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

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FOREWORD: This report presents the results of the bioassay of formulated calcium cyanamide 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 chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that a test chemical is carcinogenic for animals under the conditions of the test and indicates 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 calcium cyanamide was conducted at the NCI Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, operated for NCI (2) by Litton Bionetics, Inc.

The manager of the bioassay at FCRC was Dr. B. Ulland, the toxicologist was Dr. E. Gordon, and Drs. R. Cardy and D. Creasia compiled the data. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for management of the facilities. Mr. A. Butler performed the computer services. Histopathologic evaluations for rats and mice were performed by Dr. J. L. Stookey, and the diagnoses included in this report represent his interpretations.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (3). Statistical analyses were performed by Dr. J. R. Joiner (4) and Ms. P. L. Yong (4), using methods selected for the bioassay program by Dr. J. J. Gart (5).

The chemicals used in this bioassay were analyzed at FCRC by Dr. W. Zielinsky. The chemical analyses and narrative were reviewed and approved by Dr. W. Lijinsky (1).

This report was prepared at Tracor Jitco (4) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Owen, Ms. M. S. King, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

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. Richard A. Griesemer, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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(1) Frederick Cancer Research Center, P.O. Box B, Frederick, Maryland.

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

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

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

(5) 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.

SUMMARY

A bioassay of formulated calcium cyanamide for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered a commercial formulation containing 63% calcium cyanamide in the diet at one of two doses, either 100 or 200 ppm for the males and either 100 or 400 ppm for the females, for 107 weeks. Groups of 50 mice of each sex were administered the test chemical at one of two doses, either 500 or 2,000 ppm, for 100 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the dosed rats and mice were only slightly lower than those of corresponding controls, except for the low-dose female mice, whose mean body weights were unaffected by the test chemical. Mortality was dose related only in the male mice. Survival was 70% or greater in all dosed and control groups of each species and sex at the end of the bioassay, and sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors. Both rats and mice may have been able to tolerate higher doses.

No tumors occurred in the dosed rats of either sex at incidences that could clearly be related to administration of the calcium cyanamide. However, in the subchronic studies performed with the rats, calcium cyanamide was found to cause diffuse follicular hyperplasia of the thyroid, with periglandular fibrosis and prominent periglandular vascularity.

In male mice, hemangiosarcomas were dose related in the males ($P = 0.006$); however, in direct comparisons, incidences in the individual dosed groups were not significantly higher than those in the control group (controls 1/20 (5%); low-dose 2/50 (4%); high-dose 10/50 (20%)). The incidence of these tumors in historical-control male B6C3F1 mice was (13/323 (4%)), and the highest incidence observed was 2/19 (10%). In the female mice, lymphomas or leukemias were dose related ($P = 0.009$), and in a direct comparison the incidence of these tumors in the high-dose group was significantly higher ($P = 0.006$) than that in the control group (controls 1/20 (5%); low-dose 11/46 (24%); high-dose 18/50 (36%)); however, the incidence of the lymphomas or leukemias in historical-control female B6C3F1 mice was 67/324

(21%), suggesting that the incidence of these tumors in the matched-control group of the present bioassay may have been abnormally low. Thus, neither the incidences of hemangiosarcomas of the circulatory system in the male mice nor of lymphomas or leukemias in the female mice can clearly be related to administration of the test chemical.

It is concluded that under the conditions of this bioassay, the test formulation of calcium cyanamide was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

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I. INTRODUCTION

Calcium cyanamide (CAS 156-62-7; NCI C02937) was first synthesized in 1898 and became one of the earliest successes in nitrogen fixation (Hardesty and Hein, 1974). The commercially formulated product contains approximately 65% calcium cyanamide, which is 20 to 24% nitrogen. For most of the 20th century it has been used as a fertilizer, and also as a cotton defoliant, herbicide, and soil insecticide (EPA, 1969; Spencer, 1973; Mooney and Quin, 1965). Its use as a fertilizer has diminished in recent years due to the introduction of other compounds (Pratt, 1974), so that the chief industrial uses of calcium cyanamide today stem from the reactivity of the nitrile group. Calcium cyanamide can be dimerized to dicyandiamide, an intermediate for melamine, one of the basic ingredients in amino plastics and resins. Other products prepared from calcium cyanamide include urea, thiourea, and guanidine. Fusion of calcium cyanamide with sodium chloride produces calcium cyanide, which is required for ore processing and the production of ferrocyanides. Calcium cyanamide is added to pig iron to impart nitrogen and to remove sulfur from steel (Mooney and Quin, 1965; Noller, 1966).

CaNCN

CALCIUM CYANAMIDE

The calcium cyanamide used in the United States today is imported, mostly from Canada (U.S. Department of Commerce, 1978). Between 1975 and 1977 the total volume of imports dropped from 144 million to 3 million pounds per year (U.S. Department of Commerce, 1978).

Calcium cyanamide was tested by Innes et al. (1969) in a large-scale screen of industrial compounds for carcinogenic activity. Based on the results of this preliminary bioassay which suggest a carcinogenic effect in mice, calcium cyanamide was selected for further testing in the Carcinogenesis Testing Program.

II. MATERIALS AND METHODS

A. Chemical

One batch of calcium cyanamide (cyanamide; CaNCN) was obtained from Eastman Chemical Co., Kingsport, Tennessee, as a fine, gray-black powder. This commercial formulation also contains carbon and calcium oxide, and the manufacturer's specifications for this formulation cover the ranges of 48 to 66% calcium cyanamide, 12 to 16% calcium oxide, 11 to 13% free carbon, and 0 to 4% water. The mean values obtained by elemental analysis of the test material were 21% carbon, 0.5% hydrogen, 22% nitrogen, and by atomic absorption analysis, 47% calcium. From a material balance, these data gave a mean composition of 63% calcium cyanamide, 22% calcium oxide, 12% free carbon, and 3% water. The infrared spectrum of the test material was consistent with the presence of calcium cyanamide in the test material. Traces of selenium, nickel, and chrominum were detected by atomic absorption and x-ray spectrometric analyses.

This commercially formulated product is referred to in this report as calcium cyanamide.

B. Dietary Preparation

Test diets containing calcium cyanamide were prepared every 1 to 1-1/2 weeks in 6- to 12-kg batches at the appropriate doses. A known weight of the chemical was first mixed with an equal weight of autoclaved Wayne® Sterilizable Lab Meal containing 4% fat (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The mixing was continued with second and third additions of feed, and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly® twin-shell blender. The diets were routinely stored at 7°C until used.

C. Animals

Male and female F344 (Fischer) rats and B6C3F1 mice were obtained as 4-week-old weanlings, all within 3 days of the same age, from the NCI Frederick Cancer Research Center (Frederick, Md.). The animals were housed within the test facility for 2 weeks and then were assigned four rats of the same sex to a cage and five mice of the same sex to a cage. The male rats used in the chronic study weighed 90 to 105 g, averaging at least 100 g; for female rats, 80 to 95 g, averaging at least 90 g; for male mice, 18 to

22 g, averaging at least 19.5 g; and for female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products, Inc., Garfield, N.J.), 19 x 10-1/2 x 8 inches for the rats and 11-1/2 x 7-1/2 x 5 inches for the mice. The cages were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven polyester-fiber 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri® hardwood chips (Northeastern Products, Inc., Warrenburg, N.Y.). The feed supplied was presterilized Wayne® Sterilizable Lab Meal containing 4% fat, provided ad libitum in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied ad libitum from glass bottles with sipper tubes (Lab Products, Inc.) through the tops of the cages.

The contaminated bedding was disposed of through an enclosed vacuum line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized

twice per week and the feed hoppers twice per month at 82 to 88°C in a tunnel-type cagewasher (Industrial Washing Corp., Mataway, N. J.), using the detergents, Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.).

The water bottles and sipper tubes were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The animal rooms were maintained at 22 to 24°C and 45 to 55% relative humidity. Fresh air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and was expelled without recirrculation through a "Z"-type roughing filter of 30% efficiency and a bag system of 90 to 95% efficiency (American Air Filters, Louisville, Ky.; Mine Safety Appliances, Pittsburgh, Pa.). Room air was changed 15 times per hour. The air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided automatically on a 12-hour-per-day cycle.

Rats administered calcium cyanamide and their corresponding controls were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 3165-93-3) 4-chloro-o-toluidine hydrochloride
(CAS 86-30-6) N-nitrosodiphenylamine

Mice administered calcium cyanamide and their corresponding controls were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 999-81-5) (2-chloroethyl)trimethylammonium chloride (CCC)
(CAS 95-80-7) 2,4-diaminotoluene
(CAS 19010-66-3) lead dimethyldithiocarbamate
(CAS 86-30-6) N-nitrosodiphenylamine
(CAS 88-96-0) phthalamide
(CAS 120-62-7) piperonyl sulfoxide
(CAS 137-17-7) 2,4,5-trimethylaniline

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of calcium cyanamide, 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. Groups of five rats and five mice of each sex were fed diets containing calcium cyanamide at one of several doses, and groups of five control animals of each species and sex were administered basal diet only. Two separate studies were

performed for the rats. The test chemical was administered for 7 weeks, followed by 1 week of additional observation. Each animal was weighed twice per week. Tables 1 and 2 show the doses fed, the survival of animals in each dosed group at the end of the study, and the mean body weights of dosed animals at week 7, expressed as percentages of mean body weights of controls.

At the end of the subchronic studies, all animals were killed using CO_2 and necropsied. During histopathologic examination, trace to moderate amounts of bile-duct hyperplasia were observed in the livers of the male and female rats dosed at 1,500, 3,000, and 4,000 ppm. There were no hepatic lesions at 1,200 ppm. A very slight to moderate increase in extramedullary hematopoiesis was found in the spleens of rats of each sex.

