	(47)
GRANULAR-CELL TUMOR, BENIGN		1 (2%)	

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
† NONNEOPLASTIC PROLIFERATIVE LESION COMPOSED OF LIPOCYTES, TUBULAR STRUCTURES, AND FIBROBLASTS IN VARYING PROPORTIONS.

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE OR ANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGAN	(9)	(50)	(49)
FIBROUS HISTIOCYTOMA		1 (2%)	
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	3	4	5
MORIBUND SACRIFICE	2	12	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	5	34	36
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	26	20
TOTAL PRIMARY TUMORS	6	34	27
TOTAL ANIMALS WITH BENIGN TUMORS	2	24	10
TOTAL BENIGN TUMORS	3	30	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	4	10
TOTAL MALIGNANT TUMORS	3	4	11
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED PHOSPHAMIDON IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(10)	(50)	(49)
FIBROUS HISTIOCYTOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(10)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
C-CELL CARCINOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(50)	(49)
NEOPLASTIC NODULE		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(10)	(50)	(49)
†HAMARTOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(50)	(45)
CARCINOMA,NOS	2 (20%)		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
† NONNEOPLASTIC PROLIFERATIVE LESION COMPOSED OF LIPOCYTES, TUBULAR STRUCTURES, AND FIBROBLASTS IN VARYING PROPORTIONS.

