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TRISODIUM ETHYLENEDIAMINETETRAACETATE TRIHYDRATE

FOR POSSIBLE CARCINOGENICITY

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For Possible Carcinogenicity

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CONTRIBUTORS: This report presents the results of the bioassay of trisodium ethylenediaminetetraacetate trihydrate ($Na_3EDTA \cdot 3H_2O$) for possible carcinogenicity, conducted by the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted at Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., the prime contractor for the NCI carcinogen bioassay program.

The experimental design was determined, and the doses were selected, by Drs. E. K. Weisburger[1] and J. H. Weisburger[1,2]. Animal treatment and observations were supervised by Drs. B. M. Ulland[3,4] and F. M. Garner[3], with the technical assistance of Mr. J. D. Farmer[3], Ms. H. I. Ruckenbrad[3], Mr. D. J. Howard[3], and Mr. H. D. Thornett[3].

Histopathology was performed at Litton Bionetics, Inc. by Drs. A. de Paoli and J. Wosu for rats and by Dr. A. de Paoli for mice, under the supervision of Dr. F. M. Garner, and the diagnoses included in this report represent their interpretation. Pathologists at NCI and Tracor Jitco, Inc. have reviewed the pathology report and concur with the overall pathologic evaluation of the study.

Compilation of tables of individual animal survival and tables of neoplasms and of nonneoplastic lesions was performed by EG&G Mason Research Institute[6]. Statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[7].

This report was prepared at Tracor Jitco under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. J. F. Robens[5], toxicologist; Dr. S. S. Olin[5], chemist; Ms. L. A. Waitz[5], bioscience writer; and Dr. E. W. Gunberg[5], technical editor. The final report was reviewed by members of the participating organizations[1,3,5].

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SUMMARY

A bioassay of the chelating agent, trisodium ethylenediaminetetraacetate trihydrate ($Na_3EDTA \cdot 3H_2O$), for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats and B6C3F1 mice. The chemical was administered to 50 males and 50 females of each species at low and high concentrations, 3,750 and 7,500 ppm, for 103 weeks. Matched-control groups were composed of 20 males and 20 females of each species.

No compound-related signs of clinical toxicity were noted. Although a variety of tumors occurred among test and control animals of both species, no tumors were related to treatment. Since survival was satisfactory and showed no consistent variation among test and control groups, the absence of treatment-related tumors could not be attributed to early mortality.

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I. INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA) is a synthetic amino-polycarboxylic acid chelating agent. It has been used extensively as a food additive to sequester trace metals that catalyze the oxidation of oils, vitamins, and unsaturated fats and cause rancidity, flavor changes, and discoloration (Furia, 1975). Permissible levels of calcium disodium EDTA in food range from 25 to 800 ppm (Food and Drug Administration, 1974), and an acceptable daily intake of 2.5 mg/kg has been established (Joint FAO/WHO Expert Committee on Food Additives, 1974). EDTA has also been used to control the interactions of trace metals in formulations of liquid soaps, cosmetics, and pharmaceuticals, in metal working, in pulp and paper processing, in rubber and polymer chemistry, and in textile processing and dyeing (Dow Chemical Company, 1974). Calcium disodium EDTA has been used primarily in the treatment of metal poisoning, although toxicity to the renal proximal tubules necessitates precautions with this therapy (Foreman et al., 1956).

EDTA was selected for testing because of structural and functional similarity to another chelating agent, namely, nitrilotriacetic acid trisodium salt ($Na_3NTA \cdot H_2O$), which also was tested in this program.

II. MATERIALS AND METHODS

A. Chemical

The trisodium ethylenediaminetetraacetate trihydrate ($Na_3EDTA \cdot 3H_2O$) used in this study was supplied by Pfaltz & Bauer, Inc., Flushing, N. Y. The chemical is hereinafter referred to as EDTA. Its molecular weight is 410.84, of which 70% is ethylenediaminetetraacetic acid. Identity of the material was verified by infrared and nuclear magnetic resonance (NMR) spectroscopy. The NMR analysis showed that the compound was in the trihydrate form. The sodium content as determined by flame emission spectrophotometry was 17.1%, compared to a theoretical content of 16.8% for the trisodium salt trihydrate. Thin-layer chromatography in two solvent systems produced a single spot as visualized by iodine vapor, methyl red, and phenol red indicator and amine-specific ferric chloride - potassium ferricyanide spray.

B. Dietary Preparation

A 6-kilogram batch of dosed feed was prepared in a hooded twin-shell blender two times a week for mice and three times a week for rats. Preparations and chemicals were refrigerated at 4°C until they were used.

Analyses were performed, using FDA methods (Food and Drug Administration, 1965) to determine the efficiency of the mixing procedure and the stability of the test chemical in feed. Samples were taken from the bottom and two wings of the blender, and analyses were performed in triplicate; the error is reported as the standard deviation. Recoveries were found to be 90.3 $\pm$ 1.4% of the theoretical value at 7,500 ppm EDTA and 90.4 $\pm$ 3.4% of the theoretical value at 3,750 ppm. Analyses performed on samples from the same batches after 12 days of storage at ambient temperature gave 95.5 $\pm$ 2.7% of the theoretical value at 7,500 ppm and 92.1 $\pm$ 5.7% at 3,750 ppm. It was concluded from these results that the preparations contained reasonably accurate concentrations of EDTA and were mixed homogeneously, and that the chemical was stable in feed for at least a week. When concentrations of EDTA were rechecked near termination of the chronic study, the accuracy of mixing and the homogeneity of the mixtures was reconfirmed.

C. Animals

Inbred Fischer 344 rats were received from A. R. Schmidt, Madison, Wisconsin, and hybrid B6C3F1 mice from the Charles River Breeding Laboratories, Wilmington, Massachusetts, at 28 days of age. These laboratories were under contract to the Division of Cancer Treatment, NCI, to provide animals used for testing. On

arrival at the laboratory, the animals were quarantined for 14 days to acclimate them to the laboratory environment and to allow observation of their physical condition. Animals were considered acceptable for testing if they had no clinical signs of disease and were within a weight range of 19-22 g for mice and 85-110 g for rats at the end of the quarantine period. Animals were weighed individually and segregated into equal weight groups. Cage assignments were then made by choosing one animal from each such group, so that the total animal weight was the same in each cage.

D. Animal Maintenance

During the chronic study rats were housed four per cage, and mice, five per cage in solid polycarbonate cages suspended on moveable racks. All cages were lined with heat-treated hardwood chip bedding and covered with filter paper over a wire mesh screen. Clean cages and fresh bedding were provided twice a week. Water bottles were sanitized and refilled with acidulated water (pH 2.5) twice a week. Test diets were prepared with Wayne® Lab Blox Meal (Allied Mills, Inc.) that was also used as feed for the control animals. Feed was available ad libitum and replaced three times a week. Air in the animal rooms was changed 15 times per hour and exhausted through HEPA filters. Animal rooms were negatively pressurized with respect to the clean hall

and positively pressurized with respect to the dirty hall. The room air was maintained at 21-25°C and 45-55% humidity. Rooms were illuminated by fluorescent lighting for 8 hours a day.

Rats and mice were housed in separate rooms. Matched controls were housed with the respective test animals. Animals treated with EDTA were maintained in rooms with animals of the same species being treated with other chemicals as follows:

Rats

iodomethanesulfonic acid, sodium salt
3-hydroxy-(3 alpha, 5 beta)cholan-24-oic acid
3,3-dimethyl-2-oxethanone

Mice

hydroxytriphenylstannane
4,4′-diisocyanato-3,3′-dimethoxy-1,1′-biphenyl
N-(aminocarbonyl)-2-bromo-2-ethylbutanamide
N,N′-diethylthiourea
mono(2,2-dimethylhydrazine)butanedioic acid
iodomethanesulfonic acid, sodium salt
2,5-cyclohexadiene-1,4-dione, dioxime
3-hydroxy-(3 alpha, 5 beta)cholan-24-oic acid
4-amino-2-nitrophenol

E. Subchronic Studies

Feeding studies were conducted to estimate the maximum tolerated doses in order to determine the high and low concentrations (hereinafter referred to as "high doses" and "low doses") to be administered in the chronic studies. The low doses given in the chronic studies were 1/2 of the high doses. In the subchronic

studies EDTA was added to the animal feed at five dietary concentrations: 4,640, 6,800, 10,000, 14,700, and 21,600 ppm. The compound was provided in feed to experimental groups of five male and five female animals of each species for 7 weeks, followed by a 1-week period of observation.

During these studies soft stools, a sign of compound-related toxicity (Foreman, 1953; Yang, 1964), were noted at 10,000 ppm and above in male rats and at 14,700 ppm and above in female rats. After 8 weeks, body weights of the treated rats at all doses were comparable to the weights of the matched controls. Gross pathologic examinations showed no signs of organ toxicity in rats. In mice, depression of weight was noted in males at 21,600 ppm and in females at 6,800 ppm and above. One male receiving 21,600 ppm died. There were no pathologic changes in any animals detected at necropsy. The low and high doses for both species were set at 3,750 and 7,500 ppm.

F. Design of Chronic Studies

The design of the chronic studies, including both test and matched-control groups, is shown in table 1.