Thyroid glands of male and female rats dosed at 4,000 ppm were enlarged two to three times the normal size. Histologic examination disclosed marked diffuse follicular hyperplasia. The thyroid glands often showed prominent periglandular vascularity and periglandular fibrosis, the latter extending into the gland to produce partitioning or pseudolobulation (interstitial fibrosis). The greater than usual crowding of the follicular epithelium resulted in a tendency towards epithelial disorganization within the follicle. The epithelial nuclear to cytoplasmic

Table 1. Calcium Cyanamide (a) Subchronic Feeding Studies in Rats

	Male		Female	
Dose (ppm)	Survival (b)	Mean Weight at Week 7 as % of Control	Survival (b)	Mean Weight at Week 7 as % of Control
First Study				
0	5/5	100	5/5	100
1,500	5/5	76	5/5	82
3,000	5/5	53	5/5	68
4,000	5/5	46	5/5	58
8,000	0/5		0/5	
10,000	0/5		0/5	
16,000	0/5		0/5	
30,000	0/5		0/5	
Second Study				
0	5/5	100	5/5	100
400	5/5	89	5/5	97
600	5/5	84	5/5	91
800	5/5	94	5/5	96
900	5/5	82	5/5	87
1,000	5/5	76	5/5	84
1,200	5/5	94	5/5	98
1,500	5/5	70	5/5	81

(a) Commercially formulated product (see "Introduction" and "Chemical" sections, above).
(b) Number surviving/number in group.

Table 2. Calcium Cyanamide (a) Subchronic Feeding Studies in Mice

Dose (ppm)	Male		Female	
	Survival (b)	Mean Weight at Week 7 as % of Control	Survival (b)	Mean Weight at Week 7 as % of Control
0	5/5	100	5/5	100
1,500	5/5	92	5/5	87
3,000	5/5	93	5/5	87
4,000	5/5	92	5/5	85
8,000	5/5	79	5/5	83
10,000	5/5	78	5/5	83
16,000	5/5	66	5/5	78
30,000	0/5		0/5	

(a) Commercially formulated product (see "Introduction" and "Chemical" sections, above).

(b) Number surviving/number in group.

ratio was diminished in comparison with control thyroid tissue. Aggregates of tiny follicles and solid clusters of follicular epithelium were observed to penetrate the heavy layer of periglandular collagen; however, no penetration beyond the capsule was observed.

Thyroid hyperplasia was noted in rats dosed at 600, 800, 900, 1,000, 1,200, 1,500, or 3,000 ppm and was considered to be dose related. Abundant pale-staining colloid was prominent at 1,200 ppm and at lower concentrations, suggesting colloid goiter rather than true follicular hyperplasia. At 400 ppm, trace to very small amounts of hyperplasia with excess colloid formation were found in three male rats and one female.

Histologic examination of male and female mice showed trace amounts of bile-duct hyperplasia at 16,000 ppm. Periportal hepatocytes with pale-staining vacuolated cytoplasm were seen in the males. Focal hepatic necrosis occurred in four females. Livers of the groups at 10,000 ppm were essentially normal.

Ten percent depression in body weight was a major criterion for estimation of MTD's in mice. The doses required to produce this response were determined by the following procedure: first, least squares regressions of mean body weights versus days on study were

used to estimate mean body weights of each of the dosed groups at day 49. Next, probits of the percent weights of dosed groups at day 49 relative to weights of corresponding control groups were plotted against logarithms of the doses, and least squares regressions fitted to the data were used to estimate the doses required to induce 10% depression in weight.

The low and high doses for the chronic studies were set at 100 and 200 ppm for male rats, 100 and 400 ppm for female rats, and 500 and 2,000 ppm for male and female mice.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in tables 3 and 4.

G. Clinical and Pathologic Examinations

All animals were observed twice daily. Observations for sick, tumor-bearing, and moribund animals were recorded daily. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least once per month, except for

Table 3. Calcium Cyanamide Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	Calcium Cyanamide (b) in Diet (c) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	107
Low-Dose	50	100	107
High-Dose	50	200	107
Female			
Matched-Control	20	0	107
Low-Dose	50	100	107
High-Dose	50	400	107

(a) All animals were 6 weeks of age when placed on study.

(b) Commercially formulated product (see "Introduction" and "Chemical" sections, above).

(c) Test and control diets were provided ad libitum 7 days per week. The commercial formulation used contained 63% calcium cyanamide calculated from elemental analysis of the test material.

Table 4. Calcium Cyanamide Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals (a)	Calcium Cyanamide (b) in Diet (c) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	100
Low-Dose	50	500	100
High-Dose	50	2,000	100
Female			
Matched-Control	20	0	100
Low-Dose	50	500	100
High-Dose	50	2,000	100

(a) All animals were 6 weeks of age when placed on study.

(b) Commercially formulated product (see "Introduction" and "Chemical" sections, above).

(c) Test and control diets were provided ad libitum 7 days per week. The commercial formulation used contained 63% calcium cyanamide calculated from elemental analysis of the test material.

weeks 50 to 80, when weights were not recorded for the rats. Moribund animals and animals and that survived to the end of the bioassay were killed using CO_2 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. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestines, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain (cerebrum and cerebellum), and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

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, 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 appropriate 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 section.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend.

One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The

Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

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

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

obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

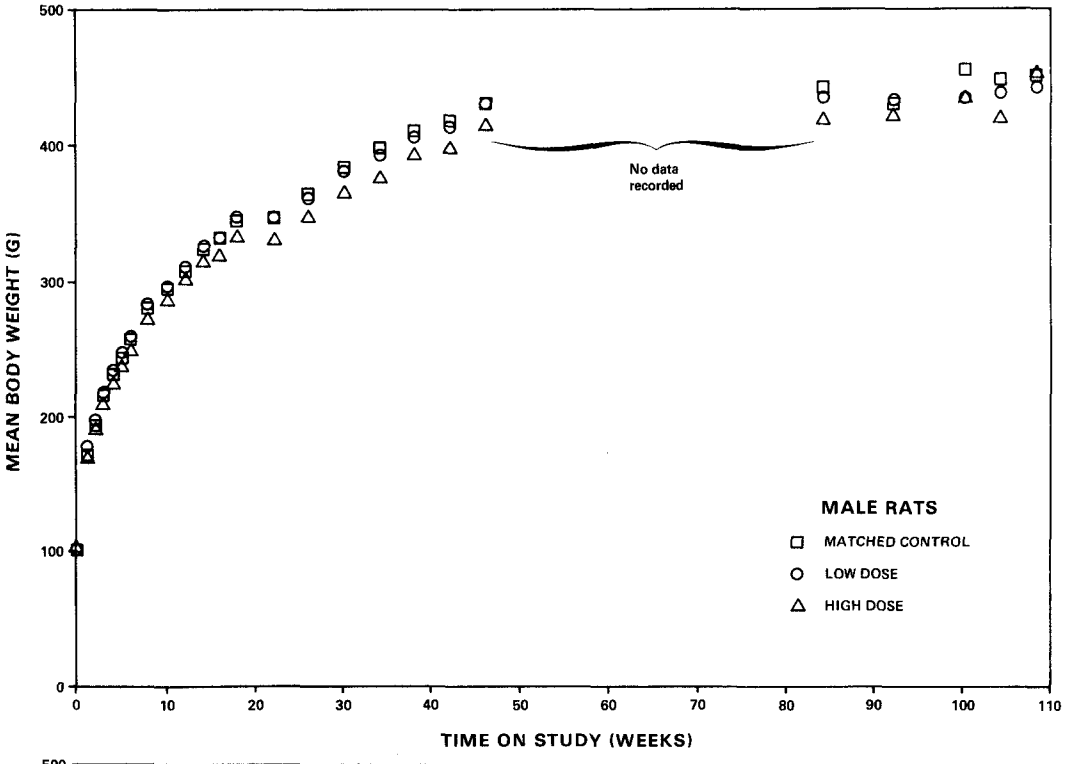
A. Body Weights and Clinical Signs (Rats)

Mean body weights of the high-dose male and female rats were slightly lower than those of the corresponding controls, but the effect on weights of the low-dose groups was slight and inconsistent (figure 1).

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered calcium cyanamide in the diet at the doses of this bioassay, with those for the matched controls, are shown by the Kaplan and Meier curves in figure 2. The result of the Tarone test for positive dose-related trend in mortality is not significant in either sex.

