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CAS No. 139-13-9 (NTA)

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BIOASSAY OF

NITRILOTRIACETIC ACID AND

NITRILOTRIACETIC ACID, TRISODIUM SALT, MONOHYDRATE

FOR POSSIBLE CARCINOGENICITY

Carcinogen Bioassay and Program Resources Branch
Carcinogenesis Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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Carcinogenesis Program, Division of Cancer Cause and Prevention
National Cancer Institute

CONTRIBUTORS: This report presents the results of carcinogen bioassays of nitrilotriacetic acid and nitrilotriacetic acid, trisodium salt, monohydrate conducted by the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassays were conducted at the Stanford Research Institute, Menlo Park, California, and Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI carcinogen bioassay program.

For the work performed at the Stanford Research Instiute (SRI)[1], the experimental design, including doses, was determined by collaborative efforts of individuals at NCI (Dr. R. R. Bates[1,8]), the University of California Medical School (Dr. R. M. Elashoff[2]), and SRI (Dr. D. C. L. Jones[1], Dr. D. P. Sasmore[1], Dr. G. W. Newell[1], and Mr. W. E. Davis[1]). The principal investigator for the contract was Dr. D. C. L. Jones; chemical analyses were performed by Dr. R. Spanggord[1]; the technical supervisor of animal treatment, observation, and data handling was Mr. W. E. Davis[1]; necropsy and tissue fixation were supervised by Dr. D. P. Sasmore[1]. Microscopic preparation and histopathologic examination were performed by Dr. W. M. Busey[3] and Dr. V. J. Rosen[4], and the diagnoses included in this report represent the interpretation of these pathologists. Dr. R. A. Squire[9] reviewed all diagnoses of tumors of the urinary tract and concurred with the overall pathologic evaluation of the SRI study.

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In studies at both laboratories, compilation of individual animal survival and summary tables was performed by EG&G Mason Research Institute[7]. Pathology tables were compiled by the different research laboratories[1,3,6]. Statistical analyses were performed by Dr. R. M. Elashoff[2].

The results of this study were reviewed and this report was prepared at Tracor Jitco. Those responsible for the report at Tracor Jitco[10] were the toxicologist, Dr. J. F. Robens; the technical editor, Dr. E. W. Gunberg; and the technical writers, Ms. L. A. Waitz, Mr. W. D. Reichardt, and Dr. G. L. Miller. The final report was reviewed by Dr. Elashoff and members of the participating organizations[1,6,9,10].

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SUMMARY

Bioassays for the carcinogenicity of nitrilotriacetic acid, trisodium salt, monohydrate ($Na_3NTA \cdot H_2O$) were conducted at Stanford Research Institute (SRI), using Fischer 344 rats, and at Litton Bionetics, Inc. (LBI), using both Fischer 344 rats, and B6C3F1 mice. Similar bioassays, using rats and mice, were conducted at LBI on the free acid, nitrilotriacetic acid (NTA). Each chemical was mixed in respective diets and administered ad libitum. The $Na_3NTA \cdot H_2O$ was tested in rats at SRI at 200, 2,000, and 20,000 ppm for a 24-month period. It was also tested in rats at LBI at 7,500 and 15,000 ppm and in mice at 2,500 and 5,000 ppm using 18-month feeding periods for both species. The NTA was tested in rats and mice at LBI at 7,500 and 15,000 ppm for the 18-month period. The numbers of animals used in tests at SRI were 24 of each sex for each dose group and for the controls; at LBI, 50 of each sex for each dose group and 20 of each sex for the controls. Since equimolar quantities of $Na_3NTA \cdot H_2O$ and NTA were not used, given concentrations of $Na_3NTA \cdot H_2O$ represented 30% less NTA than did equal concentrations of the free acid.

Average weights attained by high-dose groups of rats and mice were consistently lower than those of control groups. Less difference was observed with the low-dose groups. Survival, however, was not decreased by the compounds administered, except in rats given 20,000 ppm $Na_3NTA\ H_2O$.

Lesions of the urinary tract were found in most treated groups of both rats and mice. They were characterized, especially in the high-dose groups, by primary tumors of epithelial origin. These tumors were particularly significant since they were not found in the urinary tract of the control mice and only rarely occur spontaneously in the strains of animals on test. Lesions of the urinary tract were also characterized by hydronephrosis and/or nephritis in high-dose rats and by nephritis in both high- and low-dose mice.

Statistical evidence of the carcinogenicity of $Na_3NTA \cdot H_2O$ and NTA was provided by incidences of tumors at different sites in the urinary tract. For example, among animals given 20,000 ppm

$Na_3NTA \cdot H_2O$ at SRI, tumors of the kidney occurred in male (treated, 9/24; untreated, 0/24; P = 0.001) and female (treated, 4/24; untreated, 0/24; P = 0.054) rats; tumors of the ureter, in male (treated, 8/24; untreated, 0/24; P = 0.002) and female (treated, 6/24; untreated, 0/24; P = 0.011) rats; and tumors of the bladder, in female rats (treated, 5/24; untreated, 0/22; P = 0.031). Similarly, among animals given 15,000 ppm NTA at LBI, tumors of the bladder occurred in female rats (treated, 12/48; untreated, 0/18; P = 0.014) and tumors of the kidney occurred in male mice (treated, 24/44; untreated, 0/20; P < 0.001). Additional tests at LBI, using 15,000 and 7,500 ppm $Na_3NTA \cdot H_2O$ and 7,500 ppm NTA in male and female rats, 15,000 ppm NTA in female mice, and 7,500 ppm NTA in male mice, also induced tumors of the urinary tract, but in numbers too low to be statistically significant. Metastatic tumors, appearing to have arisen from primary tumors of the urinary tract, were found in 5/24 male and 5/24 female rats given 20,000 ppm $Na_3NTA \cdot H_2O$ at SRI and in one male rat given 15, 000 ppm NTA at LBI; none were found in rats given lower doses or in mice.

Thus, NTA and $NA_3NTA \cdot H_2O$ were shown to be carcinogenic to the urinary tracts of both rats and mice at the higher doses tested. Lower doses, as delineated in this report, did not induce significant numbers of such lesions.

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I. INTRODUCTION

Nitrilotriacetic acid (NTA) is a synthetic amino-polycarboxylic acid chelating agent used chiefly as a replacement for phosphates in detergents. NTA sequesters magnesium and calcium ions present in hard water, which would normally inhibit the activity of detergent surfactants. In December, 1970, the detergent industry voluntarily suspended such applications of NTA in the United States, following an unpublished government report indicating that the compound was teratogenic (Chernoff and Courtney, 1970). During that year the annual production of NTA was 150 million pounds, of which 86-92% was used in detergents. Major nondetergent uses, for which NTA is still being produced, include water treatment, textile treatment, metal plating and cleaning, and pulp and paper processing. To a lesser extent, NTA is used in leather tanning, photographic development, synthetic rubber production, the manufacture of pharmaceuticals, agriculture (in herbicide formulations and micronutrient solutions), and in the separation of rare-earth elements (Cureton, 1967). It has been predicted that water-soluble chelates that survive biodegradation in sewage treatment plants and natural water would be present in domestic water supplies at an average concentration of less than 25 parts per billion (Thayer and Kensler, 1973). This estimate was obtained under conditions of actual use of NTA-containing

detergents prior to 1972 and does not represent current exposure.

The trisodium salt of NTA ($Na_3NTA \cdot H_2O$), the form used in detergents, and NTA itself were both selected for testing in the carcinogenesis bioassay program because toxicological evidence of the incidence of tumors in rats administered NTA was inconclusive (Saffiotti et al., unpublished ms.) and the exposure potential to these compounds was significant. The bioassay of $Na_3NTA \cdot H_2O$ was conducted in rats at Stanford Research Instiute (SRI); bioassays of both $Na_3NTA \cdot H_2O$ and NTA were conducted at Litton Bionetics, Inc. (LBI). The combined results of these bioassays are summarized in this report.

II. MATERIALS AND METHODS - STANFORD RESEARCH INSTITUTE (SRI)

A. Chemical

The test chemical in the SRI bioassay was nitrilotriacetic acid, trisodium salt, monohydrate: $N(CH_2CO_2Na)_3 \cdot H_2O$. It was obtained from Hampshire Chemical Division of W. R. Grace and Co., Nashua, New Hampshire. The identity and purity of all batches used in the bioassay were checked at SRI by elemental analysis (C,H,N) and infrared and nuclear magnetic resonance spectrometry. No impurities were detected. Elemental composition in all cases matched the theoretical within normal error limits. Spectra were as expected for this structure.

B. Dosage Preparation and Analyses

$Na_3NTA \cdot H_2O$ was administered by mixing the compound in the feed (Lowfat Lab Chow®, Ralston Purina Co.). A stock mixture containing 40,000 ppm $Na_3NTA \cdot H_2O$ was prepared every 2 weeks by premixing the compound and the feed, adding corn oil as a dust suppressant, and then mixing by machine. Every 2 weeks diets fed to the animals were diluted from the stock mixture with control diet containing corn oil and stored in covered plastic food containers at room temperature until use. The concentration of corn oil in

the feed was 3%. The corn oil was purchased from the Staley Manufacturing Company (Orange, California).

To check the concentration of test chemical in each stock mixture immediately prior to preparation of the fed diets, SRI used the method developed by Chau & Fox (1971) involving extraction, purification, conversion to the tripropyl ester, and quantitation by gas-liquid chromotography. All batches of stock mixture were within the ±4,000 ppm (± 10%) tolerance limits of the theoretical (40,000 ppm). Dosage mixtures that were stored for 2 weeks at room temperature in a rat feeder showed no change in concentration of the test chemical.

C. Animals

Fischer 344 rats (Simonsen Laboratory; Gilroy, California) of both sexes were used in these tests. Upon arrival at the laboratory, all animals were observed, weighed, and quarantined for 2 weeks. Following quarantine, all males gained less than 25 g, females gaining less than 15 g, and all sickly animals were culled. The remaining animals were assigned to the test and control groups in the following manner: they were assigned one per cage until all cages were occupied; this procedure was repeated until cages contained three animals. Cages were numbered and assigned to test using computer-generated randomization tables.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. Incoming air was filtered at a rate of 10 changes of room air per hour. Fluorescent lighting was provided on a 12-hour-per-day cycle. The rats were housed in polycarbonate cages equipped with a disposable filter top of random-woven polyester fiber. Clean cages with autoclaved bedding (Iso-Dri® hardwood chips, manufactured by Becton, Dickinson, and Carworth, Warrensburg, New York), were provided twice each week.