Table 1. Design of Chronic Studies of EDTA

Species, Sex and Treatment Group	Initial No. of Animals	EDTA in Diet (ppm)	Time on Study	
			Treated (weeks)	Untreated (weeks)
RATS				
Male				
Matched-Control	20	0	0	104
Low-Dose	50	3,750	103	1
High-Dose	50	7,500	103	1
Female				
Matched-Control	20	0	0	104
Low-Dose	50	3,750	103	1
High-Dose	50	7,500	103	1
MICE				
Male				
Matched-Control	20	0	0	104
Low-Dose	50	3,750	103	1
High-Dose	50	7,500	103	1
Female				
Matched-Control	20	0	0	104
Low-Dose	50	3,750	103	1
High-Dose	50	7,500	103	1

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, weighed at regular intervals, and palpated for masses at each weighing. Those animals appearing moribund at the time of clinical examination were killed and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of all major tissues, organs, or gross lesions taken from killed animals and, when feasible, from animals found dead. The following tissues and organs were routinely subjected to microscopic examination: skin, lymph nodes, mammary gland, salivary gland, bone marrow, trachea, lungs and bronchi, heart, thyroids, parathyroids, esophagus, stomach, small intestine, large intestine, liver, gallbladder, pancreas, spleen, kidneys, adrenals, urinary bladder, prostate or uterus, testis or ovary, brain, and pituitary. 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. An occasional section was subjected to special staining techniques for more definitive diagnosis.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of

autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

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

Probabilities of survival were estimated by the product limit procedure of Kaplan and Meier (1958) and presented in this report in the form of graphs. Deaths due to accident or scheduled deaths are treated as censored observations and all other deaths are uncensored. Statistical tests of differences in survival between groups are compared using the method of Cox (1972) for two groups and an extension of this method by Tarone (1975) for more than two groups.

The incidence of neoplastic or nonneoplastic lesions is given as the proportion of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals examined pathologically at that site (denominator). For the organs and tissues in which most of the lesions appeared, the denominators included only those animals for which such sites were examined histologically. For tissues that required gross observation for detection of lesions (e.g., skin or mammary tumors), for lesions that appeared at several sites (e.g., lymphomas), or for tissues that were examined histologically only when lesions were detected grossly, the denominators consisted of the numbers of animals necropsied.

Statistical analysis of the incidence of tumors was made using the Fisher exact test (Cox, 1970) to compare a control group to a group of treated animals at each dose. In addition, the Armitage and Cochran test for linear trend in proportions, with continuity correction (Armitage, 1971), was used. This test, assuming a linear trend, determines if the slope of the dose-response curve is different from zero, at the 0.05 level of significance. The method also calculates the level of probability of a departure from linear trend.

A conservative adjustment, the Bonferroni inequality (Miller, 1966), was used for simultaneous comparison of several treated

groups with a control group. For the comparison of results obtained with k different test doses with those for a control, this correction requires a level of significance less than or equal to 0.05/k for the overall comparison to be significant at the 0.05 level. This adjustment was not made in the tables where the Fisher exact test results are shown but is discussed in the analysis when appropriate.

As an additional analysis, the exact 95% confidence interval for the odds ratio (Gart, 1970) between each of the treated groups and its control was calculated. The odds ratio is $p_t(1-p_c)/p_c(1-p_t)$ where p_t is the true binomial probability of tumor in a treated animal and p_c is the true spontaneous tumor probability in the controls. The hypothesis of equality between the true proportion of a specific tumor in a treated group and that in a control is expressed by an odds ratio of 1 (one). Values in excess of 1 (one) represent the condition of a larger proportion in the treated group than in the control. The confidence interval entries in the statistical tables of this report represent the conversion of each odds ratio to the difference in probabilities, p_t-p_c, where $p_t-p_c = 0$ implies an odds ratio of 1 (one).

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Average body weights of treated male and female rats were comparable to those of the matched controls throughout the study (figure 1). No significant signs were observed among test animals during the first year of the study. In the 6 months preceding termination of the test, corneal opacities, ascites, and urine stains, occurred in both treatment and control groups.

B. Survival (Rats)

Curves showing the probability of survival of treated and control rats are shown in figure 2. The male rats exhibited a negative dose-related trend in survival with the probability level of P = 0.103; the treated and control groups of male rats can thus be considered as comparable to each other in survival. The female rats also exhibited a negative dose-related trend in survival, but in this case the effect was statisically significant (P = 0.029).

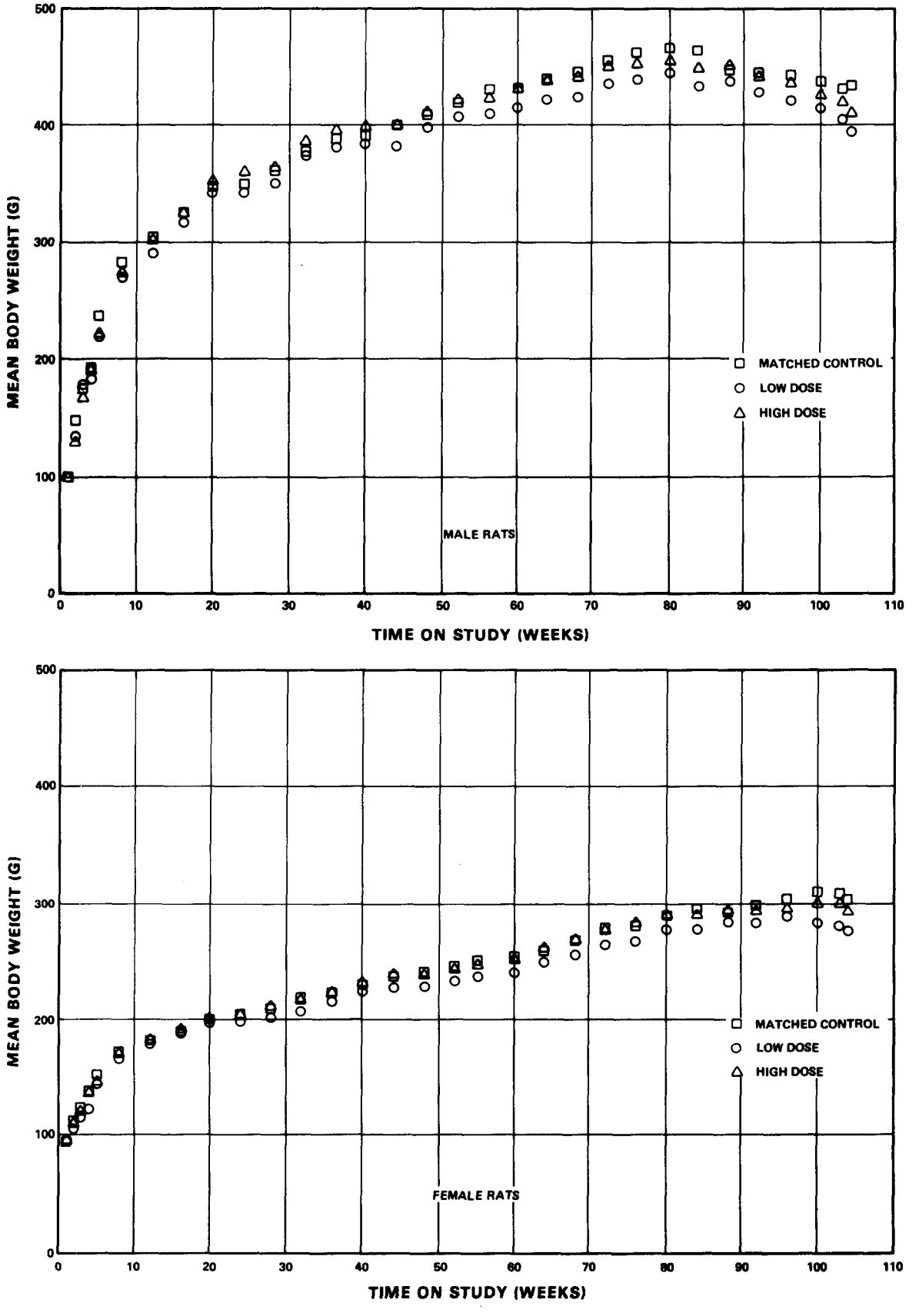
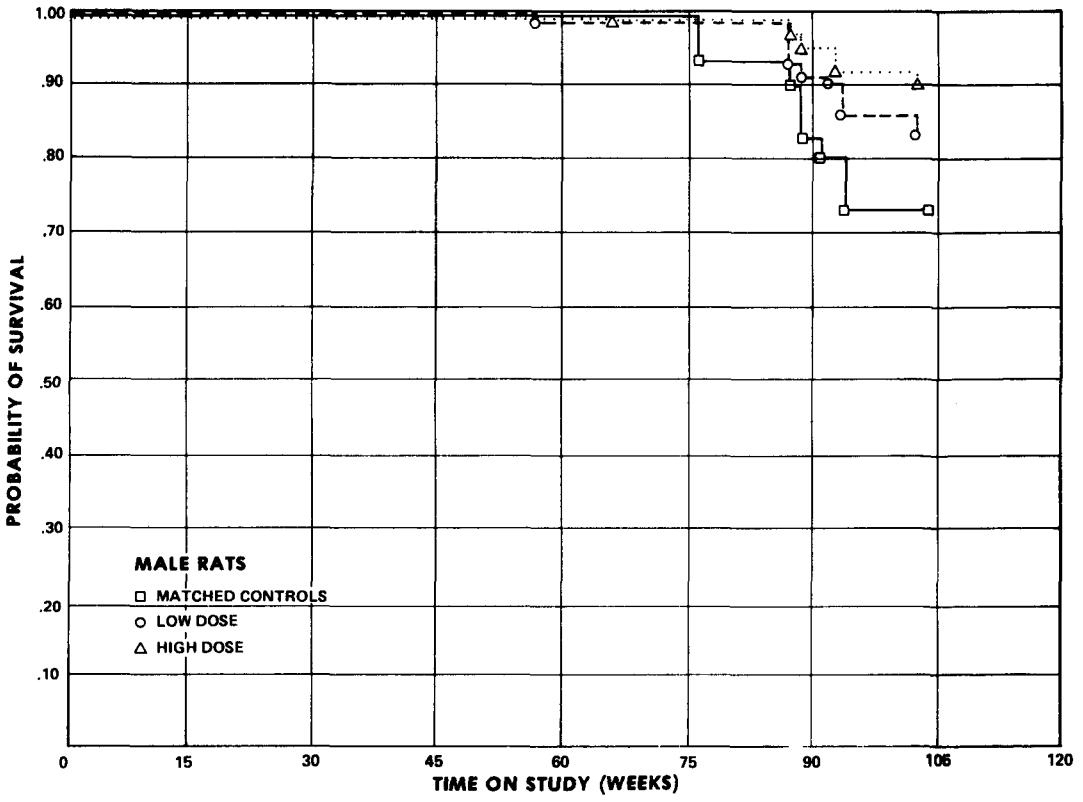