In male rats, 14/20 (70%) of the controls, 35/50 (70%) of the low-dose group, and 39/50 (78%) of the high-dose group lived to the end of the bioassay. In female rats, 18/20 (90%) of the controls, 41/50 (82%) of the low-dose group, and 41/50 (82%) of the high-dose group lived to the end of the bioassay.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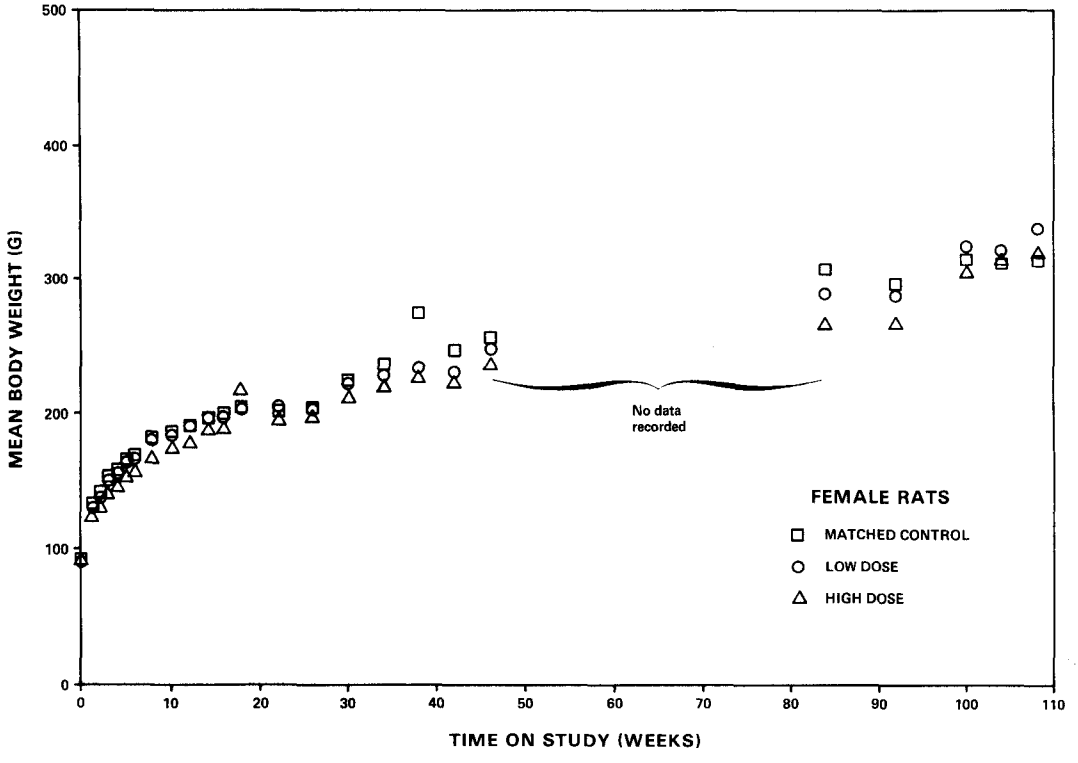
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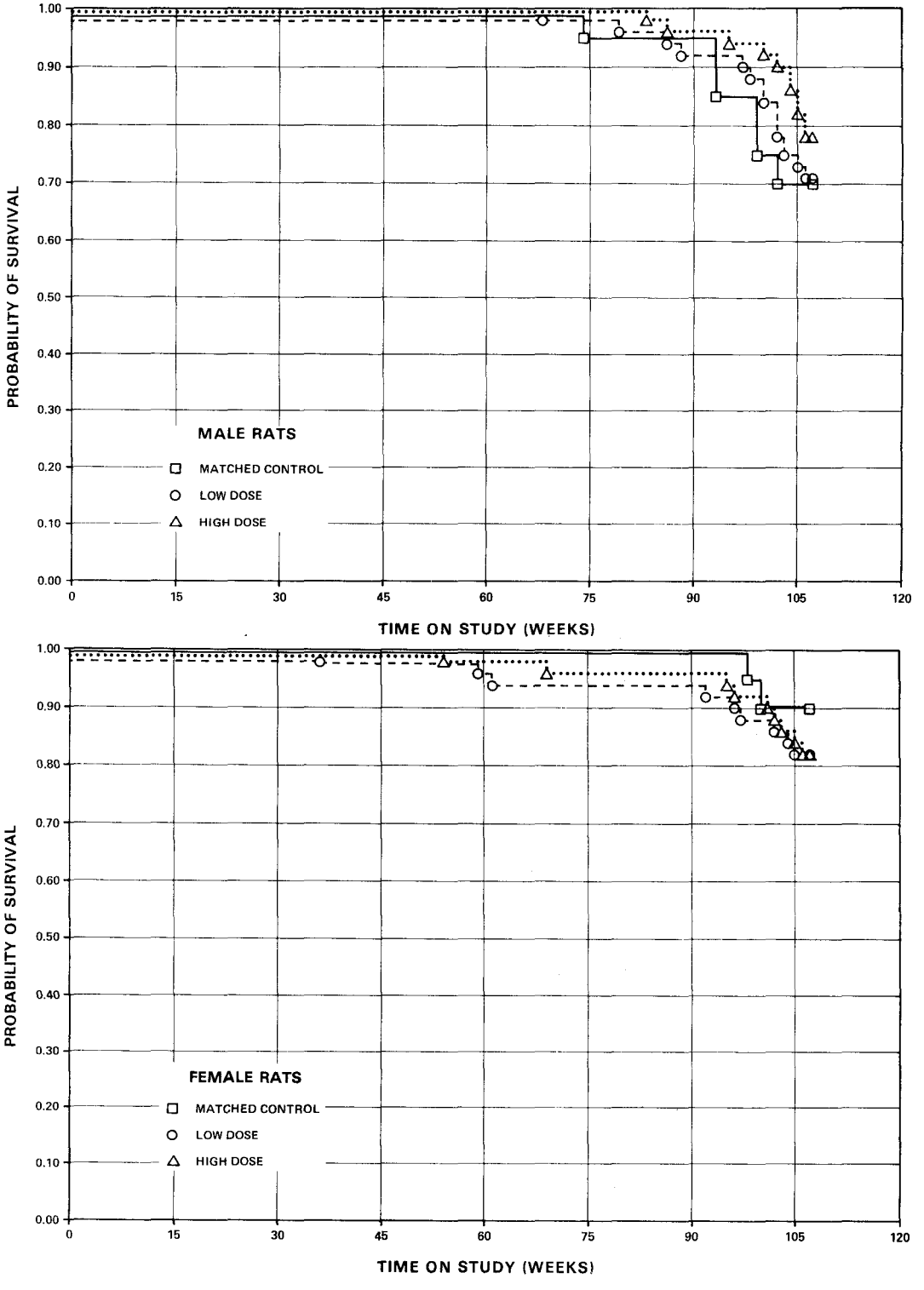

Figure 2. Survival Curves for Rats Administered Calcium Cyanamide in the Diet

Sufficient numbers of animals in all groups were at risk for the development of late-appearing tumors.

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.

The sites of neoplasms observed most frequently were the adrenal, pituitary, thyroid, and testes. The pituitary neoplasms and adenomas (and hyperplasias) of the thyroid, and interstitial-cell tumors of the testes, although high in numbers, occurred with comparable frequency in control and dosed rats. The incidences of adrenal neoplasms are summarized in the table below:

	Cortical Adenoma	Pheochromocytomas	Pheochromocytoma, Malignant
Males			
Control	0/20 (0%)	4/20 (20%)	0/20 (0%)
Low-Dose	3/49 (6%)	10/49 (20%)	0/49 (0%)
High-Dose	3/50 (6%)	15/50 (30%)	1/50 (2%)
Females			
Control	3/19 (16%)	0/19 (0%)	0/19 (0%)
Low-Dose	1/50 (2%)	4/50 (8%)	0/50 (0%)
High-Dose	7/50 (14%)	6/50 (12%)	1/50 (2%)

While the number of cortical tumors in the dosed animals appeared to be balanced by similarly affected control rats, the incidence of pheochromocytomas in the dosed females appears to be greater than the normal incidence and may be related to administration of the test chemical.

A variety of nonneoplastic lesions and disorders were encountered with regularity in both control and dosed groups of rats. Such lesions are common in aged F344 rats.

Although the incidence of adrenal medullary tumors was high in the dosed rats, the conclusion, based on the histopathologic examination, is equivocal. Calcium cyanamide is not clearly carcinogenic in F344 rats under the conditions of the 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 of one group and at an incidence of at least 5% in one or more than one group.

In male rats, the results of the Cochran-Armitage test for the

incidence of lymphomas, leukemias, or neoplasms, NOS, of the hematopoietic system does not indicate a dose-related trend; however, a departure from linear trend is indicated (P = 0.003), since the incidence of 18/50 (36%) in the low-dose group is higher than that in either the high-dose group (7/50 (14%)) or the control group (2/20 (10%)). The results of the Fisher exact test indicate a significant (P = 0.025) difference in the incidences of tumors in the low-dose and control groups. The historical records of this laboratory indicate an incidence of lymphomas, leukemias, or neoplasms, NOS, in male F344 rats of 87/416 (21%). In female rats, the incidence of this type of tumor is 6/20 (30%) in the controls, 11/50 (22%) in the low-dose group, and 9/50 (18%) in the high-dose group, and none of the statistical tests indicate significant results. Taking the historical records and the results in the high-dose male groups into account, the incidence observed in the low-dose males cannot clearly be related to the administration of the test chemical.

In female rats, the result of the Cochran-Armitage test for dose-related trend in the incidence of adenocarcinomas of the mammary gland is significant (P = 0.042), but the results of the Fisher exact test are not significant. When the incidence of either adenocarcinoma or adenoma of the mammary gland in female rats is

analyzed, the results of neither the Cochran-Armitage test nor the Fisher exact test are significant.

The incidences of adrenal pheochromocytomas in female rats were not significant either by the Cochran-Armitage test for dose-related trend or by direct comparison of the dosed groups with the controls.

The results of the Cochran-Armitage test indicate a negative trend in the combined incidence of acidophil adenoma or carcinoma of the pituitary in male rats and in the incidences of endometrial stromal polyp of the uterus in female rats. Since survivals in both male and female dosed groups were comparable to survivals in their respective control groups, these negative trends cannot be attributed to shortened survival in any group.

In summary, no tumor at any site in the rats can clearly be associated with the administration of calcium cyanamide in this bioassay.

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 calcium cyanamide, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the dosed male and female mice were slightly lower than those of the corresponding controls, except for the low-dose females, whose mean body weights were unaffected (figure 3).

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered calcium cyanamide in the diet at the doses of this bioassay, together with those for the matched controls, are shown by the Kaplan and Meier curves in figure 4. The result of the Tarone test for positive dose-related trend in mortality is significant in the male mice ($P = 0.005$) but not in the females.

In male mice, 20/20 (100%) of the control group, 45/50 (90%) of the low-dose group, and 38/50 (76%) of the high-dose group lived to the end of the bioassay. In females, 18/20 (90%) of the control group, 43/50 (86%) of the low-dose group, and 46/50 (92%) of the high-dose group lived to the end of the bioassay.