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CHROMOPHOBE ADENOMA	3 (30%)	7 (14%)	7 (16%)
#ADRENAL	(10)	(50)	(48)
CARCINOMA, NOS	1 (10%)		
CORTICAL ADENOMA	2 (20%)	6 (12%)	6 (13%)
CORTICAL CAR INOMA		1 (2%)	
#THYROID	(10)	(50)	(46)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA		8 (16%)	7 (15%)
C-CELL CARCINOMA		1 (2%)	1 (2%)
#PANCREATIC ISLETS	(10)	(49)	(48)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(49)
ADENOMA, NOS		1 (2%)	
FIBROMA			1 (2%)
FIBROADENOMA	2 (20%)	6 (12%)	3 (6%)
#UTERUS	(10)	(48)	(48)
PAPILLARY ADENOMA	1 (10%)		
LEIOMYOMA			1 (2%)
LEIOMYOSARCOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP		1 (2%)	3 (6%)
#OVARY	(10)	(49)	(49)
GRANULOSA-CELL TUMOR		1 (2%)	1 (2%)
SARCOMA, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(9)	(50)	(49)
GRANULAR-CELL TUMOR, BENIGN		1 (2%)	
OLIGODENDROGLIOMA	1 (11%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		2	4
MORIBUND SACRIFICE	1	7	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	41	39
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	29	26
TOTAL PRIMARY TUMORS	12	40	31
TOTAL ANIMALS WITH BENIGN TUMORS	5	26	23
TOTAL BENIGN TUMORS	8	34	28
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	4	2
TOTAL MALIGNANT TUMORS	4	4	2
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	1
TOTAL UNCERTAIN TUMORS		2	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 B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED PHOSPHAMIDON IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED PHOSPHAMIDON IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(9)	(49)	(49)
FIBROSARCOMA		1 (2%)	
FIBROUS HISTIOCYTOMA			1 (2%)
*SUBCUT TISSUE	(9)	(49)	(49)
FIBROMA		1 (2%)	1 (2%)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(9)	(49)	(45)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (11%)	2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(9)	(49)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(9)	(49)	(46)
HEPATOCELLULAR ADENOMA		2 (4%)	
NEOPLASTIC NODULE			2 (4%)
HEPATOCELLULAR CARCINOMA	2 (22%)	7 (14%)	5 (11%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMANGIOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE OR ANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITICN SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	1	1	5
MORIBUND SACRIFICE	2	4	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	7	45	42
ANIMAL MISSI NG			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	14	11
TOTAL PRIMARY TUMORS	3	17	11
TOTAL ANIMALS WITH BENIGN TUMORS	1	6	3
TOTAL BENIGN TUMORS	1	6	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	10	6
TOTAL MALIGN NT TUMORS	2	11	6
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECOND RY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALI NANT			2
TOTAL UNCERTAIN TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED PHOSPHAMIDON IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(10)	(50)	(49)
FIBROUS HISTIOCYTOMA, MALIGNANT			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (10%)	2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(49)
MALIGNANT LYMPHOMA, NOS			2 (4%)
LEUKEMIA,NOS			1 (2%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(10)	(49)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#LYMPH NODE	(9)	(44)	(45)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYST M			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(49)
HEPATOCELLULAR ADENOMA		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NEOPLASTIC NODULE			1 (2%)
HEPATOCELLULAR CARCINOMA		5 (10%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(48)	(42)
CHROMOPHOBE ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
#OVARY	(10)	(47)	(47)
CYSTADENOCARCINOMA, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(10)	(50)	(49)
PAPILLARY ADENOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH @		2	1
MORIBUND SACRIFICE		2	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	10	46	45
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	11	9
TOTAL PRIMARY TUMORS	1	11	10
TOTAL ANIMALS WITH BENIGN TUMORS	1	3	3
TOTAL BENIGN TUMORS	1	3	3
TOTAL ANIMALS WITH MALIGNANT TUMORS		8	6
TOTAL MALIGNANT TUMORS		8	6
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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 FED PHOSPHAMIDON IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED PHOSPHAMIDON IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(9)	(50)	(49)
GRANULOMA, NOS		2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG/ALVEOLI	(8)	(49)	(49)
CALCIFICATION, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(7)	(50)	(49)
FIBROSIS, FOCAL		3 (6%)	
INFARCT, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(8)	(50)	(49)
ARTERIOSCLEROSIS, NOS			1 (2%)
CALCIFICATION, FOCAL		1 (2%)	
#MYOCARDIUM	(8)	(50)	(49)
FIBROSIS			3 (6%)
FIBROSIS, FOCAL	1 (13%)		1 (2%)
#ENDOCARDIUM	(8)	(50)	(49)
FIBROSIS, DIFFUSE			1 (2%)
*AORTA	(9)	(50)	(49)
MEDIAL CALCIFICATION		1 (2%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(9)	(50)	(47)
FIBROSIS		2 (4%)	
#LIVER	(9)	(49)	(46)
DEGENERATION, NOS	1 (11%)		
DEGENERATION PARENCHYMATOUS	1 (11%)		
METAMORPHOSIS FATTY	1 (11%)	2 (4%)	4 (9%)
FOCAL CELLULAR CHANGE		1 (2%)	6 (13%)
ANGIECTASIS	1 (11%)		
#BILE DUCT	(9)	(49)	(46)
HYPERPLASIA, DIFFUSE		1 (2%)	
#PANCREAS	(7)	(47)	(49)
PERIARTERITIS			1 (2%)
ATROPHY, NOS			1 (2%)
#GASTRIC MUCOSA	(7)	(50)	(45)
CALCIFICATION, FOCAL		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(9)	(49)	(49)
GLOMERULONEPHRITIS, NOS	2 (22%)		
PYELONEPHRITIS, ACUTE		1 (2%)	
INFLAMMATION CHRONIC	3 (33%)	12 (24%)	18 (37%)
METAPLASIA, NOS		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(8)	(49)	(48)
CYST, NOS		2 (4%)	5 (10%)
HYPERPLASIA, NOS			2 (4%)
HYPERPLASIA, FOCAL		2 (4%)	4 (8%)
#ADRENAL	(8)	(49)	(49)
CYST, NOS		2 (4%)	
CONGESTION, NOS		1 (2%)	
HEMORRHAGE		2 (4%)	
#ADRENAL CORTEX	(8)	(49)	(49)
HYPERPLASIA, FOCAL	1 (13%)	3 (6%)	4 (8%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(8)	(48)	(47)
FOLLICULAR CYST, NOS		1 (2%)	
HYPERPLASIA, C-CELL		7 (15%)	3 (6%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	6 (13%)
#PARATHYROID	(2)	(27)	(39)
HYPERPLASIA, NOS			2 (5%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(7)	(45)	(47)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
#TESTIS	(9)	(49)	(48)
ATROPHY, NOS	1 (11%)	7 (14%)	15 (31%)
ATROPHY, FOCAL	1 (11%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(9)	(50)	(49)
PERIARTERITIS		2 (4%)	1 (2%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
FIBROSIS		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	9	4
AUTOLYSIS/NO NECROPSY	1		1