Figure 1. Growth Curves for Rats Fed EDTA in the Diet



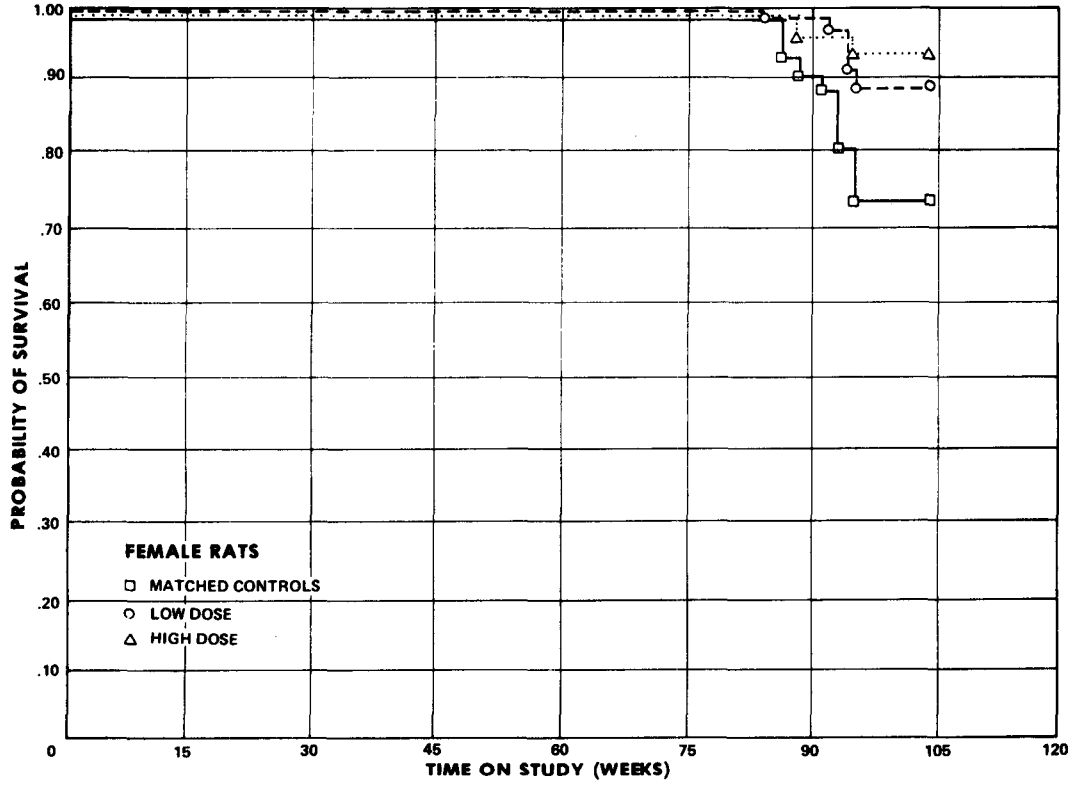


Figure 2. Survival Curves for Rats Fed EDTA in the Diet

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are tabulated in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are tabulated in Appendix C, tables C1 and C2. The incidence of neoplasms was high in the reproductive and endocrine systems and lower in the hematopoietic, respiratory, integumentary, and digestive systems. No neoplasms were observed in the nervous, musculoskeletal, or urinary systems or in organs of special sense.

Interstitial-cell tumors of the testes were observed in nearly all male rats in each feeding group. This high incidence of interstitial-cell tumors in both treated and control animals reflects this commonly occurring age-related lesion in the male Fischer 344 rat.

In females the distribution of neoplasms in the reproductive system among control and treated rats was random, the tumors occurred mainly in the uterus. The majority of these were endometrial stromal polyps. However, one adenocarcinoma and one leiomyosarcoma occurred in high-dose animals. An ovarian cystadenoma was detected in a single low-dose rat.

A variety of endocrine tumors were found, some types occurring only in treated animals. However, these tumors occurred in low

numbers and have frequently been seen in untreated animals in other studies. Therefore, they are probably unrelated to treatment.

A number of tumors occurred in other organ systems of both sexes, controls as well as treated animals. In some instances the incidence of tumors in the controls exceeded that of the treated animals. With the possible exception of endocrine tumors in the males, no clear association between the incidence of tumors, treatment, or sex could be established.

Inflammatory and degenerative changes were observed in about the same frequency in all groups. These lesions appeared to be related to age and not to the administration of the chemical.

In the judgment of the pathologist, the nature, incidence, and severity of the lesions observed in this study provide no clear evidence of carcinogenic effect in rats.

D. Statistical Analyses of Results (Rats)

Tables 2 and 3 list those tumors that occurred in more than 5% of the rats in a given treated group or appeared in a larger proportion in the treated group when compared to the matched controls. There was no other control group whose environment, period of test, and pathologic diagnosis were comparable to the

Table 2. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Rats Fed EDTA Trisodium Salt in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Leukemia, Malignant Lymphoma and Lymphocytic Leukemia[b]	3/20 (0.15)	4/50 (0.08)	4/50 (0.08)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.25,0.08)	(-0.25,0.08)
Weeks to First Observed Tumor	76	104	102
Adrenal: Pheochromocytoma[b]	2/20 (0.10)	5/49 (0.10)	4/50 (0.08)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.19,0.12)	(-0.19,0.09)
Weeks to First Observed Tumor	104	104	67

Table 2. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Rats Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	0/17 (0.00)	6/35 (0.17)	3/38 (0.08)
P Values[c]	N.S.	P = 0.080	N.S.
95% Confidence Interval[d]		(-0.07,1.00)	(-0.09,1.00)
Weeks to First Observed Tumor	--	104	104
Pituitary: Chromophobe Adenoma[b]	0/18 (0.00)	3/47 (0.06)	5/44 (0.11)
P Values[c]	P = 0.089	N.S.	N.S.
95% Confidence Interval[d]		(-0.09,1.00)	(-0.09,1.00)
Weeks to First Observed Tumor	--	88	104

Table 2. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Rats Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma and Carcinoma[b]	1/18 (0.06)	2/50 (0.04)	3/49 (0.06)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.14,0.06)	(-0.15,0.08)
Weeks to First Observed Tumor	104	95	67
Liver: Hepatocellular Adenoma and Neoplastic Nodule[b]	0/20 (0.00)	1/48 (0.02)	1/50 (0.02)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.05,1.00)	(-0.05,1.00)
Weeks to First Observed Tumor	--	104	104

Table 2. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Rats Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	19/20 (0.95)	43/50 (0.86)	44/50 (0.88)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.14,0.13)	(-0.14,0.13)
Weeks to First Observed Tumor	88	85	95

[a]Treated groups received average doses of 3,750 and 7,500 ppm in feed.

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

[c]Beneath the proportions for the matched-control group are the probability levels (P values) for the Cochran-Armitage test for dose-related trend in proportions when P is below 0.10; otherwise, N.S. (not significant) is indicated.
Beneath the proportions for the treated groups are the P values for the Fisher exact (conditional) test for the comparison of the treated groups with the matched-control group when P is below 0.05; otherwise, N.S. is indicated.

[d]95% confidence interval of the difference in proportions of treated group and matched-control group.

Table 3. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Rats Fed EDTA Trisodium Salt in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma, Leukemia and Lymphocytic Leukemia[b]	1/20 (0.05)	8/50 (0.16)	0/50 (0.00)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.11,0.18)	(-1.00,0.02)
Weeks to First Observed Tumor	104	80	--
Adrenal: Pheochromocytoma[b]	1/20 (0.05)	1/49 0.02)	3/48 (0.06)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.10,0.04)	(-0.14,0.08)
Weeks to First Observed Tumor	98	104	104

Table 3. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Rats Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	0/11 (0.00)	0/36 (0.00)	1/37 (0.03)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]			(-0.08,1.00)
Weeks to First Observed Tumor	--	--	104
Pituitary: Chromophobe Adenoma[b]	6/19 (0.32)	10/48 (0.21)	11/50 (0.22)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.37,0.13)	(-0.38,0.12)
Weeks to First Observed Tumor	95	104	104

Table 3. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Rats Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/20 (0.00)	3/48 (0.06)	2/48 (0.04)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.08,1.00)	(-0.07,1.00)
Weeks to First Observed Tumor	--	104	104
Liver: Neoplastic Nodule[b]	0/20 (0.00)	1/48 (0.02)	0/48 (0.00)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.05,1.00)	
Weeks to First Observed Tumor	--	104	--

Table 3. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Rats Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	5/20 (0.25)	6/50 (0.12)	7/50 (0.14)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.35,0.07)	(-0.34,0.09)
Weeks to First Observed Tumor	104	96	85
Mammary Gland: Fibroadenoma[b]	4/20 (0.20)	3/50 (0.06)	3/50 (0.06)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.29,0.04)	(-0.29,0.04)
Weeks to First Observed Tumor	85	96	97

[a]Treated groups received average doses of 3,750 and 7,500 ppm in feed.

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

Table 3. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Rats Fed EDTA Trisodium Salt in the Diet[a]

(continued)

[c]Beneath the proportions for the matched-control group are the probability levels (P values) for the Cochran-Armitage test for dose-related trend in proportions when P is below 0.10; otherwise, N.S. (not significant) is indicated.
Beneath the proportions for the treated groups are the P values for the Fisher exact (conditional) test for the comparison of the treated groups with the matched-control gorup when P is below 0.05; otherwise, N.S. is indicated.