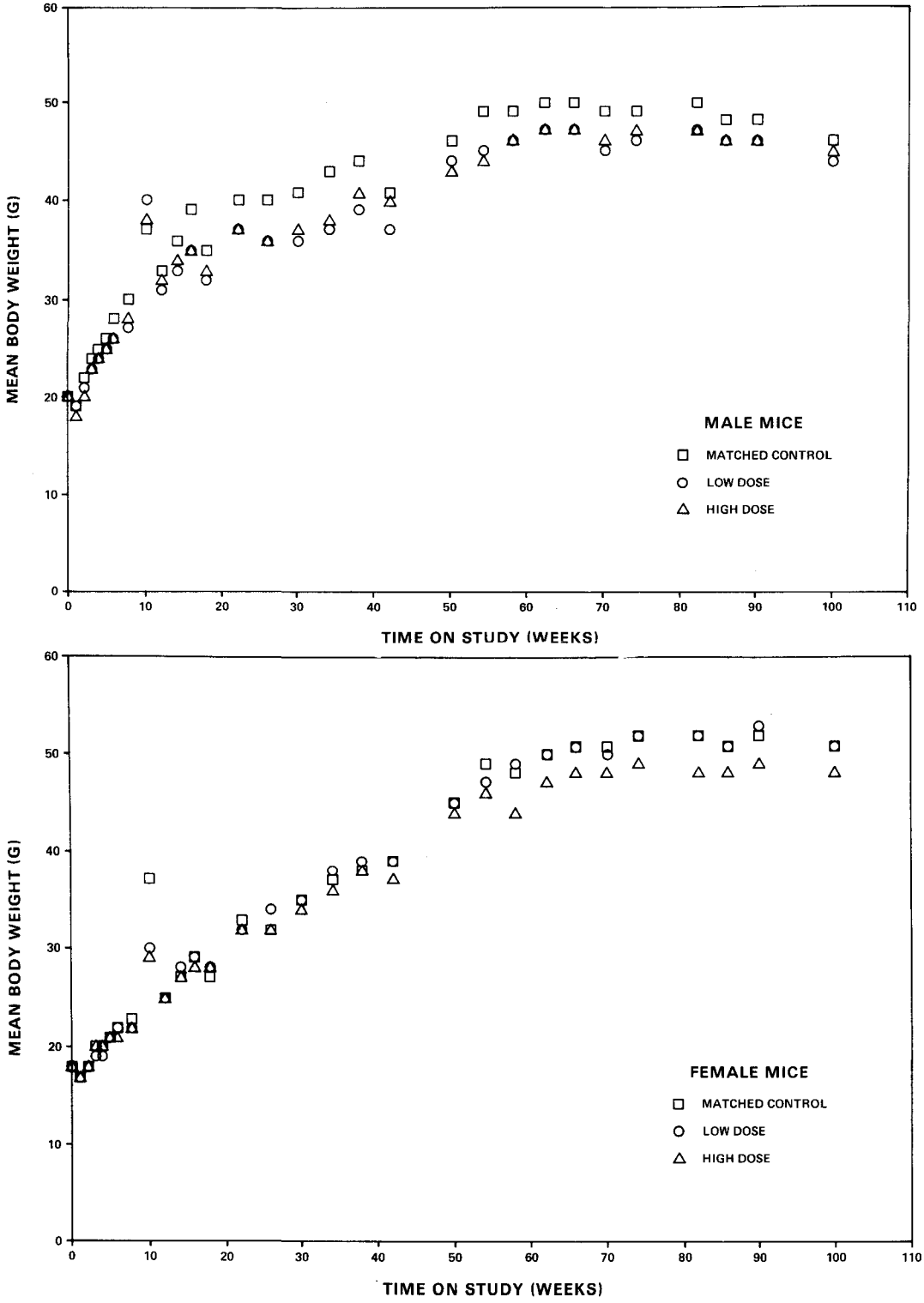

Figure 3. Growth Curves for Mice Administered Calcium Cyanamide in the Diet

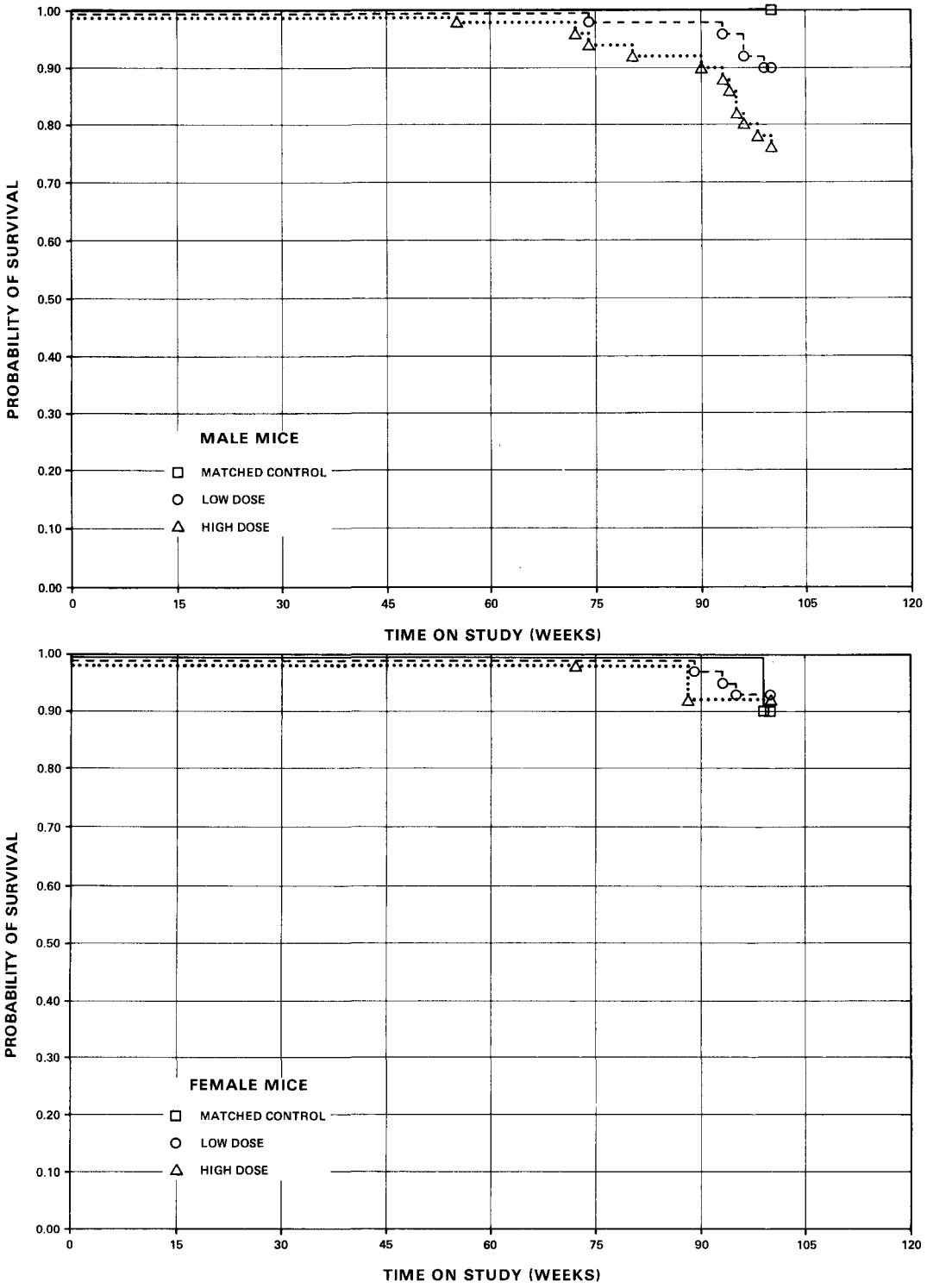

Figure 4. Survival Curves for Mice Administered Calcium Cyanamide in the Diet

Sufficient numbers of animals in all groups were at risk for the 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.

Several groups of neoplasms occurred in considerable numbers, and these included hepatocellular and lung neoplasms, hemangiosarcomas of various organs, and malignant lymphomas. With the exception of the hemangiosarcomas and the malignant lymphomas, the remaining neoplasms occur with similar frequencies in control and dosed animals or are commonly encountered in aged mice of this strain. There was an increased incidence of certain hematopoietic neoplasms in male and female mice. Hemangiosarcomas were present in 2/50 (4%) low-dose and 10/50 (20%) high-dose male mice but in only 1/20 (5%) control male mice. Malignant lymphomas was found in 11/46 (24%) low-dose, 16/50 (32%) high-dose, and 1/20 (5%) control female mice.

A variety of nonneoplastic lesions common in aged B6C3F1 mice were encountered in both control and dosed groups of mice.

Based on the histopathologic examination, calcium cyanamide may be carcinogenic in B6C3F1 mice inducing a high incidence of hemangiosarcomas in male B6C3F1 mice and malignant lymphoma in female 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.

In male mice, the results of the Cochran-Armitage test indicate a dose-related linear trend ($P = 0.006$) in the incidence of hemangiosarcomas of all sites, but the results of the Fisher exact test are not significant. The current historical records of bioassays at this laboratory indicates an incidence of hemangiosarcomas in control groups of 13/323 (4%), and the highest incidence seen in any control group is 2/19 (10%), compared with 10/50 (20%) in the high-dose male rats of this study. Overall,

the incidence of this neoplasm in the high-dose group suggests an association of these tumors with the administration of the chemical, but the absence of significant results in the Fisher exact test fails to confirm this association.

In female mice, the results of the Cochran-Armitage test indicate a dose-related trend (P = 0.009) in the incidence of lymphoma or leukemia; also, the results of the Fisher exact test establish a significantly higher incidence (P = 0.006) in the high-dose group (18/50 (36%)) than in the control group (1/20 (5%)). The incidence of lymphomas or leukemias in historical-control female B6C3F1 mice at this laboratory is 67/324 (21%). Thus, the incidences of these tumors in the matched-control group of the present bioassay may be abnormally low.