The chemical-feed mixture was supplied once per week in hopper-type feeders that were kept filled during one week and replaced the next. Water, which was softened, filtered, and sterilized with ultraviolet light, was supplied automatically to each cage. All diets and water were consumed *ad libitum*. Neither cages nor racks were routinely rotated. The animals were housed in a room in which N-nitroso-N-pentyl-1-pentamine, N-methyl-N′-nitro-N-nitrosoguanidine, and 4-(butylnitrosoamino)-1-butanol were also tested.

E. Subchronic Study and Design of Chronic Study

The study with $Na_3NTA \cdot H_2O$ at SRI was part of a larger investigation of the effects of combinations of chemicals on

their carcinogenicity. For this reason, the design of this study varied from that of bioassays conducted at other laboratories.

To estimate the maximum tolerated dose for the chronic study, a preliminary toxicity study was conducted in which $Na_3NTA \cdot H_2O$ was administered in feed for 8 weeks at four doses to 15 animals of each sex. At 20,000 ppm, the highest dose administered, there was a weight decrement of approximately 10% in males and 0% in females at 8 weeks. No mortality occurred and no other signs of toxicity were apparent. Since $Na_3NTA \cdot H_2O$ was thus determined to be relatively nontoxic, and since doses suitable for the chronic study were also based on predicted effects of combinations of compounds tested in the larger study, concentrations of 200, 2,000 and 20,000 ppm were selected for the $Na_3NTA \cdot H_2O$. These were administered throughout the chronic study (table 1). Rats were started on the test at 53 $\pm$ 2 days of age.

Table 1. Dosage Schedule for Chronic Study of $Na_3NTA \cdot H_2O$: SRI

Species	No./Sex	Concentration in Feed (ppm)	Concentration in Feed (mM[a]/kg)	Treatment Period (wks)
Fischer 344 Rat	24/M & 24/F	0	0	104
	24/M & 24/F	200	0.7	104
	24/M & 24/F	2,000	7	104
	24/M & 24/F	20,000	70	104

[a]Millimoles.

The control animals were obtained from the same source as the $Na_3NTA \cdot H_2O$-treated animals, given identical animal care, housed in the same room, and administered the same quantity of corn oil in the feed.

F. Clinical and Pathologic Examinations

All animals were observed daily for deaths and for clinical signs. They were weighed individually at the start and every 2 weeks for the initial 12 weeks of the study and every 4 weeks thereafter. They were also 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. The following tissues and organs were taken from killed animals and, where feasible, from animals found dead: all gross lesions, skin, mammary gland, mandibular lymph node, salivary gland, sternal marrow, costochondral rib, thymus, lung, heart, trachea, esophagus, thyroid, parathyroid, stomach, cecum, colon, ileum, jejunum, duodenum, pancreas, spleen, mesenteric lymph node, liver, bladder, prostate, testes, ovaries, uterus, pituitary, kidneys, adrenals, and brain. Tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, routinely stained with hematoxylin and eosin, and examined histopathologically. 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 showed early deaths. 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.

G. Data Recording and Statistical Analyses

Pertinent data for this experiment have been recorded in an automatic data processing system, the Carcinogen 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.

Survivorship analyses were made in the form of graphs for males and females using the Kaplan-Meier techniques (1958). On each graph survivorship curves were computed for each dose group and the controls. The Thomas-Breslow-Gart program (1976) was used to obtain these curves. Significance tests to compare survivorship curves for the different dose groups were carried out using the Cox (1972) and Tarone (1975) methods.

Analyses for the tumor pathology proceeded in the following order:

(1) For the incidence of tumor-bearing animals, (a) each dose group was compared with the controls using Fisher-Irwin one-tailed tests, (b) the departure from the linear-trend statistic was calculated (Cochran, 1954), (c) when the departure statistic P value was greater than 0.10, the Cox (1970) test for significance of linear trend was carried out, and (d) exact two-sided 95% confidence intervals for the odds ratio (Gart, 1970) were given when the result in (a) had a P value less than 10% (values of the odds ratio larger than 1 imply that the treated group has a higher incidence of tumors than the controls). These analyses were run on the incidence of tumors using computer programs developed at the University of California and at NCI (Thomas, 1975; Thomas et al., 1976). In precise terms, the incidence of total tumors for males is equal to the proportion of animals with at least one tumor (excluding benign testicular tumors) among the number of animals autopsied and histologically examined. The incidence of tumors for females is

defined in the same way, except that benign uterine tumors are excluded.

(2) For several sites, systems, and histology, the same analyses were carried out as those reported in (1) above. For example, analyses of the incidence of urinary-tract tumors were done where the incidence of urinary-tract tumors equaled the number of animals with at least one primary urinary-tract tumor among the animals autopsied and histologically examined. Sites or systems, or histologic types with no tumors or very few tumors, are not reported but were statistically analyzed.

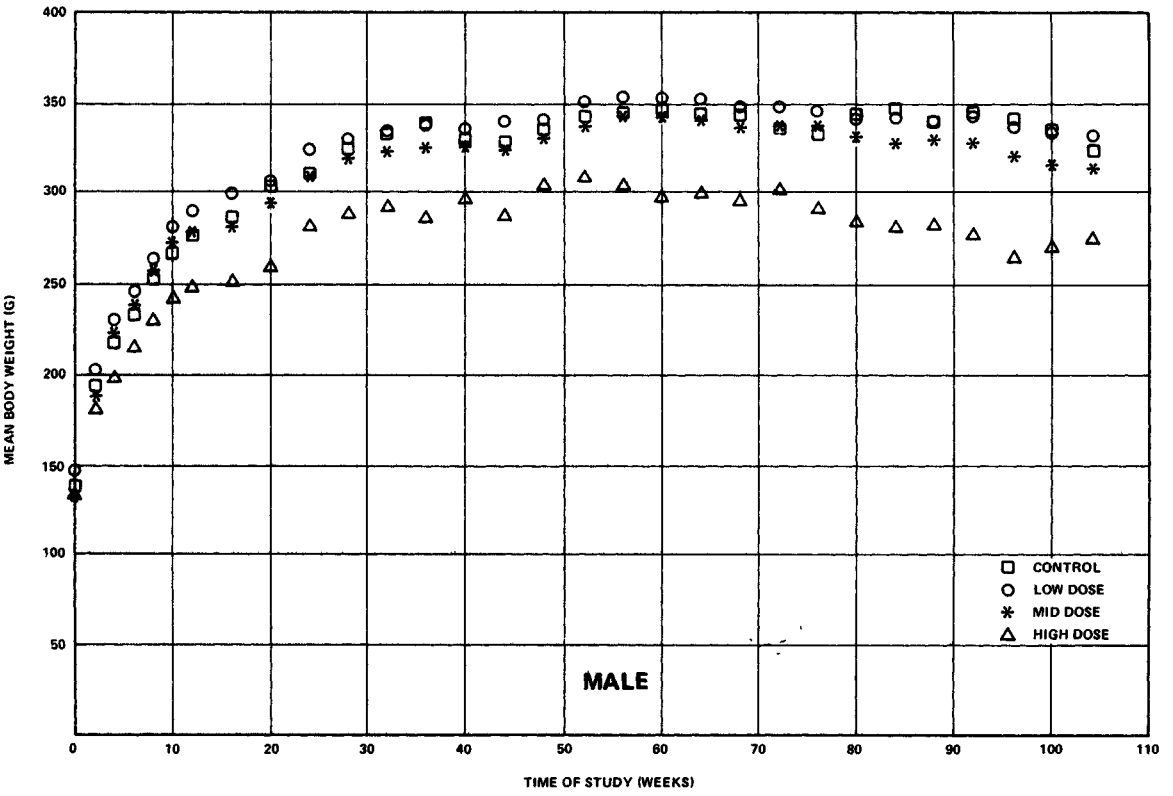
III. RESULTS - STANFORD RESEARCH INSTITUTE - $Na_3NTA \cdot H_2O$ - RATS

A. Body Weights and Clinical Signs

High-dose animals (20,000 ppm), when compared with the controls, exhibited a weight decrement of approximately 12% for males and 10% for females (figure 1). They had no clinical signs beyond those of normal aging except for enlarged and/or hard kidneys, first noted by palpation between weeks 60 and 64. By week 68, 13/22 males and 2/23 females had these renal abnormalities. High-dose animals that died before termination were generally weak, emaciated, and anemic, and had a history of the enlarged and/or hard kidneys. No unusual signs were noted, however, in the other groups. The mean survival time for the males fed 20,000 ppm $Na_3NTA \cdot H_2O$ in the diet was 92 weeks, compared with the other groups, in which the mean was not reached before termination at 104 weeks.

B. Survival

Figure 2 shows the estimated probabilities of survival of the rats in this experiment. In the males, there was a statistically significant difference ($P < 0.001$) among the four groups (three dose groups and one control). The high-dose group had earlier deaths than the other dose groups. Although 75% of the high-dose