[d]95% confidence interval of the difference in proportions of treated group and matched-control group.

matched-control group used in this analysis, so no pooled-control group was used.

No tumor appeared in a statistically significant ($P < 0.05$) positive trend in either sex. The Cox test for positive trend (unadjusted for time of appearance) has a probability level of $P = 0.089$ for chromophobe adenoma of the pituitary, and the Fisher exact test for the comparison of C-cell adenomas of the thyroid of the low-dose and untreated males had a probability level of $P = 0.08$. Except for these two results, all statistical tests showed probability levels higher than 0.10.

As an additional statistical test, the 95% confidence interval of each group was calculated and entered in the tables. The implication of this interval is that in 95/100 (95%) of a large number of similar experiments, the true difference between the tumor rate for the treated group of animals and the rate for the control group would be inside the interval calculated from the experiment. In each of the intervals shown in the tables, zero is included; this indicates the negative aspects of the results. It should also be noted that each of the intervals has a positive endpoint, indicating the theoretical possibility of tumor induction by EDTA, which was not detected under the conditions of this test.

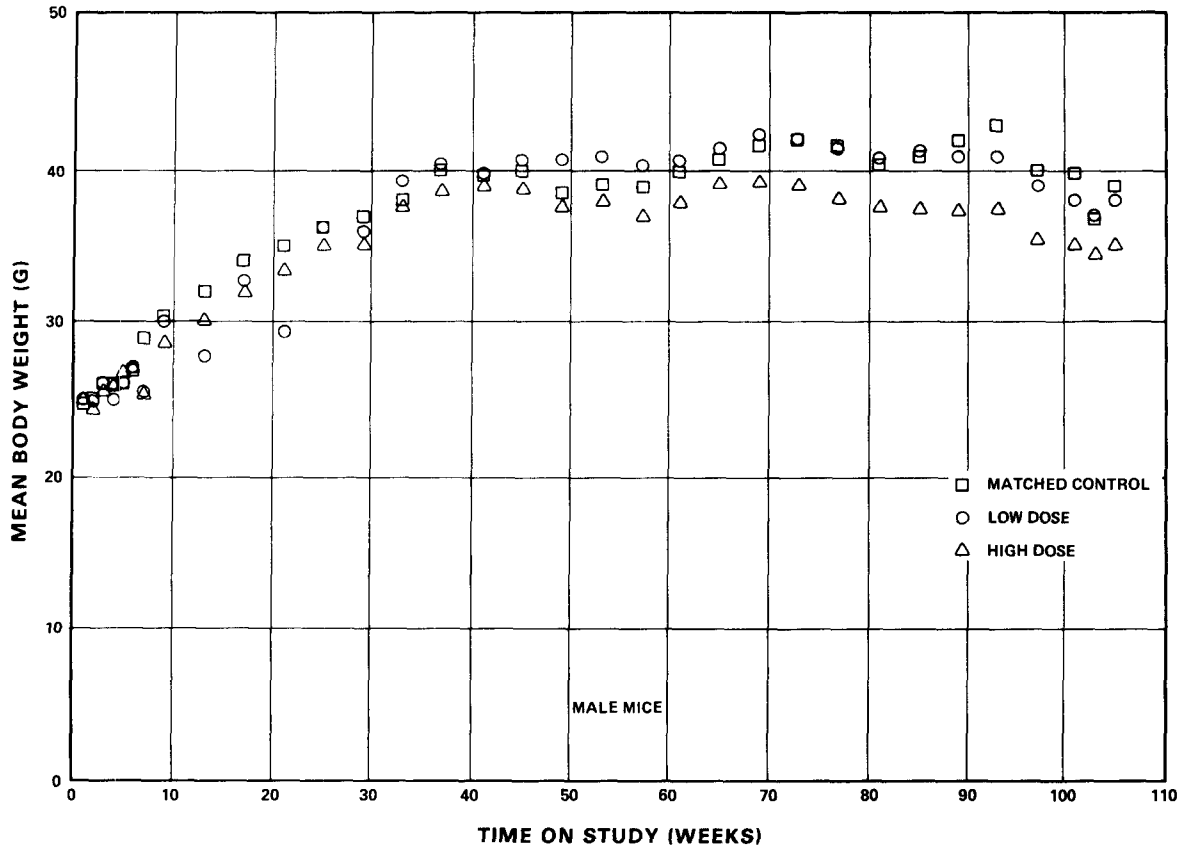
IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

In male mice only the high-dose group showed throughout most of the test period a decrease in average body weight compared to the controls (figure 3). In female mice average body weights of the treatment groups were depressed in a dose-related manner during the test period, although the effect was small (figure 3). Few clinical signs of toxicity appeared in mice during the test. Ataxia occurred in a low-dose male at 8 months, and ascites was noted in mice of both sexes during the second year of the study.

B. Survival (Mice)

Curves for the probability of survival of treated and control mice are shown in figure 4. There was no statistically significant difference in survival between the different groups, and this finding applied to both sexes. In male mice, 5/50 (10%) of the low-dose group, 2/50 (4%) of the high-dose group, and 1/20 (5%) of the matched controls were accidentally killed or missing at 26 weeks or before and are, therefore, censored at the time of death in the survival curve. While there was a higher rate of mortality in the treated male groups than in the control males,



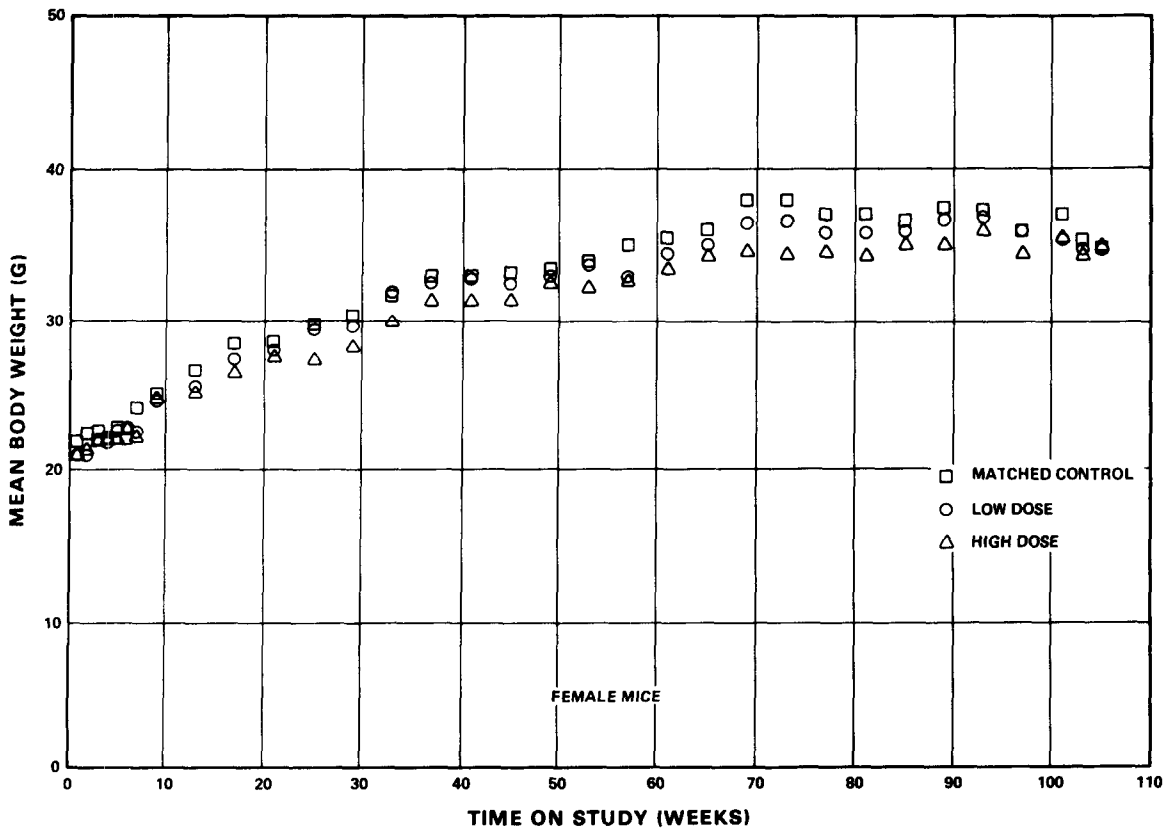
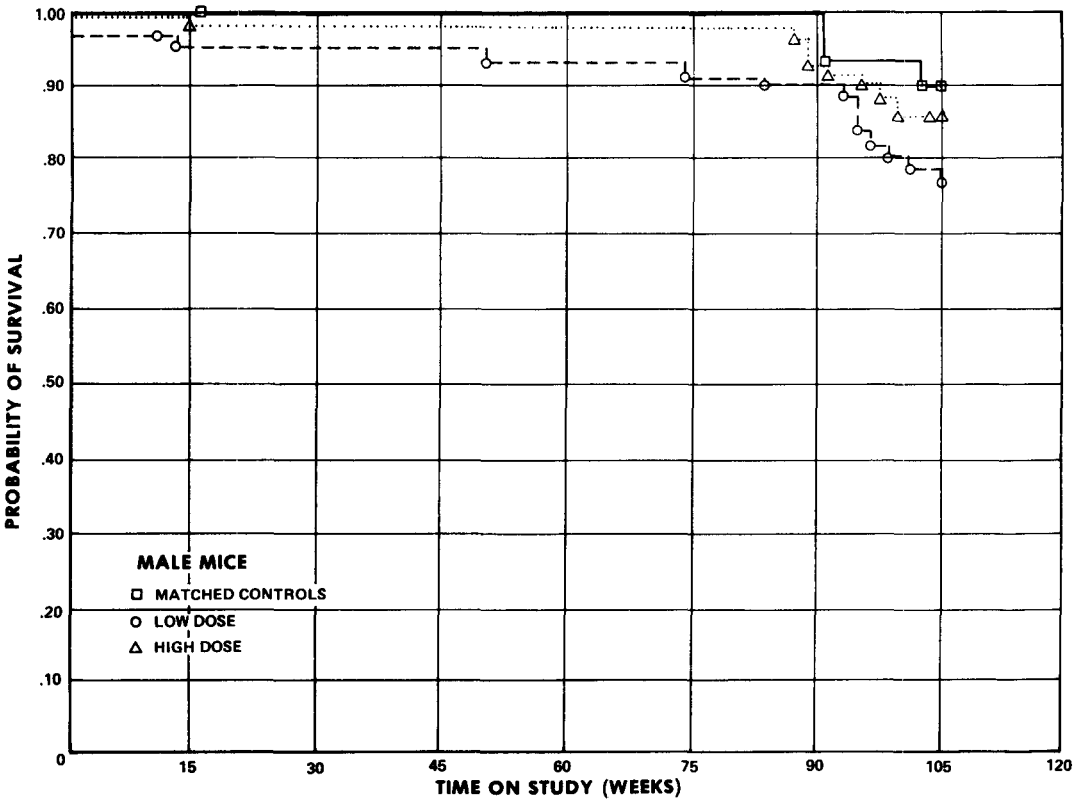


