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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
National Institutes of Health
Bethesda, Maryland 20014

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

CONTRIBUTORS: This bioassay of acetohexamide was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

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

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. Statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[7], and the results of the analyses were reviewed by Dr. C. W. Jameson[5].

This report was prepared at Tracor Jitco[5] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[6]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

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SUMMARY

A bioassay of acetohexamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 35 rats of each sex were administered acetohexamide in the diet at one of two doses, either 10,000 or 20,000 ppm, for 103 weeks and then observed for 2 to 4 additional weeks. Matched controls consisted of 15 untreated rats of each sex. All surviving rats were killed at 105 to 107 weeks.

Groups of 35 mice of each sex were administered acetohexamide at one of two doses for 103 weeks and then observed for 4 or 5 additional weeks. Time-weighted average doses were 6,359 or 12,718 ppm. Matched controls consisted of 15 untreated mice of each sex. All surviving mice were killed at 107 or 108 weeks.

Mean body weights of the dosed rats and mice of both sexes were lower than those of the corresponding matched controls throughout the study, and the depressions in weight were dose related. Except for the female mice, sufficient numbers of animals survived long enough to be at risk for development of late-appearing tumors.

In the rats, the only tumor occurring with greater incidence in dosed than in matched-control animals was leukemia (males: matched controls 0/15, low-dose 10/35, high-dose 4/35; females: matched controls 0/14, low-dose 7/35, high-dose 4/34). Only the incidence in the low-dose males was statistically significant ($P = 0.018$). All of these animals had undifferentiated (mononuclear cell) leukemia, which commonly occurs spontaneously in Fischer 344 rats, except for two with lymphocytic leukemia. The incidence of combined leukemia and lymphoma in historical-control male rats at this laboratory in the bioassay program to date is 24/235 (10.2%), which is higher than that in the matched

controls. Thus, the incidence in the low-dose males cannot be clearly associated with administration of the test chemical.

In the mice, the only neoplasms that occurred at a higher incidence in dosed groups than in matched controls were lymphomas in the males, but the incidences were not statistically significant (matched controls 1/15, low-dose 9/35, high-dose 3/34). These types of lesions are found commonly in untreated B6C3F1 mice. The incidence of lymphomas in the historical-control male mice is 28/536 (5.2%).

It is concluded that under the conditions of this bioassay, acetohexamide was not carcinogenic for either Fischer 344 rats or B6C3F1 mice.

TABLE OF CONTENTS

APPENDIXES

Page

TABLES

FIGURES

I. INTRODUCTION

Acetohexamide (CAS 968-81-0; NCI C03247) is an oral hypoglycemic agent of the arylsulfonyl-urea group with a potency between that of tolbutamide and chlorpropamide (Jackson, 1969).

These compounds function by stimulating the secretion of insulin by the pancreas, and therefore, are effective only in patients with at least minimal pancreatic function, as is the case with maturity-onset diabetics (Larner and Haynes, 1975). Although some oral hypoglycemics have been in use over the past 20 years on a chronic basis in the treatment of maturity-onset diabetes, acetohexamide has been used clinically for only about 10 years. The chief advantage of these compounds over insulin is that they may be taken orally where dietary modifications have failed to control elevated blood glucose levels. However, since controlled studies have shown that they are no more effective than dietary modifications in controlling the symptoms of maturity-onset diabetes on a long-term basis in the majority of patients and that they may be associated with an increase in cardiovascular mortality, their use in the future is expected to decline (Shen and Bressler, 1977).

Acetohexamide was selected for testing in the carcinogenesis program as part of an attempt to evaluate the carcinogenicity of drugs that may be used for prolonged periods in humans.

II. MATERIAL AND METHODS

A. Chemical

Acetohexamide (1-[(p-acetylphenyl)-sulphonyl]-3-cyclohexylurea) was obtained in two batches (Lot Nos. 6RV07 and 7AD46) from Eli Lilly and Company, Indianapolis, Indiana. The purity and identity of Lot No. 7AD46, which was used in the chronic study, was confirmed in analyses at Midwest Research Institute. The melting point was 184.5-186°C and was consistent with that of 187-189°C reported in the literature (Aumueller et al., 1962). Elemental analyses (C, H, N, S) were correct for $C_{15}H_{20}N_2O_4S$, the molecular formula of acetohexamide. Nuclear magnetic resonance, infrared, and ultraviolet spectra were in agreement with those in the literature (Salim and Hilty, 1967; Baltazar and Braga, 1966; Kuhnert-Brandstatter et al., 1970) and with the structure.

The batch used for the chronic study was stored in the original container at 5°C.

B. Dietary Preparation

Test diets were prepared every 2 weeks by mixing a known amount of sifted acetohexamide with a small amount of Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a portable mixer, then adding this mixture to the required amount of animal

meal and mixing in a twin-shell blender for 10 minutes. The prepared diets were stored at room temperature in sealed plastic containers.

The stability of acetohexamide in the feed mixture was determined at Midwest Research Institute by measuring the concentration of acetohexamide in formulated diets stored at room temperature (25°C) for a 2-week period. The results of this analysis indicated that acetohexamide mixed with animal meal is stable for 2 weeks at room temperature.

C. Animals

For the subchronic study, male Fischer 344 rats and female B6C3F1 mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

For the chronic study, Fischer 344 rats and B6C3F1 mice of both sexes were obtained from Charles River Breeding Laboratories through contracts with the Division of Cancer Treatment, National Cancer Institute. These rats and mice were received at 30 days of age and were quarantined for 3-4 weeks. Animals with no visible signs of disease were earmarked and then assigned to control and dosed groups.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. Room air was changed 15 times per hour and passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Food and water were supplied daily and were available ad libitum.

Rats and mice were housed five per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The rat cages were provided with Iso-Dri® hardwood chip bedding (Carworth, Edison, N.J.), and cage tops were covered with disposable filter bonnets; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.), and cage tops were covered with disposable filter bonnets beginning at week 56. Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week.

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

RATS

Feed Studies

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

MICE

Feed Studies

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

Gavage Studies

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

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine hydrochloride) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of acetohexamide, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. In the subchronic studies, acetohexamide was administered in the diet to male Fischer 344 rats at doses of 1,200, 3,000, 6,000, 15,000, or 30,000 ppm and to female B6C3F1

mice at doses of 2,500, 5,000, 10,000, 20,000, or 40,000 ppm. Five animals of each species were used at each dose, and 5 rats and 10 mice were maintained as untreated controls. Dosed animals were fed diets containing acetohexamide 7 days per week for 45 days and then observed for an additional 45 days.

There were no deaths in the rats at any dose administered. At the end of the 45-day administration period, mean body weight gain of the dosed animals, compared with that of the controls, was 98% at 1,200 ppm, 97% at 3,000 ppm, 87% at 6,000 ppm, 87% at 15,000 ppm, and 15% at 30,000 ppm. Mean body weight gains at 90 days were all within 15% of controls. The low and high doses for the chronic studies using rats were set at 10,000 and 20,000 ppm.