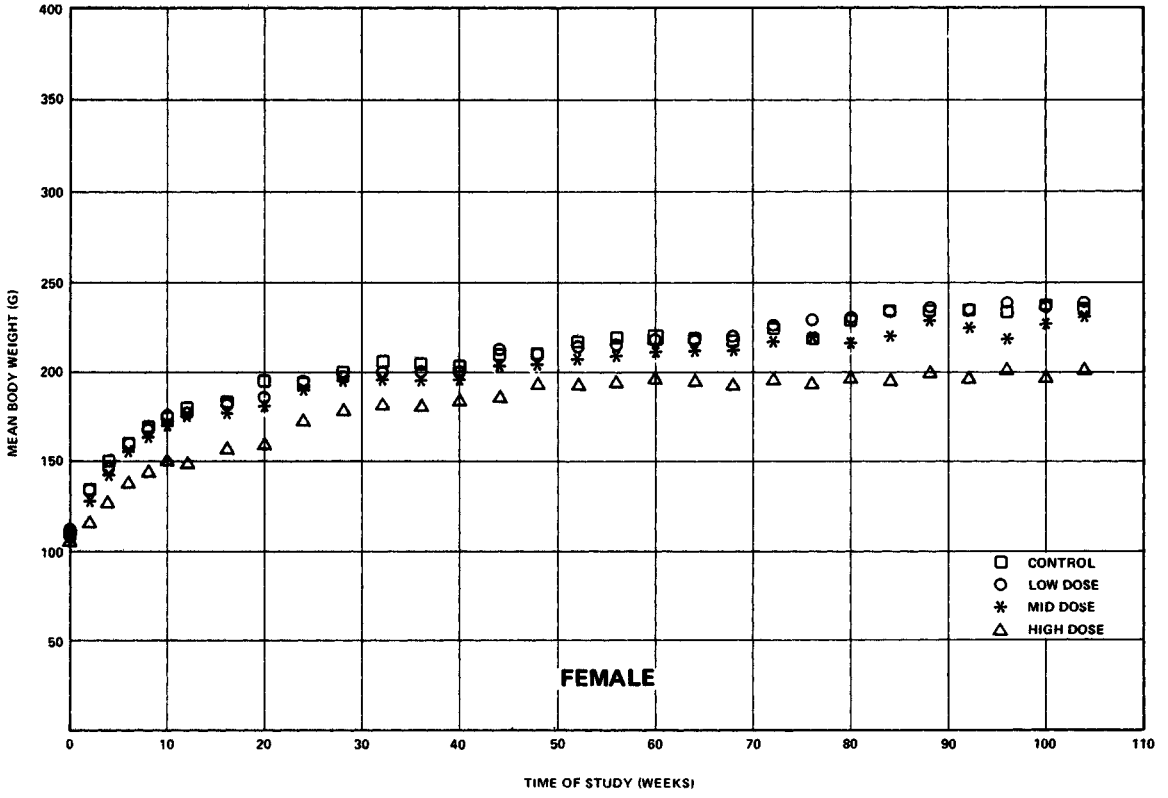


Figure 1. Growth Curves for Rats, $Na_3NTA \cdot H_2O$ - Stanford Study

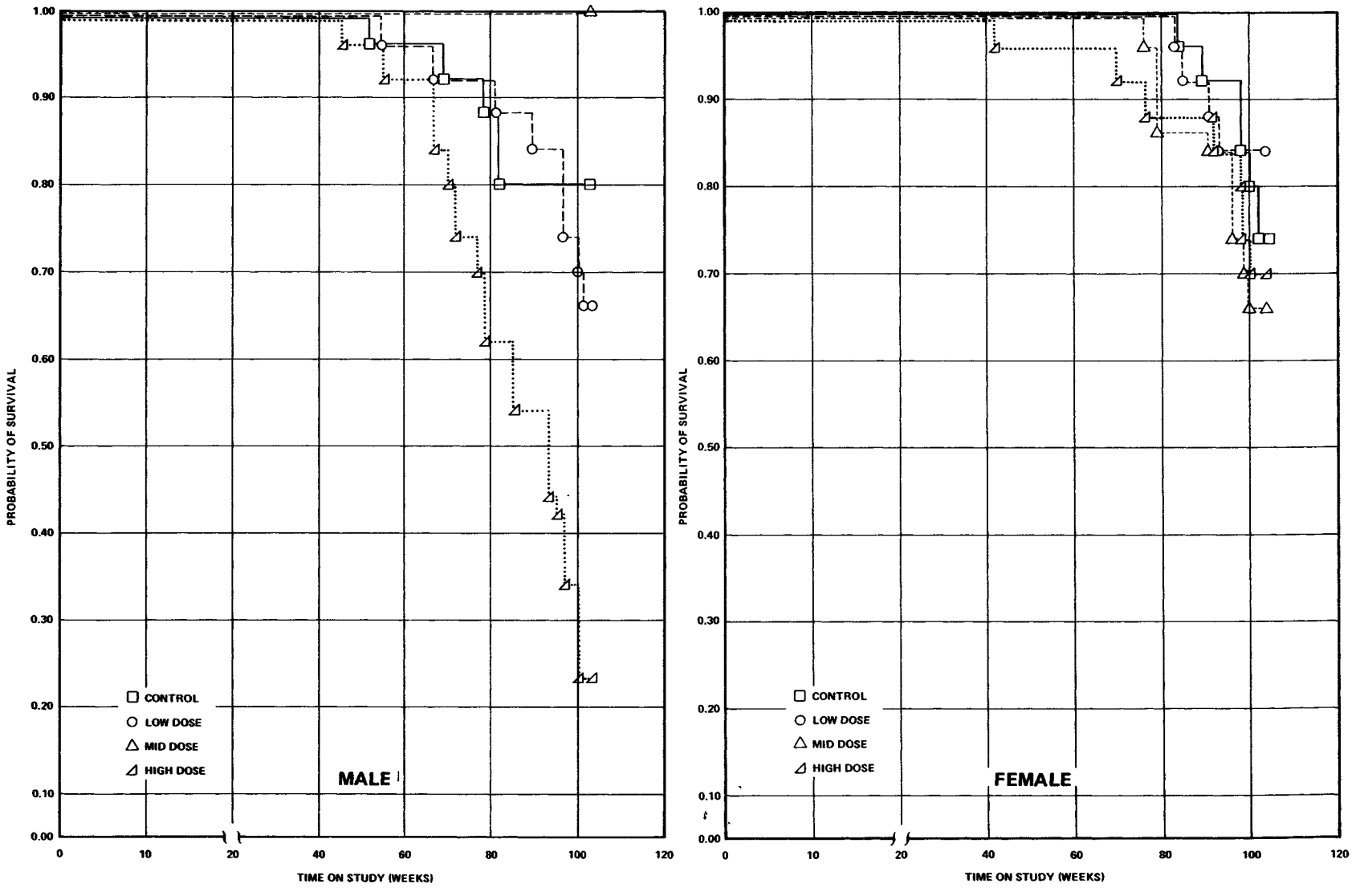


Figure 2. Survival Curves for Rats, $Na_3NTA \cdot H_2O$ - Stanford Study

group died before termination of the study, only one death occurred in the first year, and 12/24 (50%) of this group were alive for over 90 weeks. The survival curves of the female groups are comparable with each other.

C. Pathology

Histopathologic findings are tabulated in Appendix A, tables A1-A2, covering neoplasms and other proliferative lesions, and in Appendix D, tables D1-D2, covering nonneoplastic lesions.

Almost all lesions of interest occurred in the 24 male and 24 female rats that had received 20,000 ppm of $Na_3NTA \cdot H_2O$. Primary neoplasms of the urinary tract were seen in 14 high-dose males, 11 of which died before 104 weeks. Similar neoplasms were also observed in 13 high-dose females; 4 of these were among the 7 animals that died before 104 weeks. The neoplasms of the urinary tract consisted of transitional-cell carcinomas, tubular-cell carcinomas, and tubular-cell adenomas of the kidney and transitional-cell carcinomas of the ureter and urinary bladder. Of these neoplasms, the earliest observed was a transitional-cell carcinoma of the ureter in a female rat; this animal died during week 42 of the study. A papilloma of the bladder was also present in a mid-dose female.

The transitional-cell carcinomas seen in the ureter and urinary

bladder were generally characterized by sheets of proliferating neoplastic transitional-epithelial cells which invaded the basement membrane submucosa and muscular walls. Evidence of vascular invasion was present in some rats, and in a few animals there was penetration to the serosal surface of the urinary bladder and ureter. In these rats, there was a seeding of neoplastic transitional cells to the other organs of the abdominal cavity. In some instances, small foci or nests of neoplastic transitional-epithelial cells were present in the submucosa and muscular wall of the ureter. In a few animals, the transitional-cell carcinomas presented in a papillary configuration with projections of proliferating transitional-epithelial cells and their attendant stroma into the lumen of the urinary bladder.

The tubular-cell adenomas were characterized by encapsulated masses of proliferating tubules, which were generally well-differentiated. Evidence of compression was present in the surrounding normal kidney tubules. The tubular-cell carcinomas were less differentiated and consisted of anaplastic tubular epithelial cells, which appeared to be infiltrating the surrounding kidney tissue. Numerous mitotic figures were present.

Metastatic transitional-cell carcinomas were seen in five

high-dose males and five high-dose females. They appeared in the lung, lymph node, pancreas, adrenal gland, and seminal vesicle. The frequency of appearance at the different sites is shown in table 2.

Table 2. Metastases of Urinary Tract Transitional-Cell Tumors in Rats Treated with 20,000 ppm $Na_3NTA \cdot H_2O$

Organ	Male (24)	%	Female (24)	%
Lung	4	17	5	21
Lymph Node	2	8	1	4
Pancreas	2	8	0	0
Adrenal Gland	1	4	0	0
Seminal Vesicle	1	4	0	0
Total animals with lesion	5	21	5	21

The incidence of other primary neoplasms in these rats was low, with the exception of interstitial-cell tumors of the testes in most of the males. Such an occurrence is not uncommon for this strain of rat.

In most high-dose rats of both sexes, the kidneys were moderately to markedly affected by a nephritis and/or hydronephrosis. These microscopic alterations were characterized by varying degrees of glomerulosclerosis, interstitial fibrosis, tubular dilatation, protein in the lumen of the tubules, and regenerative epithelium.

In several of the urinary bladders, ureters, and renal pelves where there were no neoplasms, there was, however, some degree of transitional-epithelial dysplasia and hyperplasia.

The incidence of primary neoplasms of the urinary tract in these high-dose rats was high, whereas primary neoplasms of the urinary tract of the rat have, in general, an extremely low incidence. It can only be assumed, therefore, that the administration of 20,000 ppm of $Na_3NTA \cdot H_2O$ was responsible for this marked increase in urinary-tract neoplasia.

D. Statistical Analyses of Results

Of the 144 treated and 48 control rats entered into the study, all were examined histopathologically except one that was autolyzed. Tables 3 and 4 contain the analyses of the incidence of tumors in both males and females as defined in (1) and (2) of the preceding section. The results in table 3 suggest the following conclusions for males: The incidence of total tumors (excluding testicular tumors) was higher in the high-dose group than in the controls. The principal cause of the elevated incidence was the occurrence of primary tumors of the urinary tract of the transitional-cell type. Within the urinary tract, the sites of action were the kidney and the ureter. The endocrine system showed increased incidences of tumors in the low- and mid-dose groups relative to the controls.