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED PHOSPHAMIDON IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALL IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(49)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(10)	(50)	(49)
BRONCHOPNEUMONIA, NOS			1 (2%)
PNEUMONIA, ASPIRATION			1 (2%)
GRANULOMA, FOREIGN BODY		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(9)	(50)	(48)
CONGESTION, NOS	1 (11%)		
HEMORRHAGE	1 (11%)		
ATROPHY, FOCAL			1 (2%)
HYPERPLASIA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(10)	(50)	(49)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(10)	(50)	(49)
INFARCT, FOCAL		1 (2%)	
METAMORPHOSIS FATTY		1 (2%)	
FOCAL CELLULAR CHANGE		3 (6%)	2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANGIECTASIS	1 (10%)		
#BILE DUCT	(10)	(50)	(49)
CYST, NOS			2 (4%)
HYPERPLASIA, NOS			2 (4%)
URINARY SYSTEM			
#KIDNEY	(10)	(50)	(49)
GLOMERULONEPHRITIS, NOS	1 (10%)		
PYELONEPHRITIS, ACUTE		1 (2%)	
INFLAMMATION CHRONIC	2 (20%)	2 (4%)	2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(50)	(45)
HYPERPLASIA, NOS		2 (4%)	
HYPERPLASIA, FOCAL		6 (12%)	4 (9%)
#ADRENAL	(10)	(50)	(48)
CYST, NOS			4 (8%)
HEMORRHAGE		6 (12%)	4 (8%)
DEGENERATION, CYSTIC		5 (10%)	1 (2%)
#ADRENAL CORTEX	(10)	(50)	(48)
DEGENERATION, CYSTIC		3 (6%)	1 (2%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	4 (8%)
ANGIECTASIS	1 (10%)		
#THYROID	(10)	(50)	(46)
HYPERPLASIA, C-CELL		4 (8%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(49)
DYSPLASIA, NOS			1 (2%)
*MAMMARY LOBULE	(10)	(50)	(49)
HYPERPLASIA, NOS		4 (8%)	1 (2%)
NERVOUS SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE OR ANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	15	13
AUTOLYSIS/NO NECROPSY			1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED PHOSPHAMIDON IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED PHOSPHAMIDON IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSI D	9	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(9)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
*SUBCUT TISSUE	(9)	(49)	(49)
FIBROSIS, FOCAL		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(9)	(49)	(45)
EMPHYSEMA, NOS			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM			2 (4%)
HEMATOPOIETIC SYSTEM			
#LYMPH NODE	(9)	(44)	(39)
INFLAMMATION, HEMORRHAGIC		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(9)	(49)	(46)
GRANULOMA, NOS	1 (11%)		
#PANCREATIC ACINUS	(8)	(49)	(44)
ATROPHY, NOS			1 (2%)
#PEYERS PATCH	(9)	(47)	(44)
INFLAMMATION, NOS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*COAGULATING GLAND	(9)	(49)	(49)
DILATATION, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	32	33