In mice, all animals administered 40,000 ppm died by week 3. No deaths occurred in any other dosed group. During the administration period, mean body weight gains of animals administered 2,500, 5,000, and 10,000 ppm were depressed compared with those of controls, and at 20,000 ppm animals failed to gain weight. Final body weights of the surviving dosed animals were, however, comparable to those of controls. The low and high doses for the chronic studies using mice were set at 10,000 and 20,000 ppm.

F. Designs of Chronic Studies

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

Table 1. Design of Acetohexamide Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	Acetohexamide in Diet[b] (ppm)	Time on Studies	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	15	0		107
Low-Dose	35	10,000	103	3-4
High-Dose	35	20,000	103	2-3
Female				
Matched Control	15	0		107
Low-Dose	35	10,000	103	4
High-Dose	35	20,000	103	3

[a]Rats were 54 days of age when placed on study.

[b]The dosed animals were fed test diets 5 days per week and control diets 2 days per week.

Table 2. Design of Acetohexamide Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	Acetohexamide in Diet[b,c] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)	Time-Weighted Average Dose[d] (ppm)
Male					
Matched-Control	15	0		107	
Low-Dose	35	10,000	28		
		5,000	75	4-5	6,359
High-Dose	35	20,000	28		
		10,000	75	4	12,718
Female					
Matched-Control	15	0		107	
Low-Dose	35	10,000	28		
		5,000	75	5	6,359
High-Dose	35	20,000	28		
		10,000	75	4	12,718

[a]Mice were 55 days of age when placed on study.

[b]The dosed animals were fed test diets 5 days per week and control diets 2 days per week.

[c]Doses were lowered at week 29, because weight gains were extremely low, particularly in the females.

[d]Time-weighted average dose $= \frac{\Sigma(\text{dose in ppm} \times \text{no. of weeks at that dose})}{\Sigma(\text{no. of weeks at each dose})}$

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied. Rats and mice were weighed individually every 2 weeks for the first year and once per month for the remainder of the study. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal whenever possible. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

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

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit

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

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a

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

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

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the

first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated

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

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

indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

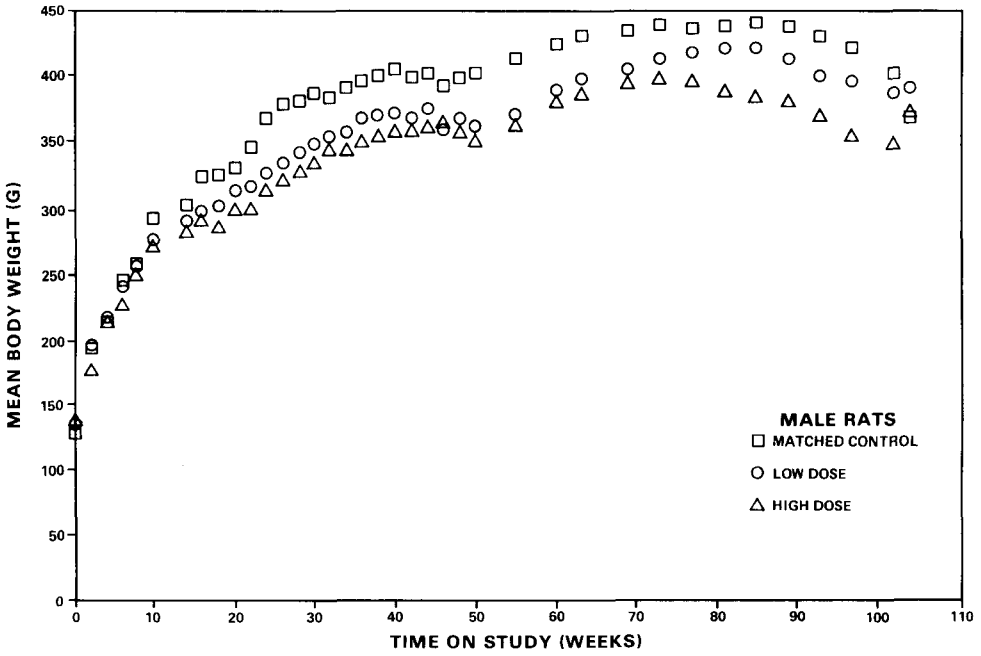
A. Body Weights and Clinical Signs (Rats)

Mean body weights of the low- and high-dose rats of both sexes were lower than those of the corresponding matched controls throughout the study, and the depressions in weight were dose related (figure 1). No other signs of toxicity related to administration of the test chemical were recorded in the rats. Rales and progressive weight loss were noted in individual animals of both the dosed and control groups.

B. Survival (Rats)

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

In each sex, the results of the Tarone test for positive dose-related trend in mortality are not significant. At least 80% of the male rats and over 70% of the female rats lived to the end of the bioassay, providing sufficient numbers of male and female rats at risk for development of late-appearing tumors.