Table 3. Incidence of Tumors in Male Rats (SRI $Na_3NTA \cdot H_2O$)

	Controls 0 ppm	Low Dose 200 ppm	Mid Dose 2,000 ppm	High Dose 20,000 ppm
Incidence of Tumors (excluding testicular tumors)	8/24 (33%)	15/23 (65%)	9/24 (38%)	18/24 (75%)
Statistical Tests[a]	P = 0.007, nonlinear	P = 0.029	N.S.	P = 0.004
95% Confidence Intervals	---	0.99 - 14.94	---	1.47 - 25.63
Weeks to First Observed Tumor	82	81	104	43
Incidence of Urinary Tumors (including primary tumors of the kidney, bladder, and ureter)	0/24 (0%)	0/23 (0%)	0/24 (0%)	14/24 (58%)
Statistical Tests[a]	P < 0.001, P < 0.001	N.S.	N.S.	P = 0.001
95% Confidence Intervals	---	---	---	7.91 - ∞
Weeks to First Observed Tumor	---	---	---	67

Table 3. Incidence of Tumors in Male Rats (SRI $Na_3NTA \cdot H_2O$)

(continued)

	Controls 0 ppm	Low Dose 200 ppm	Mid Dose 2,000 ppm	High Dose 20,000 ppm
Incidence of Tumors of the Kidney	0/24 (0%)	0/23 (0%)	0/24 (0%)	9/24 (38%)
Statistical Tests[a]	$P < 0.001$, $P < 0.001$	N.S.	N.S.	$P = 0.001$
95% Confidence Intervals	---	---	---	3.41 - ∞
Weeks to First Observed Tumor	---	---	---	56
Incidence of Transitional-cell Carcinoma of the Kidney	0/24 (0%)	0/23 (0%)	0/24 (0%)	4/24 (17%)
Statistical Tests[a]	$P = 0.006$, $P = 0.003$	N.S.	N.S.	$P = 0.054$
95% Confidence Intervals	---	---	---	0.96 - ∞
Weeks to First Observed Tumor	---	---	---	56

Table 3. Incidence of Tumors in Male Rats (SRI $Na_3NTA \cdot H_2O$)

(continued)

	Controls 0 ppm	Low Dose 200 ppm	Mid Dose 2,000 ppm	High Dose 20,000 ppm
Incidence of Tumors of the Ureter	0/24 (0%)	0/23 (0%)	0/24 (0%)	8/24 (33%)
Statistical Tests[a]	$P < 0.001$, $P < 0.001$	N.S.	N.S.	$P = 0.002$
95% Confidence Intervals	---	---	---	2.81 - ∞
Weeks to First Observed Tumor	---	---	---	56
Incidence of Primary Tumors of the Lung	1/24 (4%)	0/23 (0%)	1/24 (4%)	1/24 (4%)
Statistical Tests[a]	N.S.,N.S.	N.S.	N.S.	N.S.
95% Confidence Intervals	---	---	---	---
Weeks to First Observed Tumor	104	---	104	98

Table 3. Incidence of Tumors in Male Rats (SRI $Na_3NTA \cdot H_2O$)

(continued)

	Controls 0 ppm	Low Dose 200 ppm	Mid Dose 2,000 ppm	High Dose 20,000 ppm
Incidence of Primary Endocrine Tumors	2/24 (8%)	8/23 (35%)	7/24 (29%)	2/24 (8%)
Statistical Tests[a]	P = 0.037, nonlinear	P = 0.03	P = 0.068	N.S.
95% Confidence Intervals	---	0.94 - 61.92	0.71 - 48.64	---
Weeks to First Observed Tumor	103	98	104	78
Incidence of Primary Hematopoietic Tumors	2/24 (8%)	6/23 (26%)	1/24 (4%)	1/24 (4%)
Statistical Tests[a]	P = 0.044, nonlinear	N.S.	N.S.	N.S.
95% Confidence Intervals	---	---	---	---
Weeks to First Observed Tumor	100	81	104	104

[a]Beneath the untreated-control incidence are two quantities: (a) the P value for a 2x4 contingency table using chisquare theory; (b) the P value for the test for linear trend if a linear trend fits the data, or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tailed test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.

Table 4. Incidence of Tumors in Female Rats (SRI $Na_3NTA \cdot H_2O$)

	Controls 0 ppm	Low Dose 200 ppm	Mid Dose 2,000 ppm	High Dose 20,000 ppm
Incidence of Tumors (exclusive of benign uterine tumors)	13/24 (54%)	17/24 (71%)	15/24 (63%)	19/24 (79%)
Statistical Tests[a]	N.S. P = 0.066	N.S.	N.S.	P = 0.062
95% Confidence Intervals	---	---	---	0.78 - 14.96
Weeks to First Observed Tumor	88	83	76	42
Incidence of Primary Urinary Tumors (inclusive primary tumors of the kidney, bladder, and ureter)	0/24 (0%)	0/24 (0%)	1/24 (4%)	13/24 (54%)
Statistical Tests[a]	P < 0.001, P < 0.001	N.S.	N.S.	P = 0.001
95% Confidence Intervals	---	---	---	6.73 - ∞
Weeks to First Observed Tumor	---	---	99	42

Table 4. Incidence of Tumors in Female Rats (SRI $Na_3NTA \cdot H_2O$)

(continued)

	Controls 0 ppm	Low Dose 200 ppm	Mid Dose 2,000 ppm	High Dose 20,000 ppm
Incidence of Primary Tumors of the Kidney	0/24 (0%)	0/24 (0%)	0/24 (0%)	4/24 (17%)
Statistical Tests[a]	P = 0.006, P = 0.003	N.S.	N.S.	P = 0.054
95% Confidence Intervals	---	---	---	1.04 - ∞
Weeks to First Observed Tumor	---	---	---	104
Incidence of Tumors of the Ureter	0/24 (0%)	0/24 (0%)	0/24 (0%)	6/24 (25%)
Statistical Tests[a]	P < 0.001, P < 0.001	N.S.	N.S.	P = 0.011
95% Confidence Intervals	---	---	---	1.79 - ∞
Weeks to First Observed Tumor	---	---	---	42

Table 4. Incidence of Tumors in Female Rats (SRI $Na_3NTA \cdot H_2O$)

(continued)

	Controls 0 ppm	Low Dose 200 ppm	Mid Dose 2,000 ppm	High Dose 20,000 ppm
Incidence of Tumors of the Bladder	0/22 (0%)	0/23 (0%)	1/22 (5%)	5/24 (21%)
Statistical Tests[a]	P = 0.010, P = 0.001	N.S.	N.S.	P = 0.031
95% Confidence Intervals	---	---	---	1.23 - ∞
Weeks to First Observed Tumor	---	---	104	96
Incidence of Endocrine Tumors	3/24 (13%)	8/24 (33%)	5/24 (21%)	4/24 (17%)
Statistical Tests[a]	N.S.,N.S.	P = 0.084	N.S.	N.S.
95% Confidence Intervals	---	0.68 - 23.17	---	---
Weeks to First Observed Tumor	98	92	80	92

[a]Beneath the untreated-control incidence are two quantities: (a) the P value for a 2x4 contingency table using chisquare theory; (b) the P value for the test for linear trend if a linear trend fits the data, or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tailed test for comparison of that group with the control group when it below 0.10; or, otherwise N.S. - not significant.

For the female rats also there was a statistically significant increase of urinary tract tumors in the high-dose group. While the kidney and the ureter were the sites of action in the males, the bladder was an additional site in the females. Most of these tumors in the females, as in the males, were transitional-cell carcinomas.

IV. MATERIALS AND METHODS - LITTON BIONETICS, INC. (LBI)

A. Chemicals

The batches of NTA and $Na_3NTA \cdot H_2O$ were manufactured by the Hampshire Chemical Division of W. R. Grace and Co., Nashua, New Hampshire. Analyses for purity were not performed in the LBI study. According to the manufacturer's specifications, commercial NTA and $Na_3NTA \cdot H_2O$ contain 99.5% active ingredients. Since the samples used at LBI and samples of the trisodium salt tested at SRI (see p. 3, above) were obtained from the same manufacturer, it is expected that they would be of similar purity.

B. Dietary Preparation

A 6-kg mixture of chemical in feed (Wayne® Lab Blox Meal, Allied Mills) was prepared in a hooded twin-shell blender twice a week for mice and three times a week for rats. Feed preparations and chemicals were refrigerated until they were used. No corn oil was added to the feed.

Analyses that were conducted at the beginning of the study and 10 days later to determine the percent recovery of NTA in dosage mixtures showed that NTA was stable in feed for at least 10 days. Similar analyses of samples taken from three positions in the blender indicated that the mixture was homogeneous. Assays of

the stability of $Na_3NTA \cdot H_2O$ in feed and of the homogeneity of such mixtures were not performed.

C. Animals

Inbred Fischer 344 rats and hybrid B6C3F1 mice were obtained from A. R. Schmidt, Madison, Wisconsin. Additional mice of the same strain were procured from Charles River Breeding Laboratories, Wilmington, Massachusetts. These laboratories were under contract to the Division of Cancer Treatment, NCI, to provide the animals used for testing.

In the NTA test, half of the mice were from Charles River and half from A. R. Schmidt; in the $Na_3NTA \cdot H_2O$ study, 90% of the mice were from Charles River and 10% were from A. R. Schmidt. No information is available on how the animals from the two sources were assigned to the test groups.

Animals were received at 28 days of age and quarantined for a period of 14 days. At the end of this period, animals with no clinical signs of disease were weighed individually. Mice weighing 19-22 g and rats weighing 85-110 g were considered acceptable for testing and were then segregated into equal weight groups. Cage assignments were made by choosing one animal from each such group so that the total weights of animals in different cages were equal.

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 witn 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; feed was available *ad libitum* and supplied 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% relative humidity. Fluorescent lighting illuminated the rooms for 8 hours a day.

Rats in the NTA bioassay were maintained concurrently in a room with rats that were being treated with N-9H-fluoren-2-ylacetamide, 4,4′-diisocyanato-3,3′-dimethoxy-1,1′-biphenyl, and N-phenyl-1,4-benzenediamine hydrochloride. Rats on test with $Na_3NTA \cdot H_2O$ were in a room with animals that were given 4,4′-methylenebis(N,N′-dimethyl)benzenamine, ethenylbenzene (styrene), and 2,5-cyclohexadiene-1,4-dione, dioxime.

All mice in this study were in a room with mice receiving N-9H-fluoren-2-ylacetamide, 2-4-dichloro-1-(4-nitrophenoxy)ben-

zene, 1H-1,2,4-triazol-3-amine, ethenylbenzene (styrene), 2-nitroethenylbenzene, and 4-nitroso-N-phenylbenzenamine.

Controls were housed in the same rooms as their corresponding experimental groups.

E. Subchronic Study and Design of Chronic Study

In the bioassay at Litton Bionetics, both NTA and $Na_3NTA \cdot H_2O$ were tested. To estimate the maximum tolerated dose for the chronic study, an 8-week subchronic toxicity test was conducted in which the compounds were administered in the diet to both species at concentrations up to 31,600 ppm for rats and 21,600 ppm for mice. At these concentrations there were no deaths or gross pathologic findings at necropsy, and depression of body weight was in the range of only 10-20%.

During the chronic study, the doses of NTA and $Na_3NTA \cdot H_2O$ were as outlined below (table 5). Since equimolar quantities of these compounds were not used, given concentrations of $Na_3NTA \cdot H_2O$ represented 30% less NTA than did equal concentrations of the free acid. Table 5 therefore expresses dosages in terms of millimoles per kilogram as well as parts per million. Treatment began when animals were 6 weeks of age and continued for 18 months. At the end of that time, rats were maintained on control

diets (feed only) for an additional 6 months, and mice for 3 months.