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
AUTO/NECROPSY/HISTO PERF			1
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY	1	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 FED PHOSPHAMIDON IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALL IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(49)
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(49)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	3 (6%)
#MESENTERIC L. NODE	(9)	(44)	(45)
INFLAMMATION, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
#PANCREAS	(10)	(49)	(49)
DILATATION/DUCTS		1 (2%)	1 (2%)
#STOMACH	(10)	(49)	(48)
HYPERPLASIA, EPITHELIAL		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(10)	(48)	(49)
CYST, NOS		1 (2%)	
AMYLOIDOSIS			1 (2%)
#URINARY BLADDER	(10)	(48)	(42)
INFLAMMATION, CHRONIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(48)	(42)
CYST, NOS			1 (2%)
HYPERPLASIA, CHROMOPHOBE-CELL			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(10)	(48)	(46)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, CYSTIC			1 (2%)
#OVARY/OVIDUCT	(10)	(48)	(46)
RETENTION FLUID		1 (2%)	
#OVARY	(10)	(47)	(47)
FOLLICULAR CYST, NOS	2 (20%)	1 (2%)	1 (2%)
INFLAMMATION, NOS	5 (50%)	3 (6%)	1 (2%)
INFLAMMATION, SUPPURATIVE		2 (4%)	
INFLAMMATION CHRONIC	1 (10%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	28	32
AUTO/NECROPSY/NO HISTO		1	
AUTOLYSIS/NO NECROPSY			1

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED PHOSPHAMIDON IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors
in Male Rats Fed Phosphamidon in the Diet (a)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Spleen: Hemangioma or Hemangiosarcoma (b)	1/7 (14)	1/91 (1)	3/50 (6)	5/49 (10)
P Values (c,d)	N.S.	P = 0.012	N.S.	P = 0.020**
Relative Risk (Matched Control) (f)			0.420	0.714
Lower Limit			0.045	0.110
Upper Limit			21.638	33.104
Relative Risk (Pooled Control) (f)			5.460	9.286
Lower Limit			0.451	1.076
Upper Limit			280.710	429.368
Weeks to First Observed Tumor	110		97	111
Pituitary: Chromophobe Adenoma (b)	2/8 (25)	13/81 (16)	8/49 (16)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			0.653	0.250
Lower Limit			0.182	0.038
Upper Limit			5.823	2.777
Relative Risk (Pooled Control) (f)			1.017	0.389
Lower Limit			0.391	0.074
Upper Limit			2.434	1.324
Weeks to First Observed Tumor	95		97	101

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Phosphamidon in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Adrenal:				
Cortical Adenoma (b)	0/8 (0)	2/90 (2)	6/49 (12)	2/49 (4)
P Values (c,d)	N.S.	N.S.	P = 0.023**	N.S.
Departure from Linear Trend (e)		P = 0.016		
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.302	0.055
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			5.510	1.837
Lower Limit			1.027	0.136
Upper Limit			53.857	24.532
Weeks to First Observed Tumor	--		103	101
Thyroid: C-cell Adenoma or Carcinoma (b)	1/8 (13)	3/81 (4)	7/48 (15)	5/47 (11)
P Values (c,d)	N.S.	N.S.	P = 0.031**	N.S.
Relative Risk (Matched Control) (f)			1.167	0.851
Lower Limit			0.198	0.124
Upper Limit			51.419	39.383
Relative Risk (Pooled Control) (f)			3.937	2.872
Lower Limit			0.945	0.584
Upper Limit			22.494	17.657
Weeks to First Observed Tumor	110		110	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Phosphamidon in the Diet (a)

(continued)

(a) Dosed groups received 80 or 160 ppm.

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

(c) Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P is less than 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 (*) or with the pooled-control group (**) when P is less than 0.05 for either control group; 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 is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the specified control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Phosphamidon in the Diet (a)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS (b)	2/10 (20)	6/88 (7)	0/50 (0)	0/45 (0)
P Values (c,d)	P = 0.008 (N)	P = 0.019 (N)	P = 0.025 (N)*	P = 0.030 (N)*
Departure from Linear Trend (e)	P = 0.001			
Relative Risk (Matched Control) (f)			0.000	0.000
Lower Limit			0.000	0.000
Upper Limit			0.667	0.739
Relative Risk (Pooled Control) (f)			0.000	0.000
Lower Limit			0.000	0.000
Upper Limit			1.104	1.223
Weeks to First Observed Tumor	111		--	--
Pituitary: Chromophobe Adenoma (b)	3/10 (30)	14/88 (16)	7/50 (14)	4/45 (9)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			0.467	0.296
Lower Limit			0.143	0.066
Upper Limit			2.518	1.824
Relative Risk (Pooled Control) (f)			0.880	0.559
Lower Limit			0.319	0.140
Upper Limit			2.152	1.650
Weeks to First Observed Tumor	110		83	111