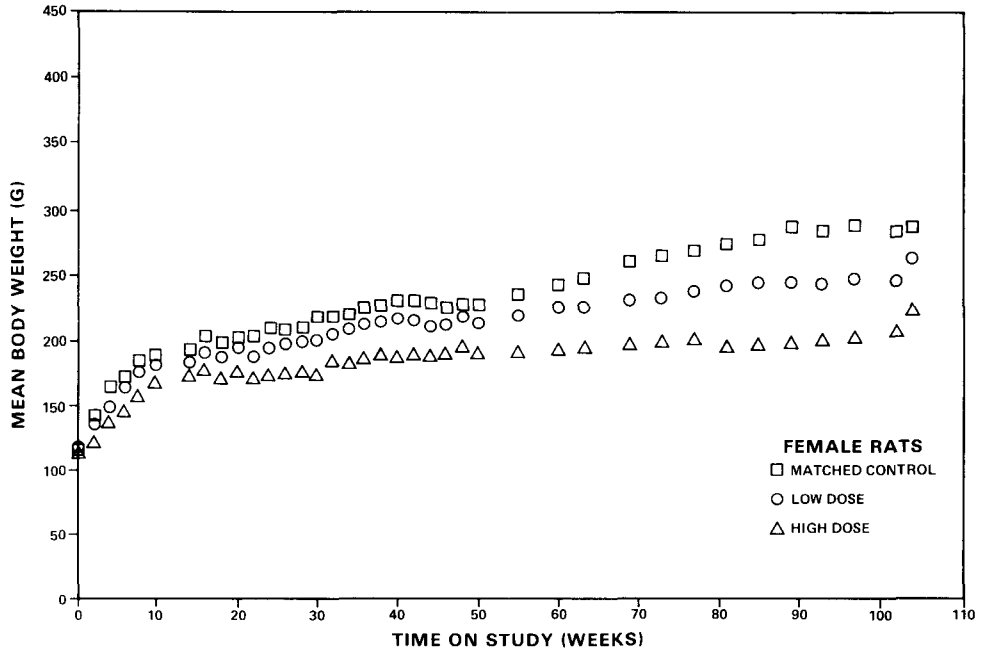


Figure 1. Growth Curves for Rats Fed Acetohexamide in the Diet

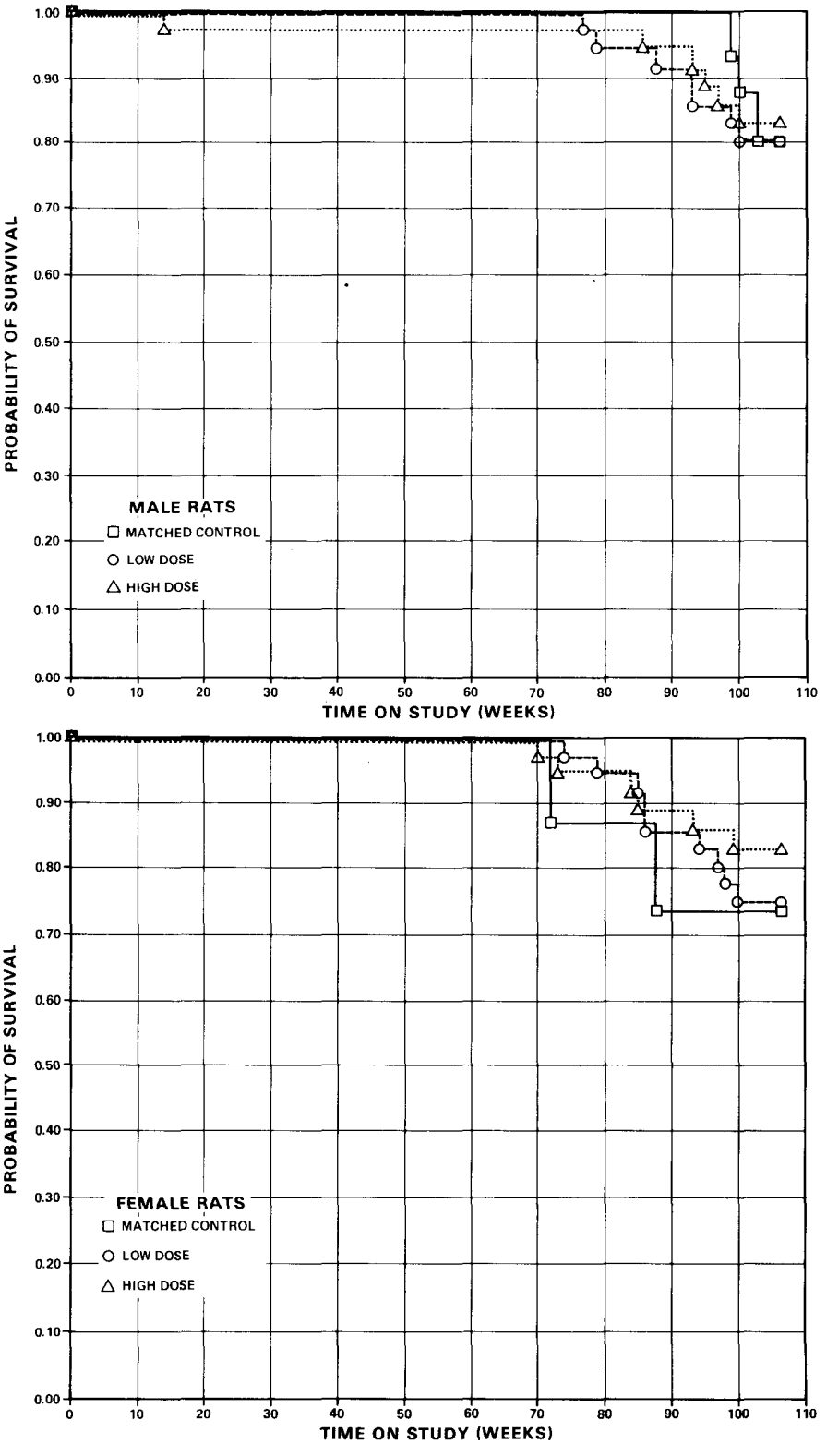


Figure 2. Survival Curves for Rats Fed Acetohexamide in the Diet

C. Pathology (Rats)

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

A variety of neoplasms occurred in both matched-control groups and dosed groups. Except for leukemias, the neoplasms listed in Appendix A appeared with approximately equal frequency in dosed and control rats or appeared in insignificant numbers. Most of these lesions are not uncommon in the Fischer 344 rat independent of any treatment.

The incidence of hematopoietic neoplasms was higher in the dosed than in the matched-control groups, and highest in the low-dose rats. The incidences of the undifferentiated and lymphocytic leukemias were as follows:

	MALES			FEMALES		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Rats Necropsied	(15)	(35)	(35)	(14)	(35)	(34)
Multiple Organs, Hematopoietic						
- undifferentiated leukemia	0	10	3	0	7	3
- lymphocytic leukemia	0	0	1	0	0	1

The undifferentiated or mononuclear leukemia (Davey and Moloney,

1970; Moloney et al., 1970) occurs spontaneously in the Fischer 344 rat with variable frequency.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals of the dosed and control groups (Appendix C). These nonneoplastic lesions are commonly seen in aged Fischer 344 rats.

Although the incidence of hematopoietic neoplasms was higher in Fischer 344 rats fed acetohexamide for 24 months at 20,000 and 10,000 ppm than in the control rats, this is considered by the pathologists to be a spontaneous occurrence not associated with administration of acetohexamide.

D. Statistical Analyses of Results (Rats)

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

In female rats, the results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for direct comparison of incidences between the matched-control group and each of the dosed groups indicated no significant increases in the tumors of the dosed groups. In male rats, the Cochran-

Armitage test result on the incidence of leukemia is not significant, but the Fisher exact test shows that the incidence in the low-dose group is significantly higher (P = 0.018) than that in the matched controls. The incidence in the high-dose group is not significant. The survivals of the groups are comparable. The incidence of combined leukemia and lymphoma in untreated-control male rats at this laboratory in this bioassay program to date is 24/235 (10.2%), which is higher than that (0/15) in the controls of this study. In females, the incidence of these tumors in the laboratory historical controls is 19/235 (8.1%).

The combined incidence of islet-cell adenoma or carcinoma is significantly higher in the control group of male rats than in either dosed group. Significant results (P = 0.003) in the negative direction are also observed in the incidence of fibroadenoma of the mammary gland in female rats.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the low- and high-dose mice of both sexes were lower than those of the corresponding matched controls throughout the entire study, and the depressions in weight were dose related (figure 3). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Progressive weight loss and rales were noted in several individual animals, mostly in the dosed groups.