Significant results in the negative direction are observed in the incidences of hepatocellular tumors in the male mice.

V. DISCUSSION

Mean body weights of the dosed rats and mice were only slightly lower than those of corresponding controls, except for the low-dose female mice, whose mean body weights were unaffected by the test chemical. Mortality was dose related only in the male mice. Survival was 70% or greater in all dosed and control groups of each species and sex at the end of the bioassay, and sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors. Both rats and mice may have been able to tolerate higher doses.

In the subchronic studies performed with the rats, calcium cyanamide was found to cause diffuse follicular hyperplasia of the thyroid, with periglandular fibrosis and prominent periglandular vascularity. The effects were considered to be dose related over the range of 600 to 4,000 ppm, and the effects of the high dose of 4,000 ppm were considered to be preneoplastic. At doses of 1,200 ppm and lower, the appearance of the lesions suggested colloid goiter. At 400 ppm, very small amounts of hyperplasia, together with excess colloid, were observed in three male rats and one female. These lesions were the basis for the doses of 100 to 400 ppm set for the chronic studies in the rats.

In the chronic studies performed with the rats, no neoplasms occurred at incidences that could clearly be associated with administration of the test chemical.

In the chronic studies performed with the mice, hemangiosarcomas were dose related in the males (P = 0.006); however, in direct comparisons, incidences in the individual dosed groups were not significantly higher than those in the control group (controls 1/20, (5%); low-dose 2/50, (4%); high-dose 10/50, (20%)). The incidence of the tumors in historical-control male B6C3F1 mice was 13/323 (4%), and the highest incidence observed was 2/19 (10%). In the female mice, lymphomas or leukemias were dose related (P = 0.009), and in a direct comparison the incidence of these tumors in the high-dose group was significantly higher (P = 0.006) than that in the control group (controls 1/20, (5%); low-dose 11/46, (24%); high-dose 18/50, (36%)); however, the incidence of the lymphomas or leukemias in historical-control female B6C3F1 mice was 67/324 (21%), suggesting that the incidence of these tumors in the matched-control group of the present bioassay may have been abnormally low. Thus, neither the incidences of hemangiosarcomas of the circulatory system in the male mice nor of lymphomas or leukemias in the female mice can clearly be related to administration of the test chemical.

In previous tests for tumorigenicity of calcium cyanamide (Innes et al., 1969; National Technical Information Service, 1968), it was reported that when technical-grade calcium cyanamide was administered by stomach tube daily for 3 weeks at 100 mg/kg, then in the diet at 240 ppm for 18 months, to hybrid mice (B6C3F1 and B6AKF1), an elevated incidence of reticulum-cell sarcomas ($P = 0.01$) was observed in the B6C3F1 hybrids.

It is concluded that under the conditions of this bioassay, the test formulation of calcium cyanamide was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

VI. BIBLIOGRAPHY

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

Berenblum. I., ed., Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of the UICC, Vol. 2. International Union Against Cancer, Geneva, 1969.

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

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

Environmental Protection Agency, EPA Compendium of Registered Pesticides, Environmental Protection Agency, Washington, D.C., 1969, p. I-C-5.

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Stat. Inst. 39:148-169, 1971.

Hardesty, J. Q. and Hein, L. B., Phosphates, phosphorus; fertilizers, potassium salts, natural organic fertilizers, urea. In: Riegel's Handbook of Industrial Chemistry, Seventh Edition, Kent, J. A., ed., Van Nostrand Reinhold Co., New York, 1974, pp. 458, 537, and 554-558.

Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I. and Peters, J., Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J. Natl Cancer Inst. 42:1101-1114, 1969.

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

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

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Mooney, R. B. and Quin, J. P., Cyanamides. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 6, Mark, H. F., McKetta, J. J., Jr., Othmer, D. F., and Standen, A., eds., John Wiley & Sons, Inc., New York, 1965, pp. 553-601.

National Technical Information Service, Evaluation of Carcinogenic, Teratogenic, and Mutagenic Activities of Selected Pesticides and Industrial Chemicals, Vol. 1, National Technical Information Service, Springfield, Va., 1968, p. 64.

Noller, C. R., Derivatives of carbonic acid and of thiocarbonic acid. In: Chemistry of Organic Compounds, 3rd Edition, W. B. Saunders Co., Philadelphia, 1966, pp. 341-343.

Pratt, C. J., Fertilizers. In: Chemical and Process Technology Encyclopedia, Considine, D. M., ed., McGraw-Hill Book Co., New York, 1974, pp. 470-475.

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

Spencer, E. Y., Calcium cyanamide. In: Guide to the Chemicals Used in Crop Protection, Publication 1093, University of Western Ontario Sub Post Office, London, Ontario, 1973, p. 73.