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Phosphamidon in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Adrenal:				
Cortical Adenoma or Carcinoma (b)	2/10 (20)	6/89 (7)	7/50 (14)	6/48 (13)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			0.700	0.625
Lower Limit			0.172	0.144
Upper Limit			6.445	5.907
Relative Risk (Pooled Control) (f)			2.077	1.854
Lower Limit			0.629	0.521
Upper Limit			7.030	6.517
Weeks to First Observed Tumor	110		94	111
Thyroid: C-cell Adenoma or Carcinoma (b)	0/10 (0)	2/82 (2)	9/50 (18)	8/46 (17)
P Values (c,d)	N.S.	P = 0.003	P = 0.003**	P = 0.004**
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.587	0.555
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			7.380	7.130
Lower Limit			1.605	1.495
Upper Limit			67.559	66.171
Weeks to First Observed Tumor	--		106	73

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Phosphamidon in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma (b)	2/10 (20)	13/94 (14)	6/50 (12)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			0.600	0.306
Lower Limit			0.138	0.044
Upper Limit			5.679	3.435
Relative Risk (Pooled Control) (f)			0.868	0.443
Lower Limit			0.285	0.084
Upper Limit			2.272	1.510
Weeks to First Observed Tumor	111		77	64
Uterus: Endometrial Stromal Polyp (b)	0/10 (0)	7/90 (8)	1/48 (2)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.012	0.139
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			0.268	0.804
Lower Limit			0.006	0.139
Upper Limit			1.985	3.323
Weeks to First Observed Tumor	--		110	64

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Phosphamidon in the Diet (a)

(continued)

(a) Dosed groups received 80 or 160 ppm.

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

(c) Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P is less than 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 (*) or with the pooled-control group (**) when P is less than 0.05 for either control group; 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 is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the specified control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED PHOSPHAMIDON IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Phosphamidon in the Diet (a)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	1/9 (11)	6/85 (7)	3/49 (6)	2/45 (4)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			0.551	0.400
Lower Limit			0.055	0.025
Upper Limit			28.360	23.103
Relative Risk (Pooled Control) (f)			0.867	0.630
Lower Limit			0.145	0.064
Upper Limit			3.845	3.332
Weeks to First Observed Tumor	90		85	91
Liver: Hepatocellular Carcinoma or Adenoma, or Neoplastic Nodule (b)	2/9 (22)	19/88 (22)	9/49 (18)	7/46 (15)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			0.827	0.685
Lower Limit			0.229	0.174
Upper Limit			7.284	6.258
Relative Risk (Pooled Control) (f)			0.851	0.705
Lower Limit			0.364	0.267
Upper Limit			1.799	1.599
Weeks to First Observed Tumor	89		85	91

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Phosphamidon in the Diet (a)

(continued)

(a) Dosed groups received 80 or 160 ppm.

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

(c) Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P is less than 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 (*) or with the pooled-control group (**) when P is less than 0.05 for either control group; 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 less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the specified control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Phosphamidon in the Diet (a)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	1/10 (10)	5/88 (6)	3/49 (6)	1/49 (2)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			0.612	0.204
Lower Limit			0.060	0.003
Upper Limit			31.507	15.723
Relative Risk (Pooled Control) (f)			1.078	0.359
Lower Limit			0.172	0.008
Upper Limit			5.262	3.070
Weeks to First Observed Tumor	90		90	91
Liver: Hepatocellular Adenoma or Carcinoma, or Neoplastic Nodule (b)	0/10 (0)	3/87 (3)	6/49 (12)	1/49 (2)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.142 (N)	P = 0.013 (N)		
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.365	0.012
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			3.551	0.592
Lower Limit			0.793	0.011
Upper Limit			20.973	7.098
Weeks to First Observed Tumor	--		86	91

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Phosphamidon in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia (b)	0/10 (0)	2/89 (2)	2/50 (4)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.065	0.206
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			1.780	3.560
Lower Limit			0.132	0.525
Upper Limit			23.790	38.057
Weeks to First Observed Tumor	--		90	79

(a) Dosed groups received 80 or 160 ppm.