To control respiratory disease, mice were given oxytetracycline in the drinking water during week 36 (0.6 mg/ml) and during week 37 (0.3 mg/ml). To suppress the spread of the disease, propylene glycol was vaporized in the mouse room during weeks 35 through 46.

B. Survival (Mice)

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

In each sex, the results of the Tarone test for positive dose-

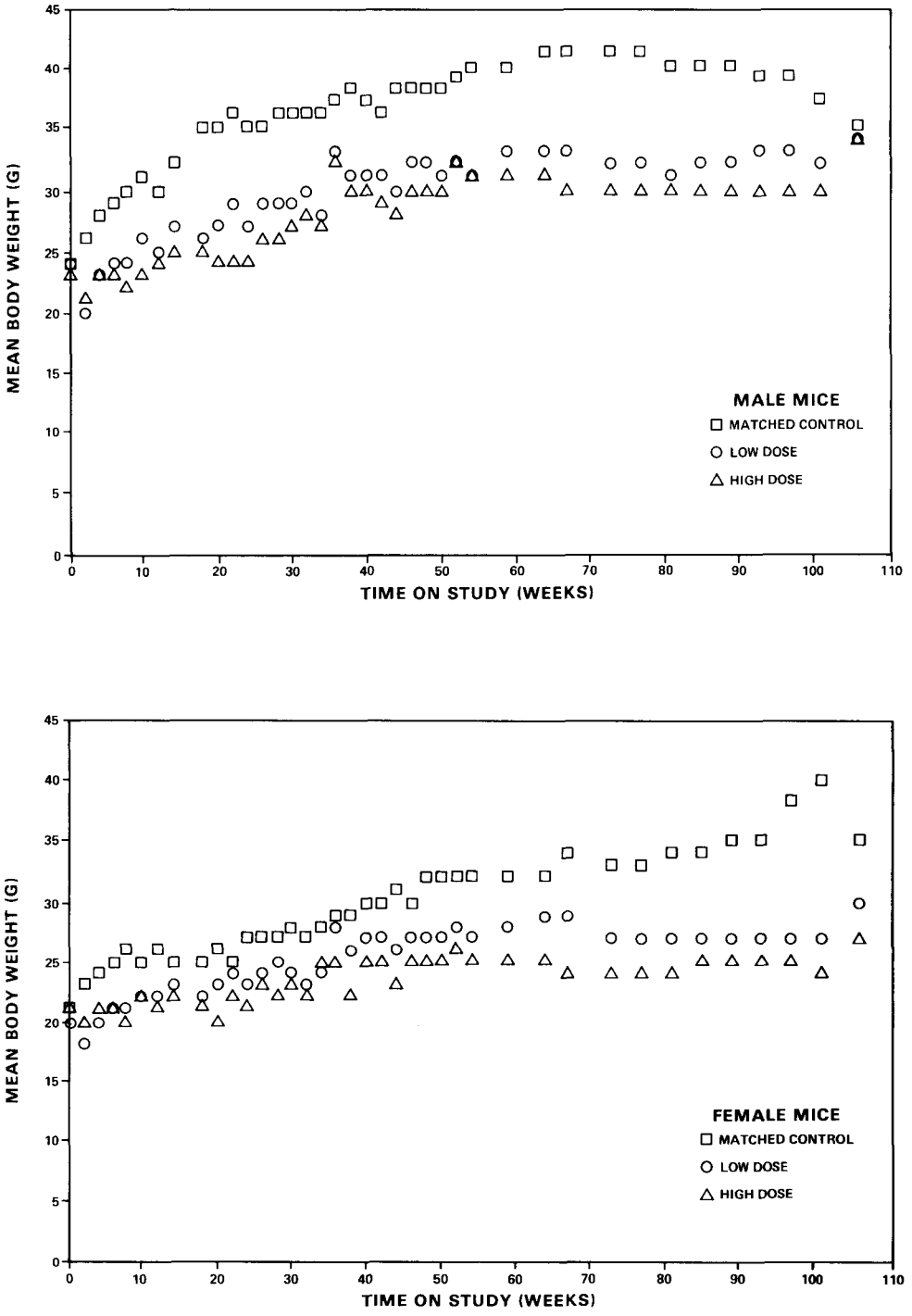


Figure 3. Growth Curves for Mice Fed Acetohexamide in the Diet

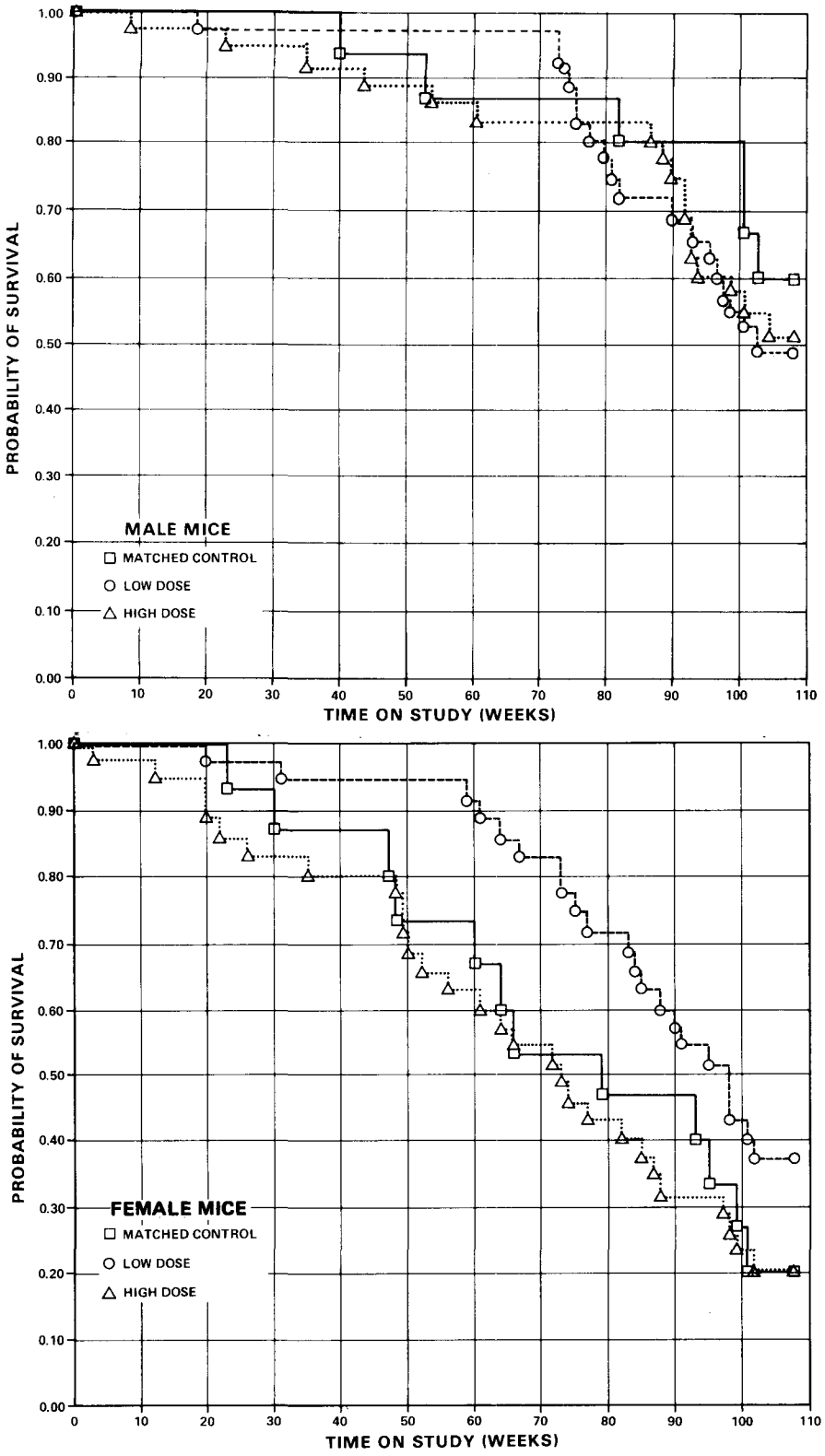


Figure 4. Survival Curves for Mice Fed Acetohexamide in the Diet

related trend in mortality over the period are not significant. In male mice, 31/35 of the high-dose group, 34/35 of the low-dose group, and 14/15 of the matched-control group lived beyond week 52 on study. In females, 24/35 of the high-dose group, 33/35 of the low-dose group, and 11/15 of the matched-control group lived at least as long as week 52 on study. Thus, sufficient numbers of mice of each sex survived to week 52 to be at risk for development of tumors appearing within this period. Sufficient numbers of the males survived to the end of the test to be at risk for development of late-appearing tumors; however, the earlier deaths of the females may have limited the development of late-appearing tumors in this sex.

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.

Excluding lymphoreticular neoplasms, the lesions listed in Appendix B appeared with approximately equal frequency in dosed and control mice or appeared in insignificant numbers. Most of these lesions are not uncommon in the B6C3F1 mouse independent of any treatment.

The incidences of lymphoreticular neoplasms were 9/35(26%) in the

low-dose males, 3/34(9%) in the high-dose males, and 1/15(7%) in the matched-control males. In female mice, the incidences were approximately equal for all three groups. The distribution and type of the lesions were as follows:

	MALES			FEMALES		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Mice Necropsied	(15)	(35)	(34)	(10)	(35)	(30)
Brain						
- malignant lymphoma, histiocytic type	0	1	0	0	0	0
Multiple Organs, Lymphoreticular						
- malignant lymphoma, lymphoblastic (undifferentiated) type	0	4	0	0	1	2
- malignant lymphoma, lymphocytic type	0	1	2	0	0	0
- malignant lymphoma, histiocytic type	1	2	1	1	2	0
Mesenteric Lymph Node						
- malignant lymphoma, histiocytic type	0	1	0	0	0	0
Number of Mice with Tumors of the Hematopoietic System	1	9	3	1	3	2

The lymphoreticular neoplasms are a common finding in B6C3F1 mice independent of any treatment, and vary in frequency from one study to the next.

In addition to the neoplastic lesions, a number of degenerative,

proliferative, and inflammatory changes were encountered in animals of the dosed and control groups (Appendix D). These nonneoplastic lesions are commonly seen in aged B6C3F1 mice; however, the severe suppurative bronchopneumonias were associated with increased deaths or decreased life spans. The incidence of bronchopneumonias was high in all the control and dosed female groups, and in the low-dose males.