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

U.S. Department of Commerce, Personal communication, 1978.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
SQUAMOUS CELL CARCINOMA		2 (4%)	3 (6%)
TRICHOEPITHELIOMA		1 (2%)	3 (6%)
*SUBCUT TISSUE	(20)	(50)	(50)
TRICHOEPITHELIOMA		1 (2%)	
FIBROMA	1 (5%)	3 (6%)	1 (2%)
LIPOMA		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	6 (12%)	4 (8%)
FIBROSARCOMA, METASTATIC	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA,NOS			1 (2%)
MONOCYTIC LEUKEMIA	2 (10%)	6 (12%)	1 (2%)
*HEMATOPOIETIC SYSTEM	(20)	(50)	(50)
NEOPLASM, NOS		12 (24%)	3 (6%)
#BONE MARROW	(20)	(50)	(43)
FIBROSARCOMA, METASTATIC	1 (5%)		
*SPLEEN	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LYMPH NODE	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR, METASTA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
HEPATOCELLULAR ADENOMA	1 (5%)	3 (6%)	1 (2%)
FIBROSARCOMA, METASTATIC	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
FIBROSARCOMA, METASTATIC	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(47)	(46)
CHROMOPHOBE ADENOMA	7 (35%)	14 (30%)	12 (26%)
CHROMOPHOBE CARCINOMA		2 (4%)	4 (9%)
ACIDOPHIL ADENOMA	2 (10%)		
ACIDOPHIL CARCINOMA		1 (2%)	
#ADRENAL	(20)	(49)	(50)
CORTICAL ADENOMA		3 (6%)	3 (6%)
PHEOCHROMOCYTOMA	4 (20%)	10 (20%)	15 (30%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(20)	(48)	(49)
C-CELL ADENOMA	5 (25%)	4 (8%)	7 (14%)
#PANCREATIC ISLETS	(20)	(49)	(50)
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
TRICHOEPITHELIOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ADENOCARCINOMA, NOS			3 (6%)
LIPOMA			2 (4%)
FIBROADENOMA	1 (5%)	1 (2%)	1 (2%)
FIBROADENOCARCINOMA			1 (2%)
#TESTIS	(19)	(50)	(50)
INTERSTITIAL-CELL TUMOR	18 (95%)	39 (78%)	40 (80%)
INTERSTITIAL-CELL TUMOR, MALIGNA			1 (2%)
LIPOMA			1 (2%)
HEMANGIOSARCOMA			1 (2%)
NERVOUS SYSTEM			
#CEREBRUM	(20)	(50)	(50)
ASTROCYTOMA		1 (2%)	
#BRAIN	(20)	(50)	(50)
ASTROCYTOMA	1 (5%)		
#CEREBELLUM	(20)	(50)	(50)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			2 (4%)
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF NECK	(20)	(50)	(50)
RHABDOMYOSARCOMA			1 (2%)
BODY CAVITIES			
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS		2 (4%)	
MESOTHELIOMA, MALIGNANT			2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
MESOTHELIOMA, MALIGNANT	1 (5%)		1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMANGIOSARCOMA			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	9	5
MORIBUND SACRIFICE	3	5	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	14	35	39
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	47	49
TOTAL PRIMARY TUMORS	44	113	121
TOTAL ANIMALS WITH BENIGN TUMORS	19	46	45
TOTAL BENIGN TUMORS	40	86	92
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	11	23
TOTAL MALIGNANT TUMORS	4	13	26
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		3
TOTAL SECONDARY TUMORS	4		3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		14	3
TOTAL UNCERTAIN TUMORS		14	3
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 A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	1 (2%)
BASAL-CELL CARCINOMA		1 (2%)	
FIBROSARCOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
LIPOMA		1 (2%)	
NEUROFIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (2%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MONOCYTIC LEUKEMIA	2 (10%)	2 (4%)	3 (6%)
*HEMATOPOIETIC SYSTEM	(20)	(50)	(50)
NEOPLASM, NOS	4 (20%)	9 (18%)	5 (10%)
#MANDIBULAR L. NODE	(19)	(50)	(50)
FIBROSARCOMA, METASTATIC		1 (2%)	
#THYMUS	(18)	(39)	(33)
ADENOMA, NOS	1 (6%)		
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
FIBROSARCOMA		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#HEPATIC SINUSOID	(19)	(50)	(50)
NEOPLASM, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(50)	(50)
HEPATOCELLULAR ADENOMA	1 (5%)		1 (2%)
LEIOMYOSARCOMA, METASTATIC	1 (5%)		
URINARY SYSTEM			
#URINARY BLADDER	(19)	(50)	(49)
LEIOMYOSARCOMA	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(49)	(48)
CHROMOPHOBE ADENOMA	9 (47%)	20 (41%)	17 (35%)
CHROMOPHOBE CARCINOMA	2 (11%)	2 (4%)	5 (10%)
ACIDOPHIL ADENOMA		1 (2%)	3 (6%)
#ADRENAL	(19)	(50)	(50)
CORTICAL ADENOMA	3 (16%)	1 (2%)	7 (14%)
PHEOCHROMOCYTOMA		4 (8%)	6 (12%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(20)	(48)	(49)
FOLLICULAR-CELL ADENOMA	1 (5%)		2 (4%)
C-CELL ADENOMA	1 (5%)	5 (10%)	6 (12%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS	4 (20%)	2 (4%)	3 (6%)
ADENOCARCINOMA, NOS	1 (5%)	1 (2%)	6 (12%)
FIBROADENOMA	1 (5%)	6 (12%)	5 (10%)
#UTERUS	(19)	(50)	(48)
ENDOMETRIAL STROMAL POLYP	4 (21%)	7 (14%)	2 (4%)
#OVARY	(19)	(50)	(47)
GRANULOSA-CELL CARCINOMA			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(50)
RHABDOMYOSARCOMA			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		6	6
MORIBUND SACRIFICE	2	3	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	41	41
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	18	40	42
TOTAL PRIMARY TUMORS	37	66	81
TOTAL ANIMALS WITH BENIGN TUMORS	14	35	33
TOTAL BENIGN TUMORS	26	48	56
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	8	17
TOTAL MALIGNANT TUMORS	7	9	19
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	4	9	6
TOTAL UNCERTAIN TUMORS	4	9	6
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 CALCIUM CYANAMIDE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
HEMANGIOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	7 (35%)	8 (16%)	8 (16%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		3 (6%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	3 (6%)	1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#SPLEEN	(20)	(50)	(50)
FIBROSARCOMA			1 (2%)
HEMANGIOSARCOMA		1 (2%)	1 (2%)
#MEDIASTINAL L.NODE	(20)	(50)	(48)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
#MESENTERIC L. NODE	(20)	(50)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
#HEPATIC SINUSOID	(20)	(50)	(50)
NEOPLASM, NOS			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
HEPATOCELLULAR ADENOMA	5 (25%)	7 (14%)	6 (12%)
HEPATOCELLULAR CARCINOMA	3 (15%)	4 (8%)	1 (2%)
HEMANGIOSARCOMA	1 (5%)		2 (4%)
#SMALL INTESTINE	(20)	(50)	(48)
ADENOCARCINOMA, NOS	1 (5%)		1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
TUBULAR-CELL ADENOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(45)	(42)
CHROMOPHOBE ADENOMA		1 (2%)	
CHROMOPHOBE CARCINOMA	1 (5%)		
#ADRENAL	(20)	(50)	(49)
CORTICAL ADENOMA	1 (5%)		3 (6%)
#THYROID	(19)	(48)	(48)
FOLLICULAR-CELL ADENOMA		1 (2%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
HEMANGIOSARCOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CYSTADENOMA, NOS	1 (5%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
LIPOMA		2 (4%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
HEMANGIOSARCOMA			7 (14%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		5	12
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	20	45	38
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	29	30
TOTAL PRIMARY TUMORS	21	35	41
TOTAL ANIMALS WITH BENIGN TUMORS	10	19	19
TOTAL BENIGN TUMORS	14	21	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	14	17
TOTAL MALIGNANT TUMORS	7	14	19
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
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 B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		4	
ANIMALS NECROPSIED	20	46	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	46	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(46)	(50)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(50)
ADENOCARCINOMA, NOS, METASTATIC	1 (5%)		
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (15%)	1 (2%)	5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(46)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	7 (15%)	10 (20%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
*BLOOD	(20)	(46)	(50)
MONOCYTIC LEUKEMIA			1 (2%)
#BONE MARROW	(20)	(46)	(48)
OSTEOSARCOMA	1 (5%)		
#SPLEEN	(19)	(45)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(20)	(46)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#THYMUS	(19)	(39)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*ROOT OF TONGUE	(20)	(46)	(50)
TRICHOEPITHELIOMA			1 (2%)
#LIVER	(20)	(46)	(49)
HEPATOCELLULAR ADENOMA		4 (9%)	3 (6%)
HEPATOCELLULAR CARCINOMA		2 (4%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(43)	(44)
CHROMOPHOBE ADENOMA	1 (5%)	3 (7%)	1 (2%)
CHROMOPHOBE CARCINOMA	1 (5%)		
#ADRENAL	(20)	(45)	(49)
CORTICAL ADENOMA		1 (2%)	
#THYROID	(20)	(42)	(46)
FOLLICULAR-CELL ADENOMA	1 (5%)	1 (2%)	
#PANCREATIC ISLETS	(20)	(45)	(50)
ISLET-CELL CARCINOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(46)	(50)
ADENOMA, NOS			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ADENOCARCINOMA, NOS	2 (10%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(46)	(50)
ADENOMA, NOS		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(46)	(50)
LIPOMA	2 (10%)	4 (9%)	1 (2%)
OSTEOSARCOMA, METASTATIC	1 (5%)		
ALL OTHER SYSTEMS			
BASE OF TAIL			
OSTEOSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	3	4
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	43	46
ANIMAL MISSING		4	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	25	27
TOTAL PRIMARY TUMORS	14	28	34
TOTAL ANIMALS WITH BENIGN TUMORS	7	13	13
TOTAL BENIGN TUMORS	7	15	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	12	18
TOTAL MALIGNANT TUMORS	7	13	20
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1	
TOTAL SECONDARY TUMORS	2	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/ALVEOLI	(20)	(50)	(50)
HISTIOCYTOSIS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(50)	(43)
HEMORRHAGE		1 (2%)	
HYPERPLASIA, DIFFUSE		3 (6%)	2 (5%)
#SPLEEN	(20)	(50)	(50)
FIBROSIS, FOCAL			1 (2%)
#MANDIBULAR L. NODE	(20)	(50)	(50)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(20)	(50)	(50)
CONGESTION, NOS	2 (10%)	1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(20)	(50)	(50)
THROMBUS, ORGANIZED		1 (2%)	2 (4%)
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	17 (85%)	41 (82%)	42 (84%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DEGENERATION, NOS			4 (8%)
*MESENTERIC ARTERY	(20)	(50)	(50)
INFLAMMATION, CHRONIC DIFFUSE			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
ATROPHY, FOCAL			1 (2%)
#LIVER	(20)	(50)	(50)
THROMBUS, ORGANIZED		1 (2%)	
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (5%)	4 (8%)	4 (8%)
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE		1 (2%)	
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, CHRONIC NECROTIZIN			1 (2%)
NECROSIS, FOCAL			1 (2%)
#LIVER/HEPATOCYTES	(20)	(50)	(50)
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, FOCAL		9 (18%)	6 (12%)
#BILE DUCT	(20)	(50)	(50)
CALCULUS, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	1 (5%)	4 (8%)	3 (6%)
#PANCREAS	(20)	(49)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
PERIARTERITIS	1 (5%)		
#PANCREATIC ACINUS	(20)	(49)	(50)
ATROPHY, FOCAL	2 (10%)	1 (2%)	5 (10%)
#STOMACH	(20)	(50)	(50)
ULCER, FOCAL			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
#GASTRIC MUCOSA	(20)	(50)	(50)
ULCER, CHRONIC			1 (2%)
#COLON	(20)	(50)	(49)
NEMATODIASIS		1 (2%)	1 (2%)
#COLONIC MUCOUS MEMBR	(20)	(50)	(49)
POLYP	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL	15 (75%)	37 (76%)	28 (56%)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
SCLEROSIS			1 (2%)
NEPHROPATHY	15 (75%)	37 (76%)	27 (54%)
NEPHROSIS, NOS		1 (2%)	
INFARCT, FOCAL		1 (2%)	
#KIDNEY/TUBULE	(20)	(49)	(50)
NEPHROSIS, TOXIC			1 (2%)
#URINARY BLADDER	(20)	(48)	(50)
CALCULUS, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(47)	(46)
CYST, NOS		3 (6%)	2 (4%)
MULTIPLE CYSTS			1 (2%)
#ADRENAL CORTEX	(20)	(49)	(50)
HYPERPLASIA, FOCAL		4 (8%)	
#ADRENAL MEDULLA	(20)	(49)	(50)
HEMORRHAGE		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(20)	(48)	(49)
HYPERPLASIA, CYSTIC		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, C-CELL		4 (8%)	3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
DILATATION/DUCTS			7 (14%)
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, DIFFUSE		3 (6%)	9 (18%)
#PROSTATE	(19)	(49)	(49)
INFLAMMATION, SUPPURATIVE			4 (8%)
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION ACTIVE CHRONIC	2 (11%)		
INFLAMMATION, ACUTE/CHRONIC	1 (5%)		
#TESTIS	(19)	(50)	(50)
ATROPHY, DIFFUSE			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(50)
HEMORRHAGE			1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(50)
CATARACT			1 (2%)
*EYE/CORNEA	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
*EYE/RETINA	(20)	(50)	(50)
DEGENERATION, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*EPICARDIUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*MESENTERY	(20)	(50)	(50)
INFLAMMATION, FOCAL GRANULOMATOU			2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
HEMORRHAGE		1 (2%)	
ADIPOSE TISSUE			
INFLAMMATION, NECRO GRAN			2
SPECIAL MORPHOLOGY SUMMARY			
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 CALCIUM CYANAMIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
ULCER, FOCAL			1 (2%)
EROSION		1 (2%)	
ACANTHOSIS		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(19)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#LUNG	(19)	(50)	(50)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(18)	(49)	(47)
HYPOPLASIA, NOS			1 (2%)
HYPERPLASIA, DIFFUSE	4 (22%)	6 (12%)	2 (4%)
#SPLEEN	(19)	(50)	(50)
FIBROSIS, FOCAL			1 (2%)
INFARCT, FOCAL			1 (2%)
HEMOSIDEROSIS	1 (5%)		1 (2%)
#MANDIBULAR L. NODE	(19)	(50)	(50)
CONGESTION, NOS	1 (5%)		
#MESENTERIC L. NODE	(19)	(50)	(50)
CONGESTION, NOS		1 (2%)	1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID		2 (4%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	17 (85%)	37 (74%)	44 (88%)
#CARDIAC VALVE	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(49)	(50)
INFLAMMATION, CHRONIC FOCAL			2 (4%)
#LIVER	(19)	(50)	(50)
THROMBOSIS, NOS			1 (2%)
METAMORPHOSIS FATTY	2 (11%)	1 (2%)	1 (2%)
#LIVER/CENTRILOBULAR	(19)	(50)	(50)
NECROSIS, DIFFUSE			1 (2%)
#LIVER/HEPATOCYTES	(19)	(50)	(50)
NECROSIS, FOCAL	1 (5%)		
HYPERPLASIA, FOCAL	1 (5%)	13 (26%)	6 (12%)
#BILE DUCT	(19)	(50)	(50)
HYPERPLASIA, FOCAL			1 (2%)
#PANCREATIC ACINUS	(19)	(50)	(50)
ATROPHY, FOCAL	1 (5%)	1 (2%)	4 (8%)
#GASTRIC SUBMUCOSA	(19)	(50)	(50)
INFLAMMATION, DIFFUSE	1 (5%)		
INFLAMMATION, CHRONIC FOCAL			1 (2%)
INFLAMMATION, CHRONIC DIFFUSE	1 (5%)		
#COLON	(19)	(50)	(50)
NEMATODIASIS			2 (4%)
#COLONIC MUCOUS MEMBR	(19)	(50)	(50)
POLYP		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(19)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	7 (37%)	12 (24%)	7 (14%)
NEPHROPATHY	6 (32%)	10 (20%)	6 (12%)
#URINARY BLADDER	(19)	(50)	(49)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
#U.BLADDER/SUBMUCOSA	(19)	(50)	(49)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(49)	(48)
CYST, NOS		1 (2%)	
MULTIPLE CYSTS	2 (11%)	3 (6%)	
#ADRENAL	(19)	(50)	(50)
HEMORRHAGE		1 (2%)	
#ADRENAL CORTEX	(19)	(50)	(50)
HEMORRHAGE			1 (2%)
DEGENERATION, NOS			1 (2%)
HYPERPLASIA, FOCAL	3 (16%)	2 (4%)	2 (4%)
#THYROID	(20)	(48)	(49)
CYST, NOS		1 (2%)	
HYPERPLASIA, C-CELL	3 (15%)	3 (6%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
DILATATION/DUCTS		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
HYPERPLASIA, DIFFUSE		1 (2%)	1 (2%)
#UTERUS	(19)	(50)	(48)
HEMORRHAGE			1 (2%)
PYOMETRA			1 (2%)
#UTERUS/ENDOMETRIUM	(19)	(50)	(48)
CYST, NOS		1 (2%)	2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MULTIPLE CYSTS	1 (5%)		1 (2%)
PYOMETRA			1 (2%)
#UTERUS/MYOMETRIUM	(19)	(50)	(48)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#OVARY	(19)	(50)	(47)
FOLLICULAR CYST, NOS		1 (2%)	1 (2%)
ABSCESS, CHRONIC			1 (2%)
NERVOUS SYSTEM			
#CEREBRAL VENTRICLE	(19)	(50)	(50)
HYDROCEPHALUS, NOS		1 (2%)	
#BRAIN	(19)	(50)	(50)
HEMORRHAGE	1 (5%)		2 (4%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
INFARCT, NOS	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		2	1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
HEMORRHAGE			1 (2%)
PERIVASCULAR CUFFING			1 (2%)
ALVEOLAR MACROPHAGES			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID	5 (25%)	3 (6%)	
#LYMPH NODE	(20)	(50)	(48)
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	
HEMATOPOIESIS	1 (5%)		
#MESENTERIC L. NODE	(20)	(50)	(48)
CONGESTION, NOS	13 (65%)	25 (50%)	5 (10%)
CONGESTION, ACUTE			1 (2%)
HYPERPLASIA, LYMPHOID		4 (8%)	2 (4%)
HEMATOPOIESIS	1 (5%)	5 (10%)	5 (10%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(48)
INFLAMMATION, CHRONIC FOCAL	8 (40%)	15 (30%)	6 (13%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
#LIVER	(20)	(50)	(50)
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			2 (4%)
INFLAMMATION, CHRONIC FOCAL		2 (4%)	
NECROSIS, DIFFUSE			1 (2%)
METAMORPHOSIS FATTY	1 (5%)		
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
NECROSIS, DIFFUSE			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
#LIVER/HEPATOCYTES	(20)	(50)	(50)
HYPERPIASIA, FOCAL	2 (10%)		
#BILE DUCT	(20)	(50)	(50)
CYST, NOS		1 (2%)	
#PANCREATIC ACINUS	(20)	(50)	(50)
ATROPHY, FOCAL		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
HEMATOMA, NOS			1 (2%)
PYELONEPHRITIS SUPPURATIVE			1 (2%)
PYELONEPHRITIS, CHRONIC	3 (15%)		1 (2%)
INFLAMMATION, CHRONIC FOCAL	3 (15%)	1 (2%)	
#KIDNEY/PELVIS	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
*URETHRA	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(45)	(42)
CYST, NOS	1 (5%)		
#THYROID	(19)	(48)	(48)
INFLAMMATION, CHRONIC FOCAL	2 (11%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(47)	(47)
INFLAMMATION, SUPPURATIVE			3 (6%)
*EPIDIDYMIS	(20)	(50)	(50)
GRANULOMA, SPERMATIC			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(20)	(50)	(50)
FIBROSIS, FOCAL			1 (2%)
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
CYST, NOS		1 (2%)	
INFLAMMATION, NECRO GRAN			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	4	8