Table 5. Dosage Schedule for Chronic Studies of NTA and $Na_3NTA \cdot H_2O$: LBI

Species	No./Sex	Concentration in Feed (ppm)	(mM/kg)	Treatment Period (months)
		NTA		
Fischer 344 Rat	20/M & 20/F	0	0	18
	50/M & 50/F	7,500 (1/2 MTD)	40	18
	50/M & 50/F	15,000 (MTD)	80	18
B6C3F1 Mouse	20/M & 20/F	0	0	18
	50/M & 50/F	7,500 (1/2 MTD)	40	18
	50/M & 50/F	15,000 (MTD)	80	18
		$Na_3NTA \cdot H_2O$		
Fischer 344 Rat	20/M & 20/F	0	0	18
	50/M & 50/F	7,500 (1/2 MTD)	27	18
	50/M & 50/F	15,000 (MTD)	55	18
B6C3F1 Mouse	20/M & 20/F	0	0	18
	50/M & 50/F	2,500 (1/2 MTD)	9	18
	50/M & 50/F	5,000 (MTD)	18	18

F. Clinical and Pathologic Examinations

Treatment and control groups were observed twice a day, and any animal that was moribund or had large masses was killed and necropsied to minimize the risk of autolysis. Cage-mates were

weighed as a group each week for the first 6 weeks, biweekly for the next 6 weeks, and monthly thereafter. Palpation for tumors was performed routinely when the animals were weighed. At termination animals were killed by CO_2-induced asphyxiation and necropsied.

The following tissues were examined during necropsy and processed for histopathologic examination: skin, trachea, lung, heart, salivary gland, esophagus, stomach, duodenum, jejunum, ileum, colon, liver, pancreas, kidney, urinary bladder, thyroid, parathyroid, adrenal, pituitary, mandibular and mesenteric lymph nodes, bone marrow, spleen, mammary gland, seminal vesicle, prostate, testis, ovary, uterus, brain, and rib, as well as any tissue mass. Large tissue masses were weighed, described morphologically, and sectioned. All tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, routinely stained with hematoxylin and eosin, and examined histopathologically. 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 showed early deaths. Also, some animals were missing, cannibalized, or judged to be in 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.

G. Data Recording and Statistical Analyses

Data obtained from Litton Bionetics were handled as described earlier for data obtained from Stanford.

V. RESULTS - LITTON BIONETICS, INC.- NTA

A. RATS (NTA)

1. Body Weights and Clinical Signs

The average weights of both male and female rats were depressed in a dose-related manner when compared with those of the controls (figure 3). No significant signs or lesions were observed until the sixth month of the study, when the first lesion occurred in a high-dose male. A crusted mass appeared on the back and remained until the termination of the study. In the second year of the study an insignificant number of palpable tissue masses and cataracts appeared in a few of the animals.

2. Survival

Estimates of the probabilities of survival of rats are shown in figure 4. Survivals of treated and control groups of both sexes are comparable. The Tarone test of male-rat survival did not indicate a dose-related trend ($P > 0.10$), but in female rats the dose-related statistic approached significance ($P = 0.057$). In every group of both sexes, more than 94% of the animals survived for a year or more.

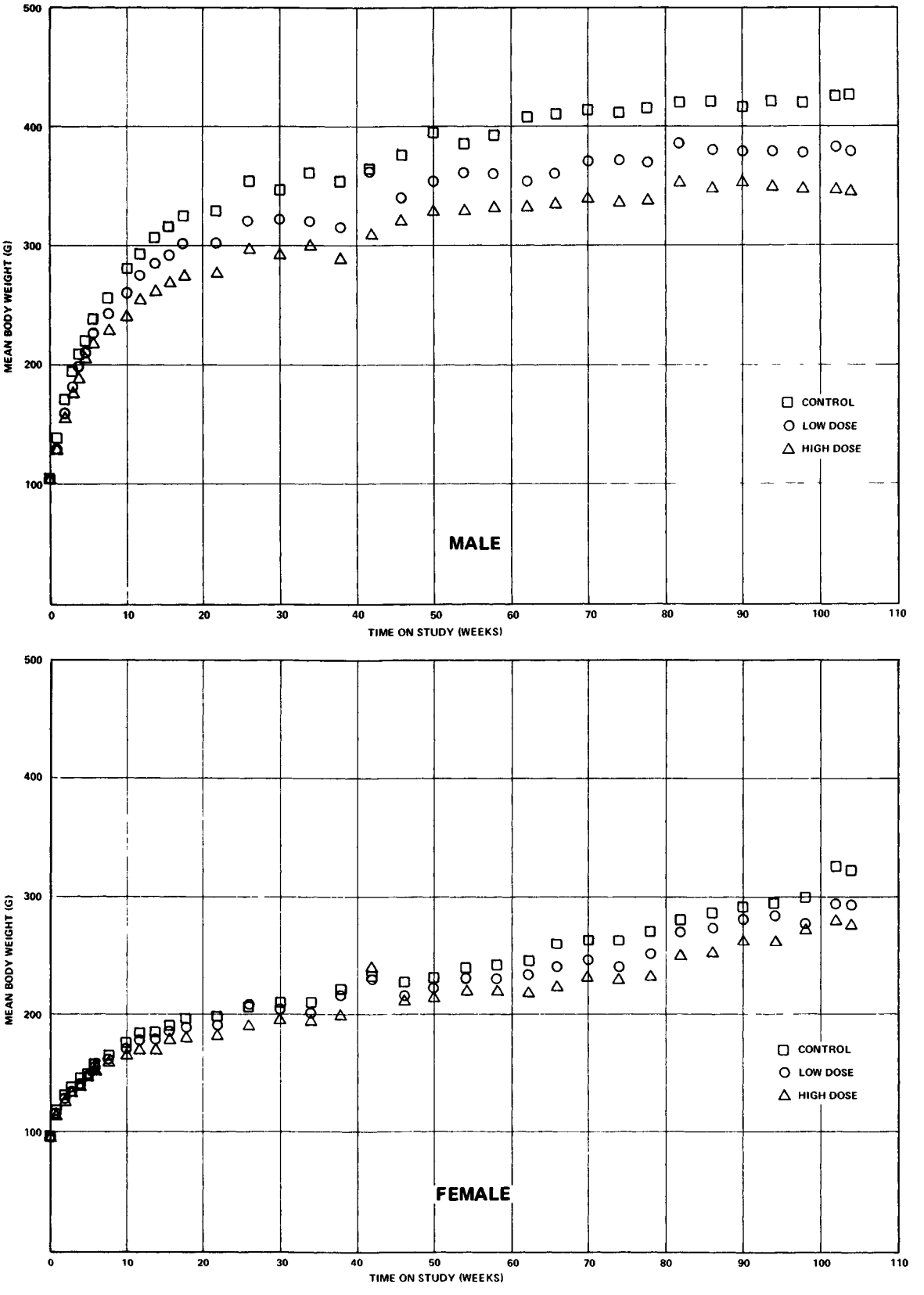


Figure 3. Growth Curves for Rats, NTA - Litton Study

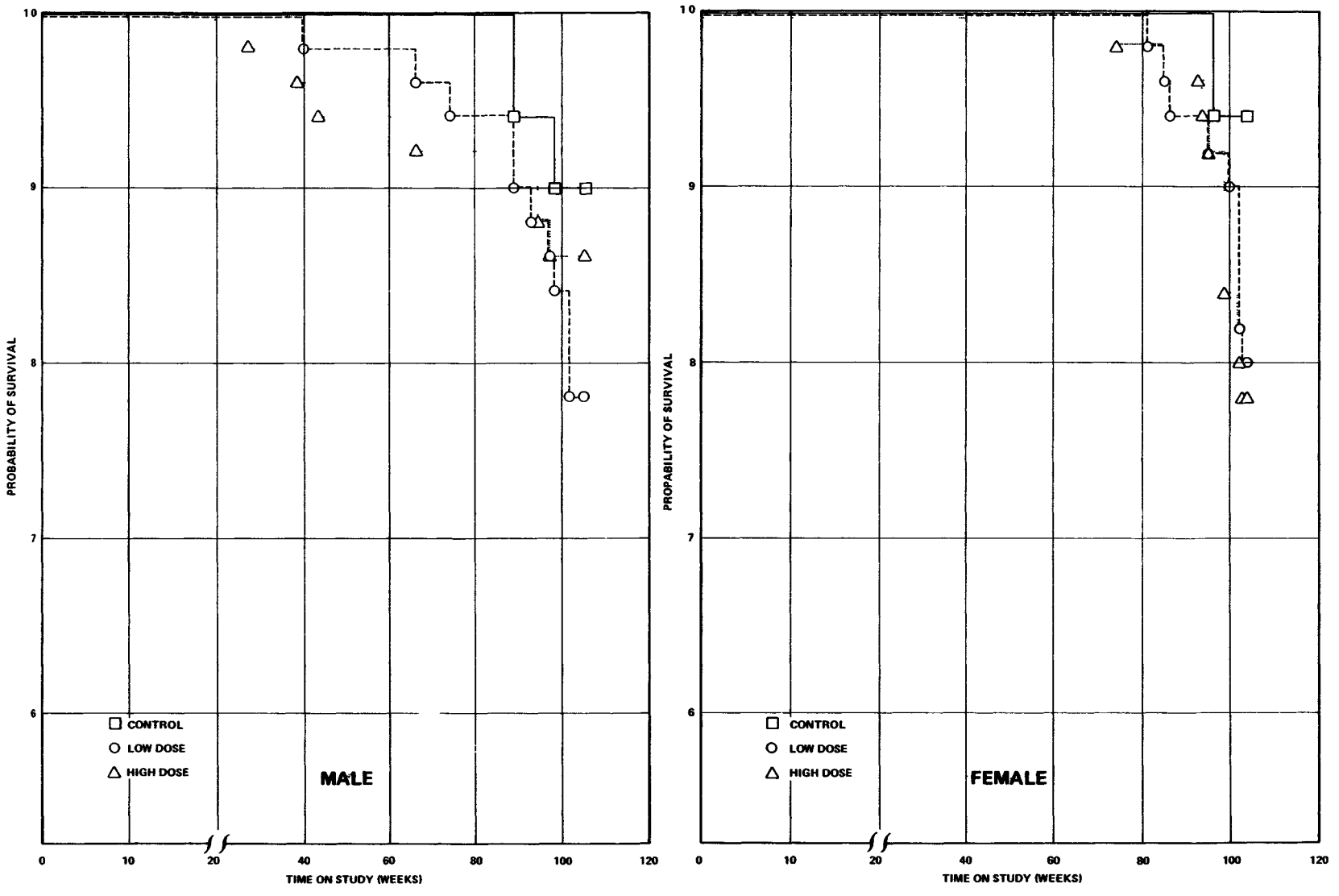


Figure 4. Survival Curves for Rats, NTA - Litton Study

3. Pathology

Histopathologic findings are tabulated in Appendix B, tables B1 and B2, covering neoplasms and other proliferative lesions, and in Appendix E, tables E1 and E2, covering nonneoplastic lesions.