Although the incidence of lymphoreticular neoplasms was higher in B6C3F1 male mice fed acetohexamide for 24 months at the original and reduced low doses (10,000 and 5,000 ppm) than in the control mice, they were considered by the pathologists to be spontaneous neoplasms not associated with administration of acetohexamide.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In each sex, the results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for direct comparison of incidences between the matched controls and each of the dosed groups are not significant. In each of the 95% confidence intervals of 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 acetohexamide, which could not be detected under the conditions of this test. In male mice, significant trends (P = 0.049) in the negative direction are observed in the incidences of liver tumors, where the incidences in the controls exceed those in the dosed animals. These significant negative results may be due to the somewhat higher mortality of the dosed animals compared with the controls in the last 20 weeks on study.

V. DISCUSSION

In this bioassay, administration of acetohexamide in feed caused a dose-related depression in mean body weights of rats and mice of both sexes. No other clinical signs related to administration of the test chemical were observed; however, some animals in all groups of mice developed severe suppurative bronchopneumonia. Except for the female mice, adequate numbers of animals survived long enough to be at risk for development of late-appearing tumors.

In the rats, the only tumor occurring with greater incidence in dosed than in matched-control animals was leukemia (males: matched controls 0/15, low-dose 10/35, high-dose 4/35; females: matched controls 0/14, low-dose 7/35, high-dose 4/34). Only the incidence in the low-dose males was statistically significant ($P = 0.018$). All of these animals had undifferentiated (mononuclear cell) leukemia, which commonly occurs spontaneously in Fischer 344 rats, except for two with lymphocytic leukemia. The incidence of combined leukemia and lymphoma in historical-control male rats at this laboratory in the bioassay program to date is 24/235, which is higher than that in the matched controls. Thus, the incidence in the low-dose males cannot be clearly associated with administration of the test chemical.

In the mice, the only lesions that appeared at a higher frequency in the dosed than in the matched-control animals were lymphomas in the males. These incidences were not statistically significant (controls 1/15, low-dose males 9/35, high-dose males 3/34) and are found commonly in untreated B6C3F1 mice. In historical-control male mice, the incidence of lymphoma is 28/536.