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		4	
ANIMALS NECROPSIED	20	46	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	46	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
*BLOOD	(20)	(46)	(50)
HYPERPLASIA, NEUTROPHILIC	1 (5%)	1 (2%)	
#SPLEEN	(19)	(45)	(50)
SCLEROSIS		1 (2%)	
HYPERPLASIA, LYMPHOID	4 (21%)	4 (9%)	2 (4%)
#LYMPH NODE	(20)	(46)	(50)
CONGESTION, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	
#MANDIBULAR L. NODE	(20)	(46)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(20)	(46)	(50)
CONGESTION, NOS	2 (10%)	2 (4%)	
HYPERPLASIA, LYMPHOID	1 (5%)		2 (4%)
#LIVER	(20)	(46)	(49)
MYELOPROLIFERATIVE DISORDER			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(46)	(50)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
*ARTERY	(20)	(46)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
*CORONARY ARTERY	(20)	(46)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(44)	(49)
INFLAMMATION, CHRONIC FOCAL	7 (35%)	5 (11%)	7 (14%)
#LIVER	(20)	(46)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	2 (10%)	3 (7%)	7 (14%)
NECROSIS, FOCAL		1 (2%)	
INFARCT, FOCAL		1 (2%)	
#PANCREAS	(20)	(45)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (5%)	2 (4%)	3 (6%)
#PANCREATIC ACINUS	(20)	(45)	(50)
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(20)	(46)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(46)	(50)
PYELONEPHRITIS, CHRONIC	5 (25%)	1 (2%)	1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)	6 (13%)	
#KIDNEY/PELVIS	(20)	(46)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*URETER	(20)	(46)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	1 (2%)
#URINARY BLADDER	(18)	(45)	(48)
INFLAMMATION, CHRONIC FOCAL		2 (4%)	4 (8%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(43)	(44)
MULTIPLE CYSTS	1 (5%)		
#THYROID	(20)	(42)	(46)
FOLLICULAR CYST, NOS		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(43)	(50)
PYOMETRA			1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(43)	(50)
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, CYSTIC	1 (5%)	5 (12%)	1 (2%)
#OVARY/OVIDUCT	(20)	(43)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
#OVARY	(20)	(46)	(50)
FOLLICULAR CYST, NOS	2 (10%)		3 (6%)
HEMORRHAGIC CYST		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(45)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*BONE	(20)	(46)	(50)
HYPERPLASIA, FOCAL		1 (2%)	
BODY CAVITIES			
*MESENTERY	(20)	(46)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(46)	(50)
HYPERPLASIA, LYMPHOID		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	8	7
ANIMAL MISSING/NO NECROPSY		4	
AUTO/NECROPSY/HISTO PERF			1

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Squamous-cell Papilloma or Carcinoma of the Skin (b)	0/20 (0)	2/50 (4)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.123	0.386
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor (g)	--	102	83
Integumentary System: Fibroma of the Subcutaneous Tissue (b)	1/20 (5)	3/50 (6)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.400
Lower Limit		0.106	0.005
Upper Limit		61.724	30.802
Weeks to First Observed Tumor (g)	107	100	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Trichoepithelioma of the Skin or Subcutaneous Tissue (b)	0/20 (0)	2/50 (4)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.123	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	107	107
Lung: Alveolar/Bronchiolar Adenoma (b)	1/20 (5)	6/50 (12)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.400	1.600
Lower Limit		0.325	0.175
Upper Limit		108.021	77.169
Weeks to First Observed Tumor	107	105	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hepatopoietic System: Lymphoma or Leukemia (b)	2/20 (10)	6/50 (12)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.800
Lower Limit		0.243	0.128
Upper Limit		11.574	8.436
Weeks to First Observed Tumor	99	86	86
Hematopoietic System: Lymphoma, Leukemia, or, Neoplasm, NOS (b)	2/20 (10)	18/50 (36)	7/50 (14)
P Values (c,d)	N.S.	P = 0.025	N.S.
Departure from Linear Trend	P = 0.003		
Relative Risk (f)		3.600	1.400
Lower Limit		0.998	0.303
Upper Limit		30.068	13.138
Weeks to First Observed Tumor	99	86	86