Figure 3. Growth Curves for Mice Fed EDTA in the Diet



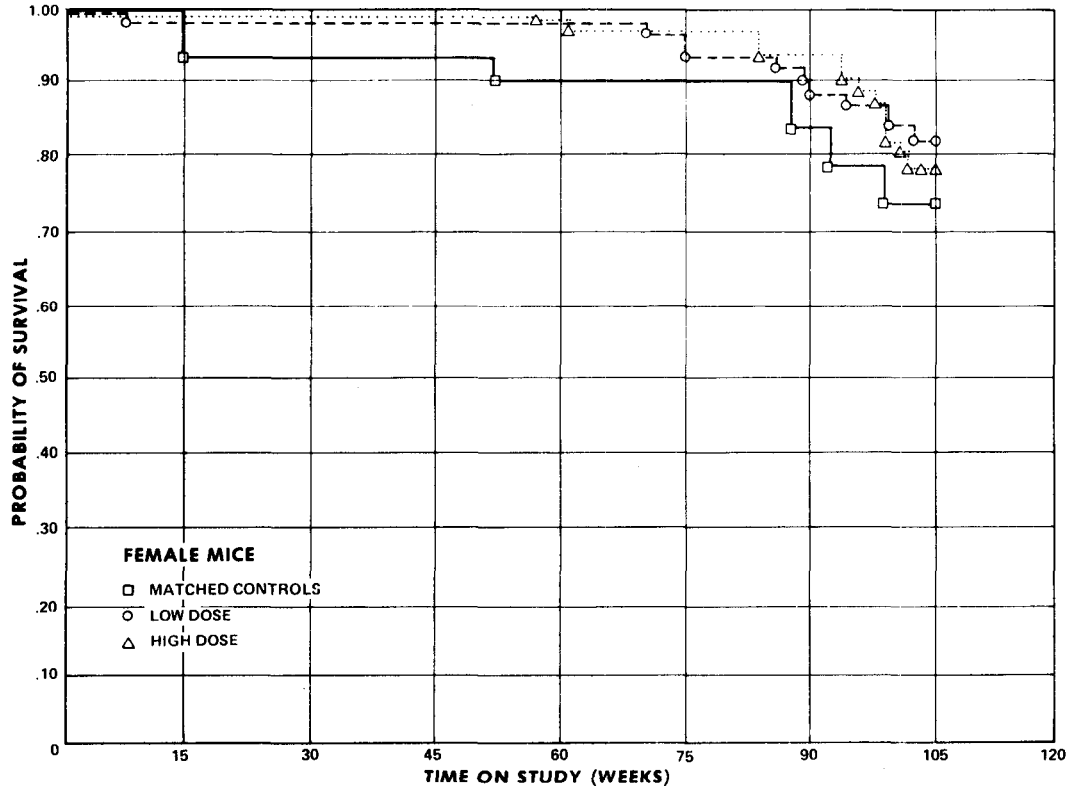


Figure 4. Survival Curves for Mice Fed EDTA in the Diet

the highest rate was observed in the low-dose group, and therefore the Tarone test for positive trend was not significant.

No low-dose female mice died accidentally (compared to the five low-dose males); however, 1/20 (5%) of the matched-control group and 3/50 (6%) of the high-dose group were lost in this way during the first 22 weeks of study and were censored from the survival curve. The Tarone test for dose-related trend in the female mice resulted in a nonsignificant probability.

C. Pathology (Mice)

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

A variety of neoplasms was observed in both treated and control animals. Each type observed has been encountered previously as a spontaneous lesion in the mouse. However, the incidence of neoplasms in all groups was high in the hematopoietic, endocrine, digestive, and respiratory systems. The incidence of neoplasms in other systems was variable.

With the exception of a splenic hemangioma in a control female and a low-dose male, all of the tumors of the hematopoietic system were malignant lymphomas or leukemias.

The distribution of endocrine tumors varied little between treated and control mice; the bulk of such tumors were of the pituitary gland, affecting 12 females and 2 males.

The incidence of hepatic neoplasms was considerably higher in males than in females. The males appeared to have a significant number of hepatocellular adenomas. However, the incidence of these neoplasms in the low-dose (9/44 [20%]) and high-dose (6/47 [13%]) male groups was approximately the same as in the male controls (2/19 [11%]).

Primary neoplasms of the respiratory system were observed in both treated and control groups and were most common in the high-dose males. Even though the greatest incidence of pulmonary neoplasms were found in the high-dose male mice (control 2/18 [11%], low-dose 8/44 [18%], and high-dose 12/45 [26%]), the frequent spontaneous occurrence of neoplasms in the lungs of mice suggest that the distribution observed in this study is probably not related to treatment.

In the judgment of the pathologist, the nature, incidence, and severity of the lesions observed in this study provide no clear evidence of carcinogenic effect of EDTA in mice.

D. Statistical Analyses of Results (Mice)

Tables 4 and 5 list those tumors which occurred in more than 5%

of the mice in a given treated group or appeared in a larger proportion in the treated group when compared to the matched controls. There was no other control group available whose environment, period of test, and pathologic diagnosis were comparable to the matched-control group used in this analysis, so no pooled-control group was used. Tumors of the hematopoietic system, lung, and liver were observed in over 10% of the matched-control males, while tumors of the hematopoietic system and pituitary gland were seen in over 10% of the matched-control females. Although this relatively high incidence in the matched controls may have concealed statistical evidence of carcinogenicity, it should be noted (1) that there were no significant differences in incidence among different treated groups and (2) that these tumors have appeared spontaneously in matched-control groups in other studies.

The only tumor system with a positive dose-related probability level less than 0.10 was alveolar/bronchiolar carcinoma or adenoma of the lung ($P = 0.096$). The Fisher exact test had a probability level of over 0.10 in all comparisons between treated and control groups. There is, therefore, no statistical evidence of carcinogenicity of EDTA at the concentrations administered.

As an additional statistical test, the 95% confidence interval of each group was calculated and entered in the tables. The

Table 4. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Mice Fed EDTA Trisodium Salt in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma[b]	2/20 (0.10)	7/46 (0.15)	7/48 (0.15)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.18,0.19)	(-0.19,0.16)
Weeks to First Observed Tumor	91	73	87
Lung: Alveolar/Bronchiolar Adenoma and Carcinoma[b]	2/18 (0.11)	8/44 (0.18)	12/45 (0.27)
P Values[c]	P = 0.096	N.S.	N.S.
95% Confidence Interval[d]		(-0.18,0.20)	(-0.12,0.28)
Weeks to First Observed Tumor	105	99	96

Table 4. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Mice Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	1/13 (0.08)	0/19 (0.00)	1/26 (0.04)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-1.00,0.05)	(-0.15,0.07)
Weeks to First Observed Tumor	105	--	105
Liver: Hepatocellular Adenoma and Carcinoma[b]	3/19 (0.16)	10/44 (0.23)	10/47 (0.21)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.20,0.23)	(-0.21,0.22)
Weeks to First Observed Tumor	103	84	105

Table 4. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Mice Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma and Carcinoma[b]	0/10 (0.00)	1/29 (0.03)	1/33 (0.03)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.09,1.00)	(-0.09,1.00)
Weeks to First Observed Tumor	--	104	105

[a]Treated groups received average doses of 3,750 and 7,500 ppm in feed.

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

[c]Beneath the proportions for the matched-control group are the probability levels (P values) for the Cochran-Armitage test for dose-related trend in proportions when P is below 0.10; otherwise, N.S. (not significant) is indicated.
Beneath the proportions for the treated groups are the P values for the Fisher exact (conditional) test for the comparison of the treated groups with the matched-control group when P is below 0.05; otherwise, N.S. is indicated.

[d]95% confidence interval of the difference in proportions of treated group and matched-control group.