It is concluded that under the conditions of this bioassay, acetohexamide was not carcinogenic for either Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

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

TABLE A1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	35
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(35)	(35)
SARCOMA, NOS		1 (3%)	
FIBROMA	1 (7%)		
RESPIRATORY SYSTEM			
#LUNG	(15)	(34)	(35)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	1 (3%)	1 (3%)
C-CELL CARCINOMA, METASTATIC			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(35)	(35)
UNDIFFERENTIATED LEUKEMIA		10 (29%)	3 (9%)
LYMPHOCYTIC LEUKEMIA			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(35)	(35)
HEPATOCELLULAR ADENOMA			1 (3%)
#STOMACH	(14)	(35)	(35)
SQUAMOUS CELL PAPILLOMA		1 (3%)	
#COLON	(14)	(35)	(35)
ADENOMATOUS POLYP, NOS	1 (7%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(15)	(35)	(35)
MIXED TUMOR, MALIGNANT		1 (3%)	
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(33)	(33)
CHROMOPHOBE ADENOMA		1 (3%)	4 (12%)
CHROMOPHOBE CARCINOMA		2 (6%)	1 (3%)
#ADRENAL	(15)	(35)	(35)
PHEOCHROMOCYTOMA, MALIGNANT	1 (7%)	1 (3%)	
#THYROID	(14)	(35)	(35)
FOLLICULAR-CELL ADENOMA	1 (7%)		
C-CELL ADENOMA	1 (7%)	2 (6%)	4 (11%)
C-CELL CARCINOMA	1 (7%)	2 (6%)	2 (6%)
#PANCREATIC ISLETS	(14)	(35)	(35)
ISLET-CELL ADENOMA	2 (14%)		
ISLET-CELL CARCINOMA	1 (7%)		1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(35)	(35)
FIBROADENOMA	1 (7%)		
*PREPUTIAL GLAND	(15)	(35)	(35)
CARCINOMA,NOS			1 (3%)
ADENOMA, NOS			1 (3%)
#TESTIS	(15)	(35)	(35)
INTERSTITIAL-CELL TUMOR	15 (100%)	34 (97%)	29 (83%)
SARCOMA, NOS			1 (3%)
NERVOUS SYSTEM			
*PERIPHERAL NERVE	(15)	(35)	(35)
NEUROFIBROSARCOMA			1 (3%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(15)	(35)	(35)
ADENOMA, NOS		1 (3%)	1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS	(15)	(35)	(35)
MESOTHELIOMA, NOS			1 (3%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	2	
MORIBUND SACRIFICE	1	5	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	28	29
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	35	33
TOTAL PRIMARY TUMORS	26	57	53
TOTAL ANIMALS WITH BENIGN TUMORS	15	34	31
TOTAL BENIGN TUMORS	23	40	41
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	15	11
TOTAL MALIGNANT TUMORS	3	17	11
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 A2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(14)	(35)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (6%)	1 (3%)
C-CELL CARCINOMA, METASTATIC		1 (3%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(35)	(34)
UNDIFFERENTIATED LEUKEMIA		7 (20%)	3 (9%)
LYMPHOCYTIC LEUKEMIA			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#STOMACH	(14)	(34)	(34)
SQUAMOUS CELL CARCINOMA			1 (3%)
URINARY SYSTEM			
#URINARY BLADDER	(14)	(35)	(34)
TRANSITIONAL-CELL CARCINOMA	1 (7%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(12)	(27)	(30)
CHROMOPHOBE ADENOMA	1 (8%)	7 (26%)	5 (17%)
CHROMOPHOBE CARCINOMA	1 (8%)	3 (11%)	2 (7%)
#THYROID	(13)	(32)	(34)
C-CELL ADENOMA		1 (3%)	1 (3%)
C-CELL CARCINOMA	1 (8%)	1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(35)	(34)
PAPILLARY ADENOCARCINOMA			1 (3%)
FIBROADENOMA	4 (29%)	4 (11%)	
#UTERUS	(14)	(33)	(34)
ADENOCARCINOMA, NOS		1 (3%)	
SARCOMA, NOS			1 (3%)
ENDOMETRIAL STROMAL POLYP	1 (7%)	5 (15%)	4 (12%)
ENDOMETRIAL STROMAL SARCOMA	2 (14%)	1 (3%)	
#OVARY	(14)	(33)	(34)
GRANULOSA-CELL CARCINOMA			1 (3%)
NERVOUS SYSTEM			
#BRAIN	(13)	(35)	(34)
ASTROCYTOMA			1 (3%)
*TRIGEMINAL NERVE	(14)	(35)	(34)
CHROMOPHOBE CARCINOMA, METASTATI			1 (3%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(14)	(35)	(34)
SQUAMOUS CELL CARCINOMA		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(14)	(35)	(34)
SARCOMA, NOS, METASTATIC			1 (3%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(14)	(35)	(34)
C-CELL CARCINOMA, METASTATIC	1 (7%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	3	1	1
MORIBUND SACRIFICE	1	8	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	11	26	29
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	23	18
TOTAL PRIMARY TUMORS	11	33	22
TOTAL ANIMALS WITH BENIGN TUMORS	5	15	10
TOTAL BENIGN TUMORS	6	19	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	13	11
TOTAL MALIGNANT TUMORS	5	14	11
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	2
TOTAL SECONDARY TUMORS	1	1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

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

TABLE B1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(15)	(35)	(33)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	1 (3%)	2 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (7%)	2 (6%)	
HEMATOPOIETIC SYSTEM			
#BRAIN	(15)	(35)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
*MULTIPLE ORGANS	(15)	(35)	(34)
MALIG.LYMPHOMA, UNDIFFER-TYPE		4 (11%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	2 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)	2 (6%)	1 (3%)
#MESENTERIC L. NODE	(14)	(32)	(29)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(34)
HEPATOCELLULAR ADENOMA	1 (7%)	1 (3%)	1 (3%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA	2 (13%)	1 (3%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(15)	(35)	(34)
PAPILLARY ADENOMA		1 (3%)	
PAPILLARY CYSTADENOMA, NOS		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	10	8
MORIBUND SACRIFICE	4	8	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	17	18
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	14	5
TOTAL PRIMARY TUMORS	6	16	6
TOTAL ANIMALS WITH BENIGN TUMORS	2	4	3
TOTAL BENIGN TUMORS	2	4	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	11	3
TOTAL MALIGNANT TUMORS	4	12	3
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	10	35	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	35	30
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(34)	(29)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(35)	(30)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (3%)	2 (7%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (10%)	2 (6%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(35)	(30)
HEPATOCELLULAR ADENOMA			1 (3%)
HEPATOCELLULAR CARCINOMA	1 (10%)	1 (3%)	
#DUODENUM	(8)	(34)	(28)
SARCOMA, NOS		1 (3%)	
URINARY SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(10)	(35)	(30)
ENDOMETRIAL STROMAL POLYP		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKEIETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	10	5	13
MORIBUND SACRIFICE	2	17	15
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	3	13	7
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	7	3
TOTAL PRIMARY TUMORS	2	7	3
TOTAL ANIMALS WITH BENIGN TUMORS		2	1
TOTAL BENIGN TUMORS		2	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	5	2
TOTAL MALIGNANT TUMORS	2	5	2
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED ACETOHEXAMIDE IN THE DIET