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Carcinoma (b)	0/20 (0)	2/47 (4)	4/46 (9)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.131	0.420
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	97	95
Pituitary: Chromophobe Carcinoma or Adenoma (b)	7/20 (35)	16/47 (34)	16/46 (35)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.973	0.994
Lower Limit		0.467	0.478
Upper Limit		2.421	2.469
Weeks to First Observed Tumor	93	97	95

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Acidophil Carcinoma or Adenoma (b)	2/20 (10)	1/47 (2)	0/46 (0)
P Values (c,d)	P = 0.040 (N)	N.S.	N.S.
Relative Risk (f)		0.213	0.000
Lower Limit		0.004	0.000
Upper Limit		3.909	1.459
Weeks to First Observed Tumor	107	102	--
Adrenal: Cortical Adenoma (b)	0/20 (0)	3/49 (6)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.255	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	107	95

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	4/20 (20)	10/49 (20)	16/50 (32)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.020	1.600
Lower Limit		0.346	0.613
Upper Limit		4.068	5.950
Weeks to First Observed Tumor	107	97	105
Liver: Hepatocellular Adenoma (b)	1/20 (5)	3/50 (6)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.400
Lower Limit		0.106	0.005
Upper Limit		61.724	30.802
Weeks to First Observed Tumor	107	107	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma (b)	5/20 (25)	4/48 (8)	7/49 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.333	0.571
Lower Limit		0.076	0.184
Upper Limit		1.412	2.068
Weeks to First Observed Tumor	74	107	107
Mammary Gland: Adenocarcinoma (b)	0/20 (0)	0/50 (0)	3/50 (6)
P Values (c,d)	N.S.	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.250
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	102

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma, Leukemia, or Neoplasm, NOS (b)	6/20 (30)	11/50 (22)	9/50 (18)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.733	0.600
Lower Limit		0.300	0.229
Upper Limit		2.148	1.829
Weeks to First Observed Tumor	100	36	102
Pituitary: Chromophobe Carcinoma (b)	2/19 (11)	2/49 (4)	5/48 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.388	0.990
Lower Limit		0.031	0.184
Upper Limit		5.108	9.880
Weeks to First Observed Tumor	98	107	69

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor (b)	18/19 (95)	39/50 (78)	40/50 (80)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.823	0.844
Lower Limit		0.762	0.782
Upper Limit		1.090	1.110
Weeks to First Observed Tumor	74	68	102
All Sites: Mesothelioma (b)	1/20 (5)	2/50 (4)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.800	1.200
Lower Limit		0.045	0.106
Upper Limit		46.273	61.724
Weeks to First Observed Tumor	102	107	106

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

(a) Dosed groups received 100 or 200 ppm in the diet.

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

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

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

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

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

(g) Weeks to first observed tumor is based on time of death with tumor.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Calcium Cyanamide in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	2/19 (11)	1/50 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.190	0.570
Lower Limit		0.003	0.073
Upper Limit		3.494	6.511
Weeks to First Observed Tumor	107	107	107
Hematopoietic System: Lymphoma or Leukemia (b)	2/20 (10)	2/50 (4)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.400	0.800
Lower Limit		0.032	0.128
Upper Limit		5.277	8.436
Weeks to First Observed Tumor	107	36	102

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Carcinoma or Adenoma (b)	11/19 (58)	22/49 (45)	22/48 (46)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.776	0.792
Lower Limit		0.483	0.493
Upper Limit		1.450	1.477
Weeks to First Observed Tumor	98	92	69
Pituitary: Acidophil Adenoma (b)	0/19 (0)	1/49 (2)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.021	0.248
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	107	107

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Cortical Adenoma (b)	3/19 (16)	1/50 (2)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.041		
Relative Risk (f)		0.127	0.887
Lower Limit		0.003	0.234
Upper Limit		1.487	4.945
Weeks to First Observed Tumor	107	107	103
Adrenal: Pheochromocytoma (b)	0/19 (0)	4/50 (8)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.368	0.771
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	59	107

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma (b)	1/20 (5)	5/48 (10)	6/49 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.083	2.449
Lower Limit		0.259	0.332
Upper Limit		96.358	110.166
Weeks to First Observed Tumor	107	96	107
Mammary Gland: Adenocarcinoma (b)	1/20 (5)	1/50 (2)	6/50 (12)
P Values (c,d)	P = 0.042	N.S.	N.S.
Relative Risk (f)		0.400	2.400
Lower Limit		0.005	0.325
Upper Limit		30.802	108.021
Weeks to First Observed Tumor	107	92	107

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Adenocarcinoma, NOS, or Adenoma, NOS (b)	5/20 (25)	3/50 (6)	9/50 (18)
P Values (c,d)	N.S.	P = 0.038 (N)	N.S.
Departure from Linear Trend (e)	P = 0.026		
Relative Risk (f)		0.240	0.720
Lower Limit		0.042	0.257
Upper Limit		1.131	2.470
Weeks to First Observed Tumor	98	92	107
Mammary Gland: Fibroadenoma (b)	1/20 (5)	6/50 (12)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.400	2.000
Lower Limit		0.325	0.249
Upper Limit		108.021	92.596
Weeks to First Observed Tumor	107	107	95

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	4/19 (21)	7/50 (14)	2/48 (4)
P Values (c,d)	P = 0.021 (N)	N.S.	N.S.
Relative Risk (f)		0.665	0.198
Lower Limit		0.198	0.020
Upper Limit		2.837	1.279
Weeks to First Observed Tumor	107	107	107

(a) Dosed groups received 100 or 400 ppm in the diet.

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

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

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

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED CALCIUM CYANAMIDE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Calcium Cyanamide in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	0/20 (0)	3/50 (6)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	96	100
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	7/20 (35)	11/50 (22)	11/50 (22)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.629	0.629
Lower Limit		0.272	0.272
Upper Limit		1.683	1.683
Weeks to First Observed Tumor	100	96	96

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	1/20 (5)	4/50 (8)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.600	1.200
Lower Limit		0.175	0.106
Upper Limit		77.169	61.724
Weeks to First Observed Tumor	100	99	80
All Sites: Hemangiosarcoma (b)	1/20 (5)	2/50 (4)	10/50 (20)
P Values (c,d)	P = 0.006	N.S.	N.S.
Relative Risk (f)		0.800	4.000
Lower Limit		0.045	0.642
Upper Limit		46.273	169.457
Weeks to First Observed Tumor	100	93	93

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	3/20 (15)	4/50 (8)	1/50 (2)
P Values (c,d)	P = 0.036 (N)	N.S.	N.S.
Relative Risk (f)		0.533	0.133
Lower Limit		0.102	0.003
Upper Limit		3.410	1.568
Weeks to First Observed Tumor	100	100	100
Liver: Hepatocellular Carcinoma or Adenoma (b)	8/20 (40)	11/50 (22)	7/50 (14)
P Values (c,d)	P = 0.025 (N)	N.S.	P = 0.022 (N)
Relative Risk (f)		0.550	0.350
Lower Limit		0.250	0.132
Upper Limit		1.373	0.972
Weeks to First Observed Tumor	100	100	100

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Cortical Adenoma (b)	1/20 (5)	0/50 (0)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.000	1.224
Lower Limit		0.000	0.108
Upper Limit		7.475	62.958
Weeks to First Observed Tumor	100	--	74

(a) Dosed groups received 500 or 2,000 ppm in the diet.

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

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

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

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Calcium Cyanamide in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	3/20 (15)	1/46 (2)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.145	0.800
Lower Limit		0.003	0.195
Upper Limit		1.700	4.615
Weeks to First Observed Tumor	100	100	100
Hematopoietic System: Lymphoma or Leukemia (b)	1/20 (5)	11/46 (24)	18/50 (36)
P Values (c,d)	P = 0.009	N.S.	P = 0.006
Relative Risk (f)		4.783	7.200
Lower Limit		0.786	1.294
Upper Limit		200.216	290.984
Weeks to First Observed Tumor	100	89	88

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Adenoma (b)	0/20 (0)	6/46 (13)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.725	0.255
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	100	100
Pituitary: Chromophobe Carcinoma or Adenoma (b)	2/20 (10)	3/43 (7)	1/44 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.698	0.227
Lower Limit		0.088	0.004
Upper Limit		7.937	4.167
Weeks to First Observed Tumor	100	100	100

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Calcium Cyanamide in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Adenocarcinoma (b)	2/20 (10)	0/46 (0)	0/50 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.011		
Relative Risk (f)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.459	1.345
Weeks to First Observed Tumor	100	--	--
Mesentery: Lipoma (b)	2/20 (10)	4/46 (9)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.870	0.200
Lower Limit		0.139	0.004
Upper Limit		9.144	3.681
Weeks to First Observed Tumor	100	100	100

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Calcium Cyanamide in the Diet (a)

(continued)

(a) Dosed groups received 500 or 2,000 ppm in the diet.

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

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

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

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

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

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

December 13, 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 on the Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. 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 Calcium Cyanamide.

The reviewer for the report on the bioassay of Calcium Cyanamide agreed that the compound was not carcinogenic under the conditions of test. After a brief description of the experimental design, he commented on several shortcomings, including the lack of data on the stability and content of the compound in the diet mix, the omission of body weight data for rats between the fifteenth and eighteenth week, and the inadequate numbers of matched controls. He pointed out the greater total tumor incidence among high-dose treated male rats and suggested that it be evaluated against historic controls. Assuming that the studies' shortcomings did not affect the results of the bioassay, the reviewer said that Calcium Cyananide would not appear to pose a risk of cancer to human beings.

The reviewer moved that the report on the bioassay of Calcium Cyanamide be accepted with the notation of the shortcomings mentioned in his critique. The motion was seconded and approved without objection.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center

Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical USA
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

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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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