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

(c) Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P is less than 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 (*) or with the pooled-control group (**) when P is less than 0.05 for either control group; otherwise, not significant (N.S.) is indicated.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Phosphamidon in the Diet (a)

(continued)

(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 is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the specified control group.

APPENDIX G

ANALYSES OF FORMULATED DIETS FOR CONCENTRATIONS OF PHOSPHAMIDON

APPENDIX G

Analysis of Formulated Diets for Concentrations of Phosphamidon

A 10-g sample of the dosage mixture to be analyzed was shaken with 125 ml benzene at room temperature for 16 hours, then filtered through Celite with benzene washes and reduced in volume to 10 ml. After appropriate dilutions, the solution was quantitatively analyzed for phosphamidon by gas-liquid chromatography (electron capture detector, 10% DC-200 on Gas Chrom Q column). Recoveries were checked with spiked samples, and external standards were used for calibration.

Theoretical Concentrations in Diet (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
80	42	81.7	4.7%	74-89
160	40	161.5	3.9%	150-174

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

July 25, 1977

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 Phosphamidon for carcinogenicity.

The primary reviewer for the report on the bioassay of Phosphamidon said that the survival of the rats and mice was adequate. No significant difference in tumor incidence was found between treated mice and control animals. In treated rats, however, a statistically significant increase was reported in the incidence of hemangiosarcomas of the spleen and in C-cell adenomas of the thyroid. The primary reviewer pointed out that the incidence of C-cell adenomas was at least as high in control rats from the Dichlorvos bioassay (Technical Report No. 10), as well as in controls from another study reported in the literature. The Dichlorvos control rats also had an incidence of hemangiosarcomas of the spleen approaching the incidence found in the treated Phosphamidon animals. The primary reviewer said that the data were inadequate to classify Phosphamidon as a carcinogen. He suggested, however, that Phosphamidon might promote the incidence of spontaneously occurring tumors.

A Program staff member commented that the finding of two different tumor types of borderling significance, as opposed to only one, took on greater meaning and was the basis for the conclusion that Phosphamidon

was carcinogenic in rats. Another Program staff member said that the difference between the Dichlorvos and Phosphamidon control rats could be related to variations between diagnoses made by the pathologists. It also was suggested that a difference in tumor incidence could have resulted from random variations in animals or from missed tumors when tissues were selected or processed. Although pathologists may use varying terminology for the same lesion, a staff pathologist said that a difference in incidence probably was not related to variations in the ability of diagnostic pathologists to detect tumors.

A Subgroup member questioned the significance of the dose-related trend reported for tumors found in treated rats. He emphasized the need to compare the treated animals with a program pool of control data. He noted that it is only in this way that low incidence tumors can be properly evaluated. Another Subgroup member suggested that questionable studies could be reexamined after the control data are accumulated and analyzed.

It was moved that the results from the bioassay should be considered to be equivocal and, therefore, no conclusion drawn on the carcinogenicity of Phosphamidon. The motion was seconded and approved unanimously.

Members present were:

Gerald Wogan (Chairman), Massachusetts Institute of Technology
Arnold Brown, Mayo Clinic
E. Cuyler Hammond, American Cancer Society
Henry C. Pitot, McArdle Laboratory for Cancer Research
Verald K. Rowe, Dow Chemical USA
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation
Sidney Wolfe, Health Research Group

---

* 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.

☆U.S. GOVERNMENT PRINTING OFFICE: 1978-281-217/3272