Table 5. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Mice Fed EDTA Trisodium Salt in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma[b]	5/19 (0.26)	11/49 (0.23)	12/47 (0.26)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.30,0.19)	(-0.29,0.21)
Weeks to First Observed Tumor	85	99	93
Lung: Alveolar/Bronchiolar Adenoma[b]	0/19 (0.00)	3/47 (0.06)	4/45 (0.09)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.09,1.00)	(-0.09,1.00)
Weeks to First Observed Tumor	--	105	102

Table 5. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Mice Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	2/12 (0.17)	6/34 (0.18)	4/29 (0.14)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.31,0.19)	(-0.32,0.16)
Weeks to First Observed Tumor	105	105	105
Liver: Hepatocellular Adenoma and Carcinoma[b]	0/19 (0.00)	1/46 (0.02)	1/47 (0.02)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.05,1.00)	(-0.05,1.00)
Weeks to First Observed Tumor	--	105	105

Table 5. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Mice Fed EDTA Trisodium Salt in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma and Carcinoma[b]	1/12 (0.08)	3/33 (0.09)	1/34 (0.03)
P Values[c]	N.S.	N.S.	N.S.
95% Confidence Interval[d]		(-0.23,0.12)	(-0.16,0.05)
Weeks to First Observed Tumor	105	99	105

[a]Treated groups received average doses of 3,750 and 7,500 ppm in feed.

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

[c]Beneath the proportions for the matched-control group are the probability levels (P values) for the Cochran-Armitage test for dose-related trend in proportions when P is below 0.10; otherwise, N.S. (not significant) is indicated.
Beneath the proportions for the treated groups are the P values for the Fisher exact (conditional) test for the comparison of the treated groups with the matched-control group when P is below 0.05; otherwise, N.S. is indicated.

[d]95% confidence interval of the difference in proportions of treated group and matched-control group.

implication of this interval is that in 95/100 (95%) of a large number of similar experiments, the true difference between the tumor rate for the treated group of animals and the rate for the control group would be inside the interval calculated from the experiment. In each of the intervals shown in the tables, zero is included; this indicates the negative aspects of the results. It should also be noted that each of the intervals has a positive endpoint, indicating the theoretical possibility of tumor induction by EDTA, which was not detected under the conditions of this test.

V. DISCUSSION

EDTA and its salts are poorly absorbed following oral administration, due to dissociation and subsequent precipitation caused by gastric pH (Foreman et al., 1953). EDTA salts have shown relatively little toxicity in feeding studies, perhaps because of the poor absorption. Toxicity varies according to the specific salt: disodium EDTA is more toxic than either calcium disodium EDTA or lead disodium EDTA (Reuber and Schmieler, 1962), and chromium EDTA is less toxic than calcium EDTA (Ahrens and Aronson, 1971).

Early in its clinical use, EDTA showed potential nephrotoxicity, specifically tubular hydropic degeneration, following parenteral administration (Foreman et al., 1956). However, Doolan et al. (1967) questioned the relative hazards of this effect clinically and reported that in rats tubular vacuolization was not accompanied by significant elevation of serum creatinine or urea nitrogen. The cause of the renal vacuolization is not known. It may be a reflection of the induction of pinocytosis by $Na_3EDTA \cdot 3H_2O$ (Schwartz et al., 1970) or of the presence of a foreign nonmetabolizable substance (Doolan et al., 1967). In studies of younger rats, 4 weeks of age, Reuber (1967) reported

that hydropic change of the parenchymal cells of the liver rather than of the kidney was predominant.

The chronic toxicity of $Na_3EDTA \cdot 3H_2O$ used in this bioassay has not been previously investigated. However, in two feeding studies in rats, using concentrations of Na_2EDTA as high as 5% of the diet (50,000 ppm) over a two-year period, no treatment-related effects were found on gross or histopathologic examination of organs or tissues (Yang and Chan, 1964); toxic manifestations were limited to diarrhea in animals receiving the highest dose of EDTA, reduced weight gain in some of the treated groups, and increased blood coagulation in one of the studies but not the other. The absence of adverse effects following oral administration was also shown for rats receiving 1,000, 2,500, and 5,000 ppm $CaNa_2EDTA \cdot H_2O$ in feed for two years (Oser, 1963).

In the present bioassay there was no evidence of carcinogenicity of EDTA at the concentrations administered, and no tumors or lesions of the kidney or other organs were related to the treatment. Survival of all groups of animals of both species was good; thus, the lack of appearance of treatment-related tumors could not be attributed to early mortality. It should be noted that the confidence intervals for all tumor sites include a positive value; this indicates that the possibility of tumorigenicity is not theoretically precluded. However, under

the conditions of this study, using concentrations of 3,500 ppm and 7,500 ppm in feed, $Na_3EDTA \cdot 3H_2O$ was not demonstrated to be carcinogenic in rats or mice.

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APPENDIX A

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

TABLE A1

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

	CONTROL (UNTR)	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)
SEBACEOUS ADENOMA		1 (2%)	
KERATOACANTHOMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
FIBROMA	1 (5%)	3 (6%)	
FIBROSARCOMA		1 (2%)	
FIBROADENOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(50)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (6%)	1 (2%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
CORTICAL CARCINOMA, METASTATIC			1 (2%)
NONCHROMAFFIN PARAGANGLIOMA, MET			1 (2%)
FIBROSARCOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA,NOS	3 (15%)	3 (6%)	4 (8%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(18)	(48)	(49)
ALVEOLAR/BRONCHIOLAR CARCINOMA,		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(50)
HEPATOCELLULAR ADENOMA		1 (2%)	
NEOPLASTIC NODULE			1 (2%)
#SMALL INTESTINE	(20)	(50)	(49)
LEIOMYOSARCOMA			2 (4%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(47)	(44)
CHROMOPHOBE ADENOMA		3 (6%)	5 (11%)
#ADRENAL	(20)	(49)	(50)
CORTICAL ADENOMA	1 (5%)		1 (2%)
CORTICAL CARCINOMA			1 (2%)
NONCHROMAFFIN PARAGANGLIOMA, MAL			1 (2%)
PHEOCHROMOCYTOMA	2 (10%)	5 (10%)	4 (8%)
#THYROID	(17)	(35)	(38)
FOLLICULAR-CELL ADENOMA			1 (3%)
C-CELL ADENOMA		6 (17%)	3 (8%)
#PARATHYROID	(12)	(24)	(21)
ADENOMA,NOS		1 (4%)	
#PANCREATIC ISLETS	(19)	(47)	(48)
ISLET-CELL ADENOMA		3 (6%)	2 (4%)
REPRODUCTIVE SYSTEM			
#TESTIS	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR	19 (95%)	43 (86%)	44 (88%)
NERVOUS SYSTEM			
#CEREBRUM	(20)	(50)	(50)
ASTROCYTOMA			1 (2%)

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

TABLE A1 MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
#BRAIN	(20)	(50)	(50)
GLIOMA,			2 (4%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(20)	(50)	(50)
MESOTHELIOMA,			2 (4%)
*PLEURA	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CARCINOMA,		1 (2%)	
ALL OTHER SYSTEMS			
THORACIC CAVITY			
FIBROSARCOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	6	1
MORIBUND SACRIFICE	4	4	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	40	45
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	48	47
TOTAL PRIMARY TUMORS	28	77	78
TOTAL ANIMALS WITH BENIGN TUMORS	19	47	45
TOTAL BENIGN TUMORS	25	67	64
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	7	11
TOTAL MALIGNANT TUMORS	3	10	11
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	2
TOTAL SECONDARY TUMORS		2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			3
TOTAL UNCERTAIN TUMORS			3
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 EDTA IN THE DIET

	CONTROL (UNTR)	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)
SQUAMOUS CELL CARCINOMA	1 (5%)	1 (2%)	
FIBROADENOMA		1 (2%)	2 (4%)
RESPIRATORY SYSTEM			
*LARYNX	(20)	(50)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
#LUNG	(20)	(48)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (6%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA,	1 (5%)	2 (4%)	
LEUKEMIA,NOS		4 (8%)	
LYMPHOCYTIC LEUKEMIA		2 (4%)	
#LYMPH NODE	(20)	(46)	(47)
MALIGNANT LYMPHOMA,		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
NEOPLASTIC NODULE		1 (2%)	

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

TABLE A2 FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
#SMALL INTESTINE	(20)	(50)	(50)
LEIOMYOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(48)	(50)
CHROMOPHOBE ADENOMA	6 (32%)	10 (21%)	11 (22%)
#ADRENAL	(20)	(49)	(48)
PHEOCHROMOCYTOMA	1 (5%)	1 (2%)	3 (6%)
#THYROID	(11)	(36)	(37)
FOLLICULAR-CELL CARCINOMA			1 (3%)
C-CELL ADENOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA,NOS		1 (2%)	
INTRADUCTAL PAPILLOMA	1 (5%)		
INTRADUCTAL CARCINOMA			1 (2%)
FIBROADENOMA	4 (20%)	3 (6%)	3 (6%)
#UTERUS	(20)	(50)	(50)
ADENOCARCINOMA,			1 (2%)
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	5 (25%)	6 (12%)	7 (14%)
#OVARY	(18)	(49)	(47)
CYSTADENOMA,			1 (2%)
NERVOUS SYSTEM			
#CEREBRUM	(19)	(50)	(50)
GLIOMA,	1 (5%)		
SPECIAL SENSE ORGANS			
*EAR LOBULE	(20)	(50)	(50)
NEUROFIBROMA			1 (2%)

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

TABLE A2 FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	2	1
MORIBUND SACRIFICE	3	4	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	44	47
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	28	27
TOTAL PRIMARY TUMORS	20	38	35
TOTAL ANIMALS WITH BENIGN TUMORS	13	22	27
TOTAL BENIGN TUMORS	17	27	31
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	9	4
TOTAL MALIGNANT TUMORS	3	10	4
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