All of the 200 treated rats and the 40 control rats that comprised this study were examined histopathologically with the exception of a single high-dose male that was autolyzed. Thus, where the number of animals with lesions is delineated, comparisons should be made to the respective group size, i. e., 20 control male, 50 low-dose male, 49 high-dose male, 20 control female, 50 low-dose female, and 50 high-dose female animals.

A significant number of tumors occurred in the urinary system of the treated animals. Seven high-dose male rats showed a total of eight neoplasms of the kidney and ureter; three of these neoplasms were classified as tubular-cell adenomas of the kidney, two as tubular-cell adenocarcinomas of the kidney (one widely metastasized), one as a papillary adenoma of the ureter, and two as papillomas of the ureter. A low-dose male rat showed a tubular-cell adenoma. In the females, tumors of the urinary bladder were more frequent than those of the kidney. Eleven high-dose and two low-dose females showed transitional-cell carcinomas of the bladder. An additional high-dose female showed a squamous-cell carcinoma of the bladder. Tumors of the kidney

were found only in two high-dose females that were not included among those that had shown transitional-cell carcinomas of the bladder; one of these showed a transitional-cell papilloma of the renal pelvis, the other a tubular-cell adenoma. No neoplasms of the urinary system were observed in the controls.

Microscopically, the tubular-cell adenomas were discrete expanding masses of tubules of varying sizes, usually located in the cortex. The cells lining and filling the tubules resembled normal renal epithelium, except that the nuclei tended to be more hyperchromatic than those of the normal contiguous renal epithelial cells.

In the adenocarcinomas, this tubular pattern was less distinct. Basement membranes were poorly defined and the cells tended to pile up; in some areas they appeared to be growing in sheets rather than in a tubular pattern. Rapid growth was suggested by the anaplasia, nuclear pleomorphism, frequent mitoses, and foci of necrosis.

The tumors of the bladder varied in appearance from benign, well-differentiated squamous- or transitional-cell papillomas to carcinomas. The latter were characterized by disarray in cellular polarity and arrangement as well as by subepithelial invasions by nests of pleomorphic epithelial cells. The mitotic index was low and metastases did not occur.

Hepatocellular carcinomas occurred in three of the low-dose male rats and neoplastic nodules appeared in three control, two low-dose, and two high-dose males. However, 8 of the low-dose and 22 of the high-dose females had neoplastic nodules, compared with 2 in the controls. This is a strikingly higher incidence than was noted with female rats receiving $Na_3NTA \cdot H_2O$ (Section VI, A).

Alveolar/bronchiolar adenoma and carcinoma occurred in one control, five low-dose, and five high-dose males, and in three low-dose and seven high-dose females. This is a frequently encountered neoplasm in the respiratory system of the Fischer 344 rat.

Fourteen primary tumors of the integument of various types occurred throughout all groups with a slightly higher proportion among controls. Eight of these tumors arose from adnexae. Of these, four were malignant, with two metastasizing. The remainder arose from connective tissue, of which two were classified as malignant.

In the endocrine system, pituitary tumors occurred in 1 control, 6 low-dose, and 2 high-dose males, and in 6 control, 10 low-dose, and 12 high-dose females. Among males, pheochromocytomas were present in one control, nine low-dose, and five high-dose animals. Fourteen pheochromocytomas were present in the

high-dose females, but none occurred in the low-dose. A single pheochromocytoma was present in the controls. Both of these tumors are frequently found in Fischer 344 rats.

The number and types of thyroid tumors in the treated animals were similar to those in the controls. In the male reproductive system, carcinomas of the preputial gland occurred in two low-dose, four high-dose, but no control animals. Interstitial-cell tumors of the testes occurred in nearly all male rats. This extremely high incidence is characteristic of Fischer 344 rats.

A number of different types of neoplasms occurred in various other organ systems, apparently not related to treatment.

In the urinary system, particularly the kidney, severe chronic inflammatory changes in the treatment groups far exceeded those seen in the controls. The lesions are those commonly associated with kidneys of old rats. These consist of fibrosed glomeruli, diffuse interstitial scarring, and dilated and cast-containing tubules, accompanied by focal accumulation of mononuclear inflammatory cells.

Hyperplasia of tissue in the urinary system was seen only in the treated rats. Epithelial hyperplasia of the urinary bladder was present in 2 low-dose males, 1 high-dose male, and 11 high-dose

females. One high-dose rat had a similar lesion in the ureter.

The gamut of lesions comprising the chronic murine pneumonia syndrome is responsible for the majority of the nonneoplastic lesions seen in this study. The treated males appear to have had a higher incidence of disease than did the controls. The reverse appears to have been the case with the females.

4. Statistical Analyses of Results (Rats)

As shown in table 6, tumors of the urinary tract in male rats occurred more frequently in the high-dose group than in the control group ($P = 0.076$), and a positive linear dose-response relation existed ($P = 0.006$). The low-dose group produced a higher incidence of endocrine tumors than the control group.

In female rats (table 7), there was a significant dose-related trend ($P < 0.001$) in overall incidence of tumors (excluding uterine), due mainly to differences in the incidences of the high-dose group and the controls ($P = 0.026$). This dose-response statistic was nonlinear. Further analyses revealed that the overall incidence of tumors in the high-dose group is different from that of the controls due to the higher incidence of nonmalignant tumors of the liver ($P = 0.024$) and malignant tumors of the urinary bladder ($P = 0.014$) in the high-dose group as

Table 6. Incidence of Tumors in Male Rats (Litton NTA)

	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors of the Urinary Tract	0/20 (0%)	1/49 (2%)	7/48 (15%)
Statistical Tests[a]	P = 0.021, P = 0.006	N.S.	P = 0.076
95% Confidence Intervals	---	---	0.83 - ∞
Weeks to First Observed Tumor	---	104	66
Incidence of Tumors of the Endocrine System	2/20 (10%)	16/49 (33%)	8/49 (16%)
Statistical Tests[a]	P = 0.054, nonlinear	P = 0.045	N.S.
95% Confidence Intervals	---	0.85 - 42.62	---
Weeks to First Observed Tumor	104	93	104

[a]Beneath the untreated-control incidence are two quantities: (a) the P values for a 2x3 contingency table using chisquare theory: (b) the P value for the test for linear trend if a linear trend fits the data, or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data. Beneath the dosed-group incidence is the Fisher-Irwin one-tail test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.

Table 7. Incidence of Tumors in Female Rats (Litton NTA)

	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors	14/20 (70%)	33/50 (66%)	47/50 (94%)
Statistical Tests[a]	P = 0.002 nonlinear	N.S.	P = 0.013
95% Confidence Intervals	---	---	1.20 - 45.34
Weeks to First Observed Tumor	76[b]/96	86	73
Incidence of Non-uterine Tumors	14/20 (70%)	30/50 (60%)	46/50 (92%)
Statistical Tests[a]	P < 0.001, nonlinear	N.S.	P = 0.026
95% Confidence Intervals	---	---	0.98 - 26.61
Weeks to First Observed Tumor	76[b]/96	86	73

Table 7. Incidence of Tumors in Female Rats (Litton NTA)

(continued)

	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors of the Lung (all malignant)	0/15 (0%)	3/49 (6%)	7/46 (15%)
Statistical Tests[a]	N.S. P = 0.033	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	---	104	73
Incidence of Tumors of the Liver (all nonmalignant)	2/15 (13%)	8/49 (16%)	22/49 (45%)
Statistical Tests[a]	P = 0.003, P = 0.001	N.S.	P = 0.024
95% Confidence Intervals	---	---	1.00 - 52.13
Weeks to First Observed Tumor	99[c]/104	104	93

Table 7. Incidence of Tumors in Female Rats (Litton NTA)

(continued)

	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors of the Urinary Tract	0/20 (0%)	2/50 (4%)	14/50 (28%)
Statistical Tests[a]	P < 0.001, P < 0.001	N.S.	P = 0.005
95% Confidence Intervals	---	---	2.06 - ∞
Weeks to First Observed Tumor	---	104	91
Incidence of Tumors of the Bladder (all malignant)	0/18 (0%)	2/45 (4%)	12/48 (25%)
Statistical Tests[a]	P = 0.002, P < 0.001	N.S.	P = 0.014
95% Confidence Intervals	---	---	1.56 - ∞
Weeks to First Observed Tumor	---	104	91

Table 7. Incidence of Tumors in Female Rats (Litton NTA)

(continued)

	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors of the Endocrine System	6/20 (30%)	13/50 (26%)	25/50 (50%)
Statistical Tests[a]	P = 0.036, P = 0.024	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	99[c]	101	91
Incidence of Adrenal Phenochromocytoma	1/20 (5%)	0/50 (0%)	14/48 (29%)
Statistical Tests[a]	P < 0.001, nonlinear	N.S.	P = 0.024
95% Confidence Intervals	---	---	1.01 - 347.90
Weeks to First Observed Tumor	99[c]	---	93

[a]Beneath the untreated-control incidence are two quantities; (a) the P values for a 2x3 contingency table using chisquare theory: (b) the P value for the test for linear trend if a linear trend fits the data, or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tailed test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.
[b]Animal at week 76 was accidentally killed; the first natural death with a tumor was at week 96.
[c]Animal at week 99 was a scheduled sacrifice, not a natural death.

compared with the controls. Also, a positive linear dose-response relation exists for the incidence of nonmalignant tumors of the liver ($P = 0.001$) and malignant tumors of the urinary bladder ($P < 0.001$). The incidence of tumors in the endocrine system shows a positive linear dose-response relation ($P = 0.024$). Finally, the incidence of adrenal tumors is elevated in the high-dose group relative to the controls ($P = 0.024$).