TABLE C1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	35
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(35)	(35)
EPIDERMAL INCLUSION CYST	1 (7%)	1 (3%)	
*SUBCUT TISSUE	(15)	(35)	(35)
INFLAMMATION, SUPPURATIVE		1 (3%)	
INFLAMMATION, GRANULOMATOUS		2 (6%)	
RESPIRATORY SYSTEM			
*NASAL CAVITY	(15)	(35)	(35)
INFLAMMATION, SUPPURATIVE		1 (3%)	
#TRACHEA	(15)	(35)	(35)
INFLAMMATION, SUPPURATIVE	1 (7%)	4 (11%)	1 (3%)
INFLAMMATION, CHRONIC		1 (3%)	
#LUNG	(15)	(34)	(35)
PNEUMONIA, CHRONIC MURINE		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(15)	(33)	(34)
ATROPHY, NOS			1 (3%)
#THYMUS	(14)	(35)	(35)
HEMORRHAGE	1 (7%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
*PHARYNX	(15)	(35)	(35)
INFLAMMATION, SUPPURATIVE		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(35)	(35)
INFLAMMATION, CHRONIC	14 (93%)	29 (83%)	20 (57%)
ENDOCRINE SYSTEM			
#THYROID	(14)	(35)	(35)
HYPERPLASIA, FOLLICULAR-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(15)	(32)	(35)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
#TESTIS	(15)	(35)	(35)
HEMORRHAGE		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(15)	(35)	(35)
INFLAMMATION, CHRONIC		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(15)	(35)	(35)
STEATITIS		2 (6%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(13)	(35)	(34)
INFLAMMATION, SUPPURATIVE	1 (8%)	2 (6%)	2 (6%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(13)	(35)	(34)
HYPERPLASIA, FOCAL			1 (3%)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(14)	(35)	(34)
INFLAMMATION, CHRONIC	7 (50%)	4 (11%)	1 (3%)
ENDOCRINE SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
#UTERUS	(14)	(33)	(34)
HYDROMETRA		2 (6%)	2 (6%)
HEMORRHAGE		1 (3%)	
DECIDUAL ALTERATION, NOS		1 (3%)	
#UTERUS/ENDOMETRIUM	(14)	(33)	(34)
INFLAMMATION, SUPPURATIVE	3 (21%)		
INFLAMMATION, CHRONIC SUPPURATIV	2 (14%)	7 (21%)	1 (3%)
HYPERPLASIA, NOS	1 (7%)		
HYPERPLASIA, FOCAL		1 (3%)	
#OVARY/OVIDUCT	(14)	(33)	(34)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
#OVARY	(14)	(33)	(34)
INFLAMMATION, CHRONIC SUPPURATIV		2 (6%)	1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(14)	(35)	(34)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
*MIDDLE EAR	(14)	(35)	(34)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(14)	(35)	(34)
HYPERPLASIA, RETICULUM CELL		1 (3%)	1 (3%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	6	14
AUTCLYSIS/NO NECROPSY	1		1

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

APPENDIX D

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

TABLE D1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	34
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(35)	(34)
INFLAMMATION, PYOGRANULOMATOUS		1 (3%)	
*SUBCUT TISSUE	(15)	(35)	(34)
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(15)	(35)	(33)
HYPERPLASIA, LYMPHOID		1 (3%)	
#LUNG	(15)	(35)	(33)
BRONCHOPNEUMONIA SUPPURATIVE	3 (20%)	7 (20%)	3 (9%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (3%)	
BRONCHOPNEUMONIA ACUTE SUPPURATI	1 (7%)		1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(15)	(32)	(34)
HYPOPLASIA, HEMATOPOIETIC		1 (3%)	
#MESENTERIC L. NODE	(14)	(32)	(29)
HYPERPLASIA, LYMPHOID		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(34)
CYTOPLASMIC VACUOLIZATION			1 (3%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NODULAR			1 (3%)
HYPERPLASIA, LYMPHOID		1 (3%)	
#DUODENUM	(14)	(30)	(32)
HYPERPLASIA, LYMPHOID			1 (3%)
URINARY SYSTEM			
#KIDNEY	(15)	(33)	(34)
INFLAMMATION, CHRONIC FOCAL	1 (7%)		
ENDOCRINE SYSTEM			
#THYROID	(15)	(35)	(33)
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(15)	(35)	(34)
ULCER, NOS		1 (3%)	
*MIDDLE EAR	(15)	(35)	(34)
INFLAMMATION, SUPPURATIVE	1 (7%)	1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(15)	(35)	(34)
HYPERPLASIA, LYMPHOID	1 (7%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	12	21
NO NECROPSY PERFORMED			1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED ACETOHEXAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	10	35	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	35	30
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(35)	(30)
ULCER, CHRONIC	1 (10%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(10)	(34)	(29)
HYPERPLASIA, LYMPHOID	1 (10%)		
#LUNG	(10)	(34)	(29)
BRONCHOPNEUMONIA SUPPURATIVE	4 (40%)	6 (18%)	9 (31%)
BRONCHOPNEUMONIA NECROTIZING			1 (3%)
BRONCHOPNEUMONIA ACUTE SUPPURATI		4 (12%)	1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (3%)	5 (17%)
NECROSIS, FOCAL		1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (10%)		1 (3%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#DUODENUM	(8)	(34)	(28)
METAPLASIA, OSSEOUS		1 (3%)	
HYPERPLASIA, LYMPHOID		1 (3%)	
URINARY SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(10)	(35)	(30)
ANGIECTASIS	1 (10%)		
#UTERUS/ENDOMETRIUM	(10)	(35)	(30)
HYPERPLASIA, EPITHELIAL	1 (10%)		
HYPERPLASIA, CYSTIC	4 (40%)	9 (26%)	
#OVARY	(9)	(34)	(28)
INFLAMMATION, SUPPURATIVE		2 (6%)	
NERVOUS SYSTEM			
#BRAIN	(10)	(34)	(30)
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)
ABSCESS, NOS			1 (3%)
*TRIGEMINAL GANGLION	(10)	(35)	(30)
INFLAMMATION, NOS		1 (3%)	
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(10)	(35)	(30)
INFLAMMATION, SUPPURATIVE	1 (10%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(35)	(30)
HYPERPLASIA, LYMPHOID	1 (10%)	2 (6%)	2 (7%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		9	8
NO NECROPSY PERFORMED			2
AUTOLYSIS/NO NECROPSY	5		3

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

APPENDIX E

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

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Acetohexamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Leukemia[b]	0/15 (0)	10/35 (29)	4/35 (11)
P Values[c,d]	N.S.	P = 0.018	N.S.
Departure from Linear Trend[e]	P = 0.007		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		1.362	0.423
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	77	93
Pituitary: Chromophobe Carcinoma[b]	0/11 (0)	2/33 (6)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.108	0.019
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	105

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	0/11 (0)	3/33 (9)	5/33 (15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.221	0.464
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	105
Thyroid: C-cell Carcinoma[b]	1/14 (7)	2/35 (6)	2/35 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.800	0.800
Lower Limit		0.047	0.047
Upper Limit		45.853	45.853
Weeks to First Observed Tumor	107	106	106

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	2/14 (14)	4/35 (11)	6/35 (17)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.800	1.200
Lower Limit		0.135	0.257
Upper Limit		8.252	11.299
Weeks to First Observed Tumor	107	106	105
Pancreatic Islets: Islet-cell Adenoma or Carcinoma[b]	3/14 (21)	0/35 (0)	1/35 (3)
P Values[c,d]	P = 0.039(N)	P = 0.020(N)	N.S.
Departure from Linear Trend[e]	P = 0.014		
Relative Risk (Matched Control)[f]		0.000	0.133
Lower Limit		0.000	0.003
Upper Limit		0.648	1.528
Weeks to First Observed Tumor	103	--	106

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	15/15 (100)	34/35 (97)	29/35 (83)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.971	0.829
Lower Limit		0.000	0.000
Upper Limit		1.066	1.046
Weeks to First Observed Tumor	99	77	86

[a]Dosed groups received 10,000 or 20,000 ppm in feed.