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

TABLE B1

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

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		4	2
ANIMALS NECROPSIED	20	46	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	45	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(46)	(48)
NEUROFIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(44)	(45)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (11%)	5 (11%)	11 (24%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		3 (7%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(46)	(48)
MALIGNANT LYMPHOMA,		3 (7%)	5 (10%)
MALIGNANT LYMPHOMA, HISTIOCYTIC		1 (2%)	
LEUKEMIA,NOS			1 (2%)
*MEDIASTINUM	(20)	(46)	(48)
MALIGNANT LYMPHOMA,			1 (2%)
MALIGNANT LYMPHOMA, LYMPHOCYTIC	1 (5%)		
#SPLEEN	(18)	(39)	(42)
HEMANGIOMA		1 (3%)	
MALIGNANT LYMPHOMA,	1 (6%)	1 (3%)	1 (2%)
#MESENTERIC L. NODE	(18)	(38)	(42)
MALIGNANT LYMPHOMA,		1 (3%)	
#SMALL INTESTINE	(19)	(41)	(47)
MALIGNANT LYMPHOMA,		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE B1 MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(19)	(44)	(47)
HEPATOCELLULAR ADENOMA	2 (11%)	9 (20%)	7 (15%)
HEPATOCELLULAR CARCINOMA	1 (5%)	1 (2%)	3 (6%)
*ANUS	(20)	(46)	(48)
SQUAMOUS CELL CARCINOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(19)	(43)	(46)
TUBULAR-CELL ADENOCARCINOMA	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(13)	(19)	(26)
CHROMOPHOBE ADENOMA	1 (8%)		1 (4%)
#THYROID	(10)	(29)	(33)
FOLLICULAR-CELL ADENOMA		1 (3%)	1 (3%)
#PANCREATIC ISLETS	(19)	(38)	(44)
ISLET-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(19)	(42)	(44)
INTERSTITIAL-CELL TUMOR	1 (5%)		1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1 MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	10	6
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	1	1	
TERMINAL SACRIFICE	17	34	41
ANIMAL MISSING		4	2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	24	27
TOTAL PRIMARY TUMORS	10	28	35
TOTAL ANIMALS WITH BENIGN TUMORS	5	15	18
TOTAL BENIGN TUMORS	6	17	22
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	11	13
TOTAL MALIGNANT TUMORS	4	11	13
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 EDTA IN THE DIET

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		3
ANIMALS NECROPSIED	19	49	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	48	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(19)	(47)	(45)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (6%)	4 (9%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(49)	(47)
MALIGNANT LYMPHOMA,	5 (26%)	9 (18%)	9 (19%)
MALIGNANT LYMPHOMA, HISTIOCYTIC		1 (2%)	
LEUKEMIA,NOS	1 (5%)		1 (2%)
#SPLEEN	(19)	(46)	(46)
HEMANGIOMA	1 (5%)		
MALIGNANT LYMPHOMA,		1 (2%)	2 (4%)
#LIVER	(19)	(46)	(47)
MALIGNANT LYMPHOMA,			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(46)	(47)
HEPATOCELLULAR ADENOMA		1 (2%)	1 (2%)
#CECUM	(19)	(43)	(46)
LEIOMYOSARCOMA	1 (5%)		

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

TABLE B2 FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(19)	(47)	(47)
TUBULAR-CELL ADENOCARCINOMA	1 (5%)		
#URINARY BLADDER	(17)	(38)	(42)
HEMANGIOMA	1 (6%)		
ENDOCRINE SYSTEM			
#PITUITARY	(12)	(34)	(29)
CHROMOPHOBE ADENOMA	2 (17%)	6 (18%)	4 (14%)
#ADRENAL	(19)	(41)	(44)
CORTICAL ADENOMA		1 (2%)	
#THYROID	(12)	(33)	(34)
FOLLICULAR-CELL ADENOMA		3 (9%)	1 (3%)
FOLLICULAR-CELL CARCINOMA	1 (8%)		
REPRODUCTIVE SYSTEM			
#OVARY	(16)	(42)	(43)
CYSTADENOMA,		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(18)	(47)	(46)
MENINGIOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE B2 FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	7	6
MORIBUND SACRIFICE	3	3	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	40	37
ANIMAL MISSING	1		3
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	24	23
TOTAL PRIMARY TUMORS	13	27	23
TOTAL ANIMALS WITH BENIGN TUMORS	4	14	10
TOTAL BENIGN TUMORS	4	15	10
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	12	13
TOTAL MALIGNANT TUMORS	9	12	13
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 EDTA IN THE DIET

TABLE C1

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

	CONTROL (UNTR)	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)
EPIDERMAL INCLUSION CYST		1 (2%)	
FIBROSIS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(50)	(49)
INFLAMMATION, INTERSTITIAL	2 (11%)	1 (2%)	2 (4%)
PNEUMONIA, CHRONIC MURINE		2 (4%)	1 (2%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(46)	(46)
HYPERPLASIA, GRANULOCYTIC			1 (2%)
#SPLEEN	(20)	(48)	(49)
FIBROSIS, FOCAL		1 (2%)	
FIBROSIS, DIFFUSE	1 (5%)		
HYPERPLASIA, LYMPHOID		1 (2%)	
#MANDIBULAR L. NODE	(19)	(45)	(46)
HYPERPLASIA, CYSTIC			1 (2%)
HYPERPLASIA, PLASMA CELL			2 (4%)
HYPERPLASIA, RETICULUM CELL	1 (5%)	4 (9%)	2 (4%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(19)	(45)	(46)
ATROPHY,			1 (2%)
HYPERPLASIA, PLASMA CELL			1 (2%)
HYPERPLASIA, RETICULUM CELL	2 (11%)	6 (13%)	4 (9%)

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

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
#THYMUS	(1)	(6)	(6)
HYPERPLASIA, RETICULUM CELL			1 (17%)
HYPERPLASIA, LYMPHOID	1 (100%)		
CIRCULATORY SYSTEM			
#HEART	(18)	(48)	(49)
PERIARTERITIS		1 (2%)	1 (2%)
#MYOCARDIUM	(18)	(48)	(49)
INFLAMMATION, FOCAL			1 (2%)
FIBROSIS	4 (22%)	10 (21%)	18 (37%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(50)
CIRRHOSIS,			1 (2%)
NECROSIS, DIFFUSE			1 (2%)
METAMORPHOSIS FATTY		4 (8%)	2 (4%)
BASOPHILIC CYTOPLASM ALTERATION		5 (10%)	1 (2%)
#PANCREAS	(19)	(47)	(48)
FIBROSIS, DIFFUSE			1 (2%)
ATROPHY,		1 (2%)	
ATROPHY, FOCAL		1 (2%)	1 (2%)
#PANCREATIC ACINUS	(19)	(47)	(48)
ATROPHY,		1 (2%)	2 (4%)
ATROPHY, FOCAL		1 (2%)	1 (2%)
#STOMACH	(20)	(50)	(49)
INFLAMMATION,		1 (2%)	
#SMALL INTESTINE	(20)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (5%)		
#DUODENUM	(20)	(50)	(49)
HYPERPLASIA, EPITHELIAL		1 (2%)	
#ILEUM	(20)	(50)	(49)
INFLAMMATION,			1 (2%)
HYPERPLASIA, LYMPHOID		3 (6%)	2 (4%)
#LARGE INTESTINE	(16)	(48)	(45)
NEMATODIASIS	1 (6%)		6 (13%)

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

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
#COLON	(16)	(48)	(45)
FIBROSIS, DIFFUSE	1 (6%)		
HYPERPLASIA, LYMPHOID		4 (8%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
PYELONEPHRITIS,		1 (2%)	
INFLAMMATION CHRONIC	11 (55%)	24 (49%)	31 (62%)
NEPHROPATHY		2 (4%)	1 (2%)
#URINARY BLADDER	(15)	(41)	(36)
INFLAMMATION,		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(20)	(49)	(50)
METAMORPHOSIS FATTY		1 (2%)	
LIPOIDOSIS		1 (2%)	4 (8%)
#ADRENAL MEDULLA	(20)	(49)	(50)
HYPERPLASIA, NODULAR		1 (2%)	
#THYROID	(17)	(35)	(38)
HYPERPLASIA, C-CELL			1 (3%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
ABSCESS,		1 (2%)	
#PROSTATE	(17)	(45)	(48)
INFLAMMATION,		2 (4%)	2 (4%)
HYPERPLASIA, EPITHELIAL	1 (6%)		
NERVOUS SYSTEM			
#CEREBRUM	(20)	(50)	(50)
HEMORRHAGIC CYST		1 (2%)	
#BRAIN	(20)	(50)	(50)
HEMORRHAGE		1 (2%)	

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

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
NECROSIS,		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMOID REACTION			2 (4%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			2

TABLE C2

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

	CONTROL (UNTR)	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			
#TRACHEA	(19)	(50)	(46)
INFLAMMATION CHRONIC			1 (2%)
#LUNG	(20)	(48)	(48)
INFLAMMATION, INTERSTITIAL	1 (5%)	2 (4%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	3 (15%)	6 (13%)	4 (8%)
HYPERPLASIA, EPITHELIAL	1 (5%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(50)	(49)
HYPERPLASIA,			1 (2%)
HYPERPLASIA, GRANULOCYTIC	1 (5%)	1 (2%)	
#LYMPH NODE	(20)	(46)	(47)
HYPERPLASIA, RETICULUM CELL			1 (2%)
#MANDIBULAR L. NODE	(20)	(46)	(47)
INFLAMMATION CHRONIC			1 (2%)
HYPERPLASIA, PLASMA CELL			1 (2%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	6 (13%)
HYPERPLASIA, LYMPHOID	1 (5%)		3 (6%)
#MESENTERIC L. NODE	(20)	(46)	(47)
HYPERPLASIA, CYSTIC		1 (2%)	1 (2%)
HYPERPLASIA, RETICULUM CELL		2 (4%)	8 (17%)
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	1 (2%)