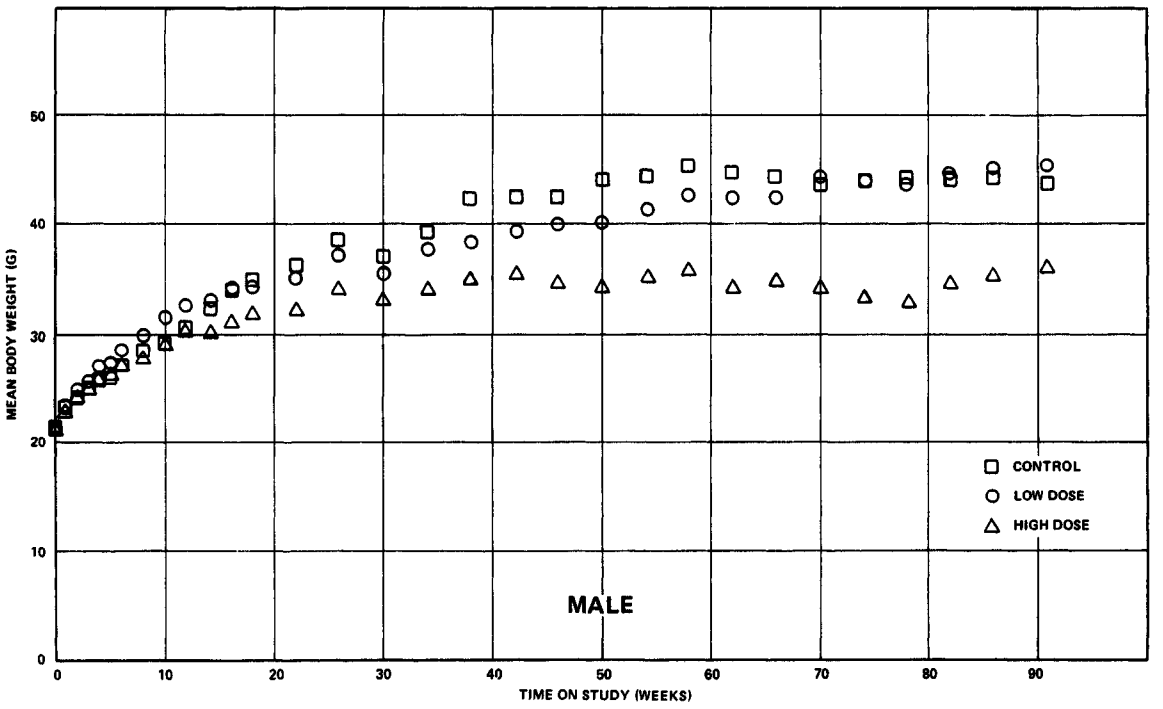
B. MICE (NTA)

1. Body Weights and Clinical Signs

The average weights of the high-dose male mice and the high- and low-dose female mice were depressed when compared with those of the controls (figure 5). No significant gross lesion or palpable mass was observed in this group of animals during the treatment period.

2. Survival

The survival curves of the treated and control groups of both sexes of the mice were comparable (figure 6). More than 92% of the animals survived to the end of the study, and the results can be evaluated over the entire time period for both the treated and control groups. The Tarone test for life-table analyses showed no significant difference in survival between the groups.



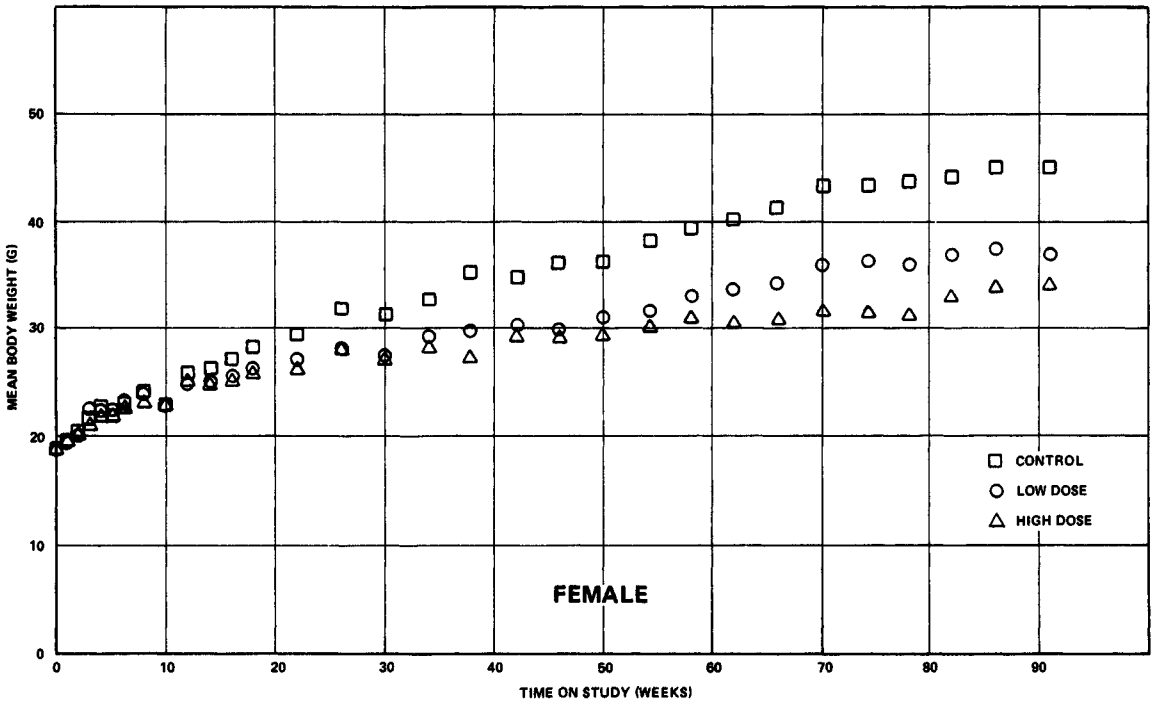


Figure 5. Growth Curves for Mice, NTA - Litton Study

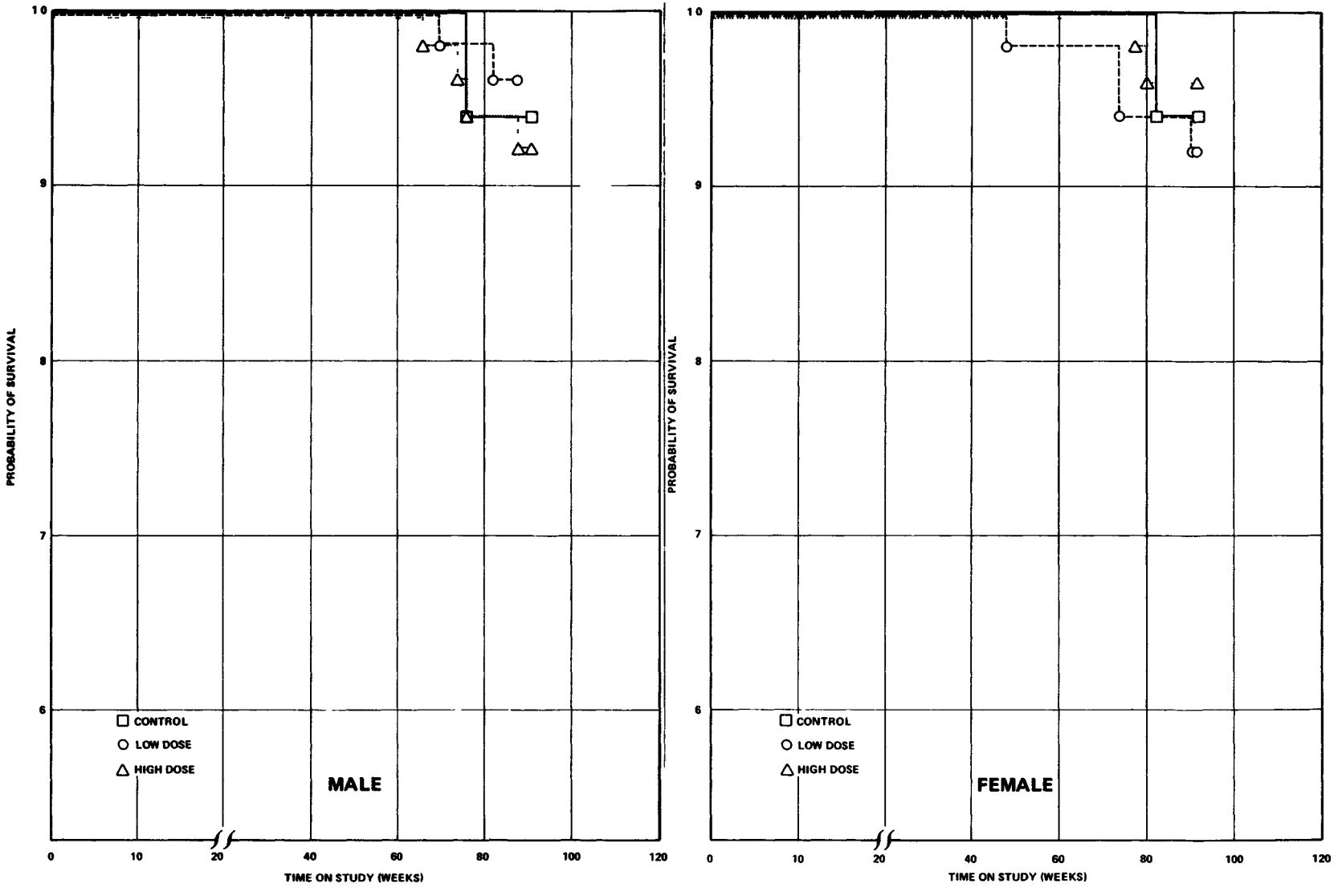


Figure 6. Survival Curves for Mice, NTA - Litton Study

3. Pathology

Histopathologic findings are tabulated in Appendix B, tables B3 and B4, covering neoplasms and other proliferative lesions, and in Appendix E, tables E3 and E4, covering nonneoplastic lesions.

The study began with 200 treated and 40 matched-control mice; of these, 222 animals received histopathologic examination. Eighteen mice escaped, including 1 low-dose male, 6 high-dose males, and 11 low-dose females. Thus, where the number of animals with lesions is delineated, comparisons should be made with regard to the respective group size, i.e., 20 control male, 49 low-dose male, 44 high-dose male, 20 control female, 39 low-dose female, and 50 high-dose female animals.

As with the rats, the urinary system was the site of treatment-related neoplasia in mice. Altogether, 32 treated mice showed urinary tract tumors. Tubular-cell adenocarcinomas, four of which were bilateral, were found in 22 high-dose males. The same tumor was also found in five low-dose males and in four high-dose females. In one of the high-dose females, the tumor was bilateral. A papilloma occurred in the renal pelvis of an additional high-dose male. No renal neoplasms were present in either the low-dose females or controls.

The pattern of these tubular-cell adenocarcinomas varied from

small cortical foci to large masses containing blood-filled cystic spaces and areas of necrosis. While ductal structures could usually be found in the tumor, large areas of neoplastic cells grew in sheets in which cell borders and basement membranes were indistinct. The individual cell types varied from fairly well-differentiated renal tubule cells to highly pleomorphic, often polygonal-shaped large cells with eosinophilic cytoplasm, frequently containing bizarre nuclei and mitotic figures.

Six high-dose, four low-dose, and two control male mice developed neoplasms of the hematopoietic system. These hematopoietic neoplasms were represented by malignant lymphomas in three of the high-dose males. Ten high-dose, seven low-dose, and four control female mice developed neoplasms of the hematopoietic system. These neoplasms were represented by malignant lymphomas in eight high-dose, three low-dose, and two control females.

In males, hepatocellular adenomas were observed in one low-dose and three control animals; and hepatocellular carcinomas occurred in two high- and two low-dose mice, but none was found in the control group. In females, only one hepatocellular carcinoma occurred, this in a low-dose animal.

The incidences of pulmonary neoplasms in treated male and female mice appear similar. In males alveolar/bronchiolar carcinomas occurred in four control, two low-dose, and four high-dose

animals; in addition, adenomas were found in three low-dose and two high-dose animals. In the females, two alveolar/bronchiolar carcinomas were seen in each treatment group, but none in the controls. Two high-dose females had adenomas. These neoplasms are frequently encountered in both sexes of mice.

Additional neoplasms were found only in the skin and in the circulatory and female reproductive systems. These occurred as single lesions or in such small numbers as to be insignificant.

The kidney was the most frequent site of nonneoplastic lesions in both male and female mice. The most frequently observed lesion, hydronephrosis, was encountered in eight high-dose and three low-dose males. Twelve high-dose females had this same lesion. Hydronephrosis was not observed in low-dose females or in any of the controls. Since epithelial hyperplasia of the kidney, renal pelvis, and ureter was described in only one female and one male of the treated mice, the cause of the hydronephrosis is obscure. However, no special diagnostic procedures were employed. No lesion of the urinary bladder was observed.

A variety of other nonneoplastic lesions occurred in both control and treated animals in insignificant numbers. The most frequently occurring lesions of this type were corpora amylacea in the brain, pneumonic changes in both males and females, inflammatory and cystic hyperplastic changes seen in the uterus, lymphoid

hyperplasia, and hyperplasia of the C-cell of the thyroid.

4. Statistical Analyses of Results

Table 8 shows that in male mice the high-dose group had a higher overall incidence of tumors than the control group (71% vs. 50%), due mainly to the higher incidence of tumors of the kidney in the high-dose group (55% vs. 0%). Thus, the important finding for male mice administered NTA was the statistically significant incidence ($P < 0.001$) of tumors of the kidney in the high-dose group. No other significant findings were observed.

In the females, tumors again appeared only in the high-dose group, but at a rate of incidence which was too low to be significant (table 9).

Table 8. Incidence of Tumors in Male Mice (Litton NTA)

	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors	10/20 (50%)	14/49 (29%)	31/44 (71%)
Statistical Tests[a]	P < 0.001 nonlinear	N.S.	P = 0.0976
95% Confidence Intervals	---	---	0.69 - 8.11
Weeks to First Observed Tumor	76	82	66
Incidence of Tumors of the Urinary Tract (all kidney tumors)	0/20 (0%)	5/49 (10%)	24/44 (55%)
Statistical Tests[a]	P < 0.001 nonlinear	N.S.	P < 0.001
95% Confidence Intervals	---	---	6.34 - ∞
Weeks to First Observed Tumor	91	88	74

[a]Beneath the untreated-control incidence are two quantities: (a) the P value for a 2x3 contingency table using chisquare theory; (b) the P value for the test for linear trend if a linear trend fits the data or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tailed test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.

Table 9. Incidence of Tumors in Female Mice (Litton NTA)

	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors of the Urinary Tract (all malignant kidney tumors)	0/20 (0%)	0/39 (0%)	4/50 (8%)
Statistical Tests[a]	P = 0.086, P = 0.041	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	---	---	91

[a]Beneath the untreated-control incidence are two quantities; (a) the P value for a 2x3 contingency table using chisquare theory; (b) the P value for the test for linear trend if a linear trend fits the data or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tail test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.

VI. RESULTS - LITTON BIONETICS, INC. - $Na_3NTA \cdot H_2O$

A. RATS ($Na_3NTA \cdot H_2O$)

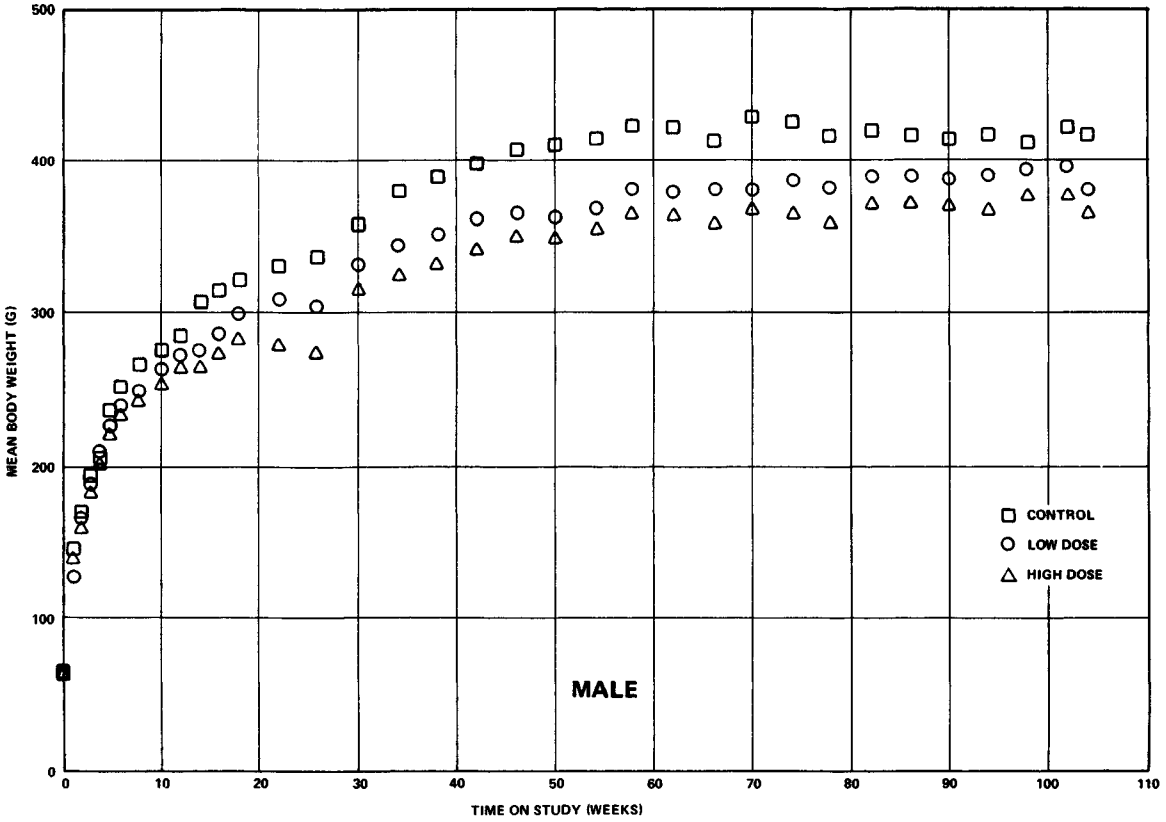
1. Body Weights and Clinical Signs

The average weights of both male and female rats were depressed in a dose-related manner when compared with those of the controls (figure 7).

No significant clinical signs were observed until late in the second year when a few animals began to show loss of weight and discharges from the eyes and nose. Near the end of the study, nearly all of the low-dose females appeared to be suffering from respiratory disease.

2. Survival

Figure 8 shows the estimated probability of survival of the rats in this study. The times to death of all groups of both sexes are comparable. While the number of early deaths increased slightly with dose, there is no statistically significant dose-related trend ($P > 0.10$). In every group more than 85% of the animals survived for 80 weeks or more. Six of the high-dose male rats and five of the high-dose female rats were accidentally killed during the test, and were examined histopathologically.



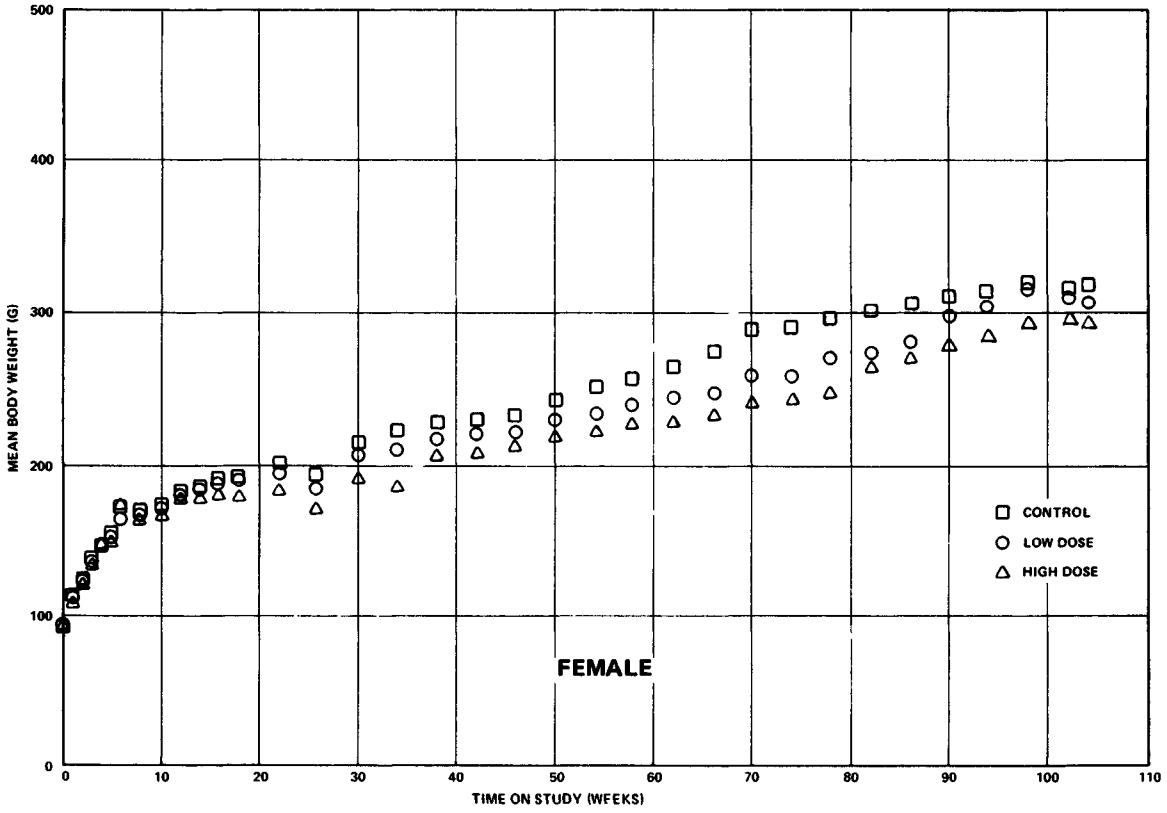


Figure 7. Growth Curves for Rats, $Na_3NTA \cdot H_2O$ - Litton Study