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

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

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

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

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Acetohexamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/14 (0)	2/35 (6)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.125	0.023
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	86	106
Hematopoietic System: Leukemia[b]	0/14 (0)	7/35 (20)	4/34 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.834	0.410
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	79	73

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Carcinoma[b]	1/14 (7)	0/35 (0)	0/34 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		7.421	7.633
Weeks to First Observed Tumor	88	--	--
Pituitary: Chromophobe Carcinoma[b]	1/12 (8)	3/27 (11)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.333	0.800
Lower Limit		0.125	0.047
Upper Limit		67.223	45.610
Weeks to First Observed Tumor	72	85	70

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	2/12 (17)	10/27 (37)	7/30 (23)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		2.222	1.400
Lower Limit		0.600	0.334
Upper Limit		18.632	12.617
Weeks to First Observed Tumor	72	85	70
Thyroid: C-cell Carcinoma[b]	1/13 (8)	1/32 (3)	0/34 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.406	0.000
Lower Limit		0.006	0.000
Upper Limit		30.928	7.090
Weeks to First Observed Tumor	107	86	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	1/13 (8)	2/32 (6)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.813	0.382
Lower Limit		0.048	0.005
Upper Limit		46.435	29.166
Weeks to First Observed Tumor	107	86	106
Mammary Gland: Fibroadenoma[b]	4/14 (29)	4/35 (11)	0/34 (0)
P Values[c,d]	P = 0.003(N)	N.S.	P = 0.005(N)
Relative Risk (Matched Control)[f]		0.400	0.000
Lower Limit		0.091	0.000
Upper Limit		1.912	0.430
Weeks to First Observed Tumor	72	107	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	1/14 (7)	5/33 (15)	4/34 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		2.121	1.647
Lower Limit		0.279	0.190
Upper Limit		96.931	78.533
Weeks to First Observed Tumor	107	86	106
Uterus: Sarcoma, NOS (not otherwise specified), or Endometrial Stromal Sarcoma[b]	2/14 (14)	1/33 (3)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.212	0.206
Lower Limit		0.004	0.004
Upper Limit		3.821	3.714
Weeks to First Observed Tumor	107	107	99

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Acetohexamide in the Diet[a]

(continued)

[a]Dosed groups received 10,000 or 20,000 ppm in feed.

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

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

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

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

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

APPENDIX F

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

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Acetohexamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	1/15 (7)	2/35 (6)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.857	0.000
Lower Limit		0.050	0.000
Upper Limit		49.128	8.417
Weeks to First Observed Tumor	107	103	--
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	2/15 (13)	3/35 (9)	2/33 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.643	0.455
Lower Limit		0.085	0.037
Upper Limit		7.208	5.871
Weeks to First Observed Tumor	107	98	107

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma[b]	1/15 (7)	9/35 (26)	3/34 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.030		
Relative Risk (Matched Control)[f]		3.857	1.324
Lower Limit		0.627	0.120
Upper Limit		162.855	67.356
Weeks to First Observed Tumor	101	75	99
Liver: Hepatocellular Carcinoma[b]	2/15 (13)	1/33 (3)	0/34 (0)
P Values[c,d]	P = 0.041(N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.227	0.000
Lower Limit		0.004	0.000
Upper Limit		4.101	1.463
Weeks to First Observed Tumor	103	93	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	3/15 (20)	2/33 (6)	1/34 (3)
P Values[c,d]	P = 0.049(N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.303	0.147
Lower Limit		0.029	0.003
Upper Limit		2.421	1.688
Weeks to First Observed Tumor	103	93	107

[a]Dosed groups received time-weighted average doses of 6,359 or 12,718 ppm in feed.

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

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

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

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

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Acetohexamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma[b]	1/10 (10)	3/35 (9)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.857	0.667
Lower Limit		0.083	0.041
Upper Limit		43.687	38.024
Weeks to First Observed Tumor	101	88	98
Liver: Hepatocellular Carcinoma[b]	1/10 (10)	1/35 (3)	0/30 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.286	0.000
Lower Limit		0.004	0.000
Upper Limit		21.825	6.165
Weeks to First Observed Tumor	107	108	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Acetohexamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	1/10 (10)	1/35 (3)	1/30 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.286	0.333
Lower Limit		0.004	0.005
Upper Limit		21.825	25.331
Weeks to First Observed Tumor	107	108	101

[a]Dosed groups received time-weighted average doses of 6,359 or 12,718 ppm in feed.

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

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

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

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

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

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

March 6, 1978

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

The primary reviewer briefly described the experimental design and the conditions under which Acetohexamide was studied. He noted an increase in the incidence of leukemia in treated rats and lymphomas in treated mice. However, neither of the lesions was statistically significant when compared to the historical control incidence. A Program pathologist commented that lymphomas frequently present a problem to interpret since their spontaneous incidence is so variable. The tendency is to draw a conservative conclusion when a response is observed in the low dose group but not in the high dose one.

A discussion ensued on the mononuclear cell leukemia reported in treated rats. Program staff noted the variability of this lesion from one study to another, as well as between laboratories. In the laboratory conducting this particular study, it was noted that the leukemia incidence observed in treated rats was within the spontaneous tumor range.

Further discussion occurred on the probability of a false positive or negative, given the induced and spontaneous tumor rates in the mice. A Subgroup member suggested that the conclusion be reworded as follows: Under the conditions of test, a statistically significant increase in the incidence of lymphomas was found in the treated male mice; however, it cannot be determined if the increased incidence was compound related because of the spontaneous occurrence of this tumor type. It was moved and seconded that the report be accepted as modified by this statement. (No vote was taken on the motion.)

After further discussion, a Subgroup member moved that the results of the study were inadequate to reach a conclusion regarding the carcinogenicity of Acetohexamide. The motion was seconded and approved by all the members except Dr. Highland and Dr. Weisburger, who abstained.

Members present were:

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

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

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