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

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#HEART	(19)	(46)	(44)
PERIARTERITIS		1 (2%)	
#MYOCARDIUM	(19)	(46)	(44)
FIBROSIS	2 (11%)	4 (9%)	6 (14%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
BASOPHILIC CYTOPLASM ALTERATION	2 (10%)	13 (27%)	15 (31%)
#LIVER/CENTRILOBULAR	(20)	(49)	(49)
NECROSIS,	1 (5%)		
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA,		1 (2%)	1 (2%)
#PANCREAS	(18)	(48)	(49)
FIBROSIS, FOCAL	1 (6%)	1 (2%)	
ATROPHY, FOCAL		1 (2%)	
#PANCREATIC DUCT	(18)	(48)	(49)
DILATATION,			1 (2%)
#PANCREATIC ACINUS	(18)	(48)	(49)
FIBROSIS, FOCAL			1 (2%)
ATROPHY,		2 (4%)	
ATROPHY, FOCAL			1 (2%)
#SMALL INTESTINE	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID		1 (2%)	
#ILEUM	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
#LARGE INTESTINE	(19)	(46)	(48)
NEMATODIASIS	7 (37%)		
#COLON	(19)	(46)	(48)
NEMATODIASIS			1 (2%)

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

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(48)
HYDRONEPHROSIS		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION CHRONIC	2 (10%)	5 (10%)	1 (2%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HYPERPLASIA, TUBULAR CELL	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(48)	(50)
CYST,		4 (8%)	1 (2%)
MULTIPLE CYSTS		1 (2%)	
HEMORRHAGIC CYST		1 (2%)	4 (8%)
ANGIECTASIS	1 (5%)		
#ADRENAL	(20)	(49)	(48)
CYST,		1 (2%)	
HEMORRHAGE	1 (5%)		
HEMORRHAGIC CYST		1 (2%)	
METAMORPHOSIS FATTY	1 (5%)		
LIPOIDOSIS		1 (2%)	
#ADRENAL CORTEX	(20)	(49)	(48)
METAMORPHOSIS FATTY			1 (2%)
LIPOIDOSIS	1 (5%)	1 (2%)	1 (2%)
HYPERPLASIA,		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(11)	(36)	(37)
HYPERPLASIA, C-CELL	1 (9%)	1 (3%)	
#PANCREATIC ISLETS	(18)	(48)	(49)
HYPERPLASIA,			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(50)	(50)
CYST,			1 (2%)
PYOMETRA	1 (5%)		
FIBROSIS		1 (2%)	1 (2%)
#CERVIX UTERI	(20)	(50)	(50)
CYST,	1 (5%)		

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

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ABSCESS,		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(50)	(50)
INFLAMMATION,	3 (15%)	3 (6%)	3 (6%)
INFLAMMATION, SUPPURATIVE		4 (8%)	3 (6%)
INFLAMMATION, VESICULAR		3 (6%)	1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	1 (2%)
#OVARY/OVIDUCT	(20)	(50)	(50)
INFLAMMATION,	1 (5%)	1 (2%)	
#OVARY	(18)	(49)	(47)
CYST,		3 (6%)	5 (11%)
FIBROSIS			1 (2%)
PIGMENTATION,			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(19)	(50)	(50)
HYDROCEPHALUS,	1 (5%)	1 (2%)	1 (2%)
PERIVASCULAR CUFFING			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		3	4

APPENDIX D

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

TABLE D1

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

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		4	2
ANIMALS NECROPSIED	20	46	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	45	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(18)	(44)	(45)
EDEMA,		1 (2%)	
PNEUMONIA, CHRONIC MURINE		2 (5%)	5 (11%)
PERIARTERITIS	1 (6%)		
HYPERPLASIA, FOCAL		1 (2%)	
LYMPHOCYTOSIS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(36)	(45)
HYPERPLASIA, HEMATOPOIETIC		1 (3%)	
#SPLEEN	(18)	(39)	(42)
AMYLOIDOSIS		1 (3%)	
HYPERPLASIA, LYMPHOID			1 (2%)
#SPLENIC CAPSULE	(18)	(39)	(42)
FIBROSIS			1 (2%)
#LYMPH NODE	(18)	(38)	(42)
HYPERPLASIA,		2 (5%)	
#MANDIBULAR L. NODE	(18)	(38)	(42)
HYPERPLASIA, RETICULUM CELL			1 (2%)
#MESENTERIC L. NODE	(18)	(38)	(42)
HEMORRHAGE		1 (3%)	
INFLAMMATION	1 (6%)		1 (2%)

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

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HYPERPLASIA,	1 (6%)	1 (3%)	1 (2%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(19)	(43)	(45)
MINERALIZATION		1 (2%)	
#MYOCARDIUM	(19)	(43)	(45)
INFLAMMATION, FOCAL			1 (2%)
FIBROSIS, FOCAL		1 (2%)	
*PULMONARY ARTERY	(20)	(46)	(48)
HYPERTROPHY,			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(44)	(47)
INFLAMMATION, FOCAL	1 (5%)	1 (2%)	
LYMPHOCYTIC INFLAM INFILTRATE		1 (2%)	
INFLAMMATION CHRONIC			1 (2%)
NECROSIS,		2 (5%)	1 (2%)
METAMORPHOSIS FATTY	1 (5%)		
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
ANGIECTASIS		2 (5%)	1 (2%)
LYMPHOCYTOSIS			1 (2%)
*GALLBLADDER	(20)	(46)	(48)
INFLAMMATION, VESICULAR			1 (2%)
#PANCREAS	(19)	(38)	(44)
NECROSIS,			1 (2%)
#PANCREATIC ACINUS	(19)	(38)	(44)
ATROPHY, FOCAL		1 (3%)	
#STOMACH	(19)	(41)	(47)
INFLAMMATION,		1 (2%)	
INFLAMMATION, FOCAL	1 (5%)		
#SMALL INTESTINE	(19)	(41)	(47)
ABSCESS,			1 (2%)
AMYLOIDOSIS		1 (2%)	

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

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID			1 (2%)
URINARY SYSTEM			
#KIDNEY	(19)	(43)	(46)
HYDRONEPHROSIS			1 (2%)
PYELONEPHRITIS,		1 (2%)	
SCAR		1 (2%)	
AMYLOIDOSIS		1 (2%)	1 (2%)
METAPLASIA, OSSEOUS		1 (2%)	
#URINARY BLADDER	(19)	(38)	(41)
INFLAMMATION,		1 (3%)	
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(36)	(41)
AMYLOIDOSIS		1 (3%)	
#THYROID	(10)	(29)	(33)
HYPERPLASIA, FOLLICULAR-CELL		2 (7%)	4 (12%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(19)	(39)	(41)
INFLAMMATION,		1 (3%)	
NERVOUS SYSTEM			
#BRAIN	(19)	(42)	(46)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MEDIASTINUM	(20)	(46)	(48)
THROMBOSIS,		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	7	13	14
ANIMAL MISSING/NO NECROPSY PERF		4	2
AUTOLYSIS/NECROPSY PERF/NO HISTO		1	

TABLE D2

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

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		3
ANIMALS NECROPSIED	19	49	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	48	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(19)	(47)	(45)
INFLAMMATION, FOCAL	1 (5%)		
INFLAMMATION, INTERSTITIAL			1 (2%)
PNEUMONIA, CHRONIC MURINE	4 (21%)	14 (30%)	16 (36%)
METAPLASIA, OSSEOUS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(46)	(46)
FIBROSIS			1 (2%)
INFARCT,			1 (2%)
AMYLOID,			1 (2%)
#LYMPH NODE	(18)	(44)	(45)
INFLAMMATION,		1 (2%)	
HYPERPLASIA,		2 (5%)	2 (4%)
#MANDIBULAR L. NODE	(18)	(44)	(45)
ABSCESS,			1 (2%)
#MESENTERIC L. NODE	(18)	(44)	(45)
INFLAMMATION,			1 (2%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(19)	(47)	(47)
MINERALIZATION		1 (2%)	1 (2%)

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

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(19)	(47)	(47)
INFLAMMATION,		1 (2%)	
*PULMONARY ARTERY	(19)	(49)	(47)
HYPERTROPHY,	1 (5%)		
*SPLENIC ARTERY	(19)	(49)	(47)
DILATATION,			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(46)	(47)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION CHRONIC		1 (2%)	
HYPERPLASIA, NODULAR		1 (2%)	
#PANCREAS	(19)	(46)	(46)
ATROPHY,		1 (2%)	
#PANCREATIC ACINUS	(19)	(46)	(46)
CYSTIC DUCTS		1 (2%)	
#STOMACH	(19)	(46)	(46)
INFLAMMATION, ACUTE FOCAL			1 (2%)
#GASTRIC SEROSA	(19)	(46)	(46)
INFLAMMATION CHRONIC		1 (2%)	
#LARGE INTESTINE	(19)	(43)	(46)
NEMATODIASIS	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(19)	(47)	(47)
HYDRONEPHROSIS		2 (4%)	
LYMPHOCYTIC INFLAM INFILTRATE		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(19)	(41)	(44)
LIPOIDOSIS		1 (2%)	
#THYROID	(12)	(33)	(34)
HYPERPLASIA, NODULAR		1 (3%)	

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

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOCAL		1 (3%)	
HYPERPLASIA, FOLLICULAR-CELL	3 (25%)	7 (21%)	5 (15%)
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(46)	(46)
CYST,		1 (2%)	1 (2%)
#UTERUS/ENDOMETRIUM	(19)	(46)	(46)
CYST,	1 (5%)	8 (17%)	4 (9%)
#OVARY/OVIDUCT	(19)	(46)	(46)
INFLAMMATION, SUPPURATIVE			1 (2%)
ABSCESS,		1 (2%)	
#OVARY	(16)	(42)	(43)
CYST,	2 (13%)	4 (10%)	7 (16%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(19)	(49)	(47)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(19)	(49)	(47)
PERIARTERITIS			1 (2%)

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

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR) 22-2056	LOW DOSE 22-2054	HIGH DOSE 22-2052
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	6	5
ANIMAL MISSING/NO NECROPSY PERF	1		2
AUTOLYSIS/NECROPSY PERF/NO HISTO		1	
AUTOLYSIS/NO NECROPSY PERFORMED		1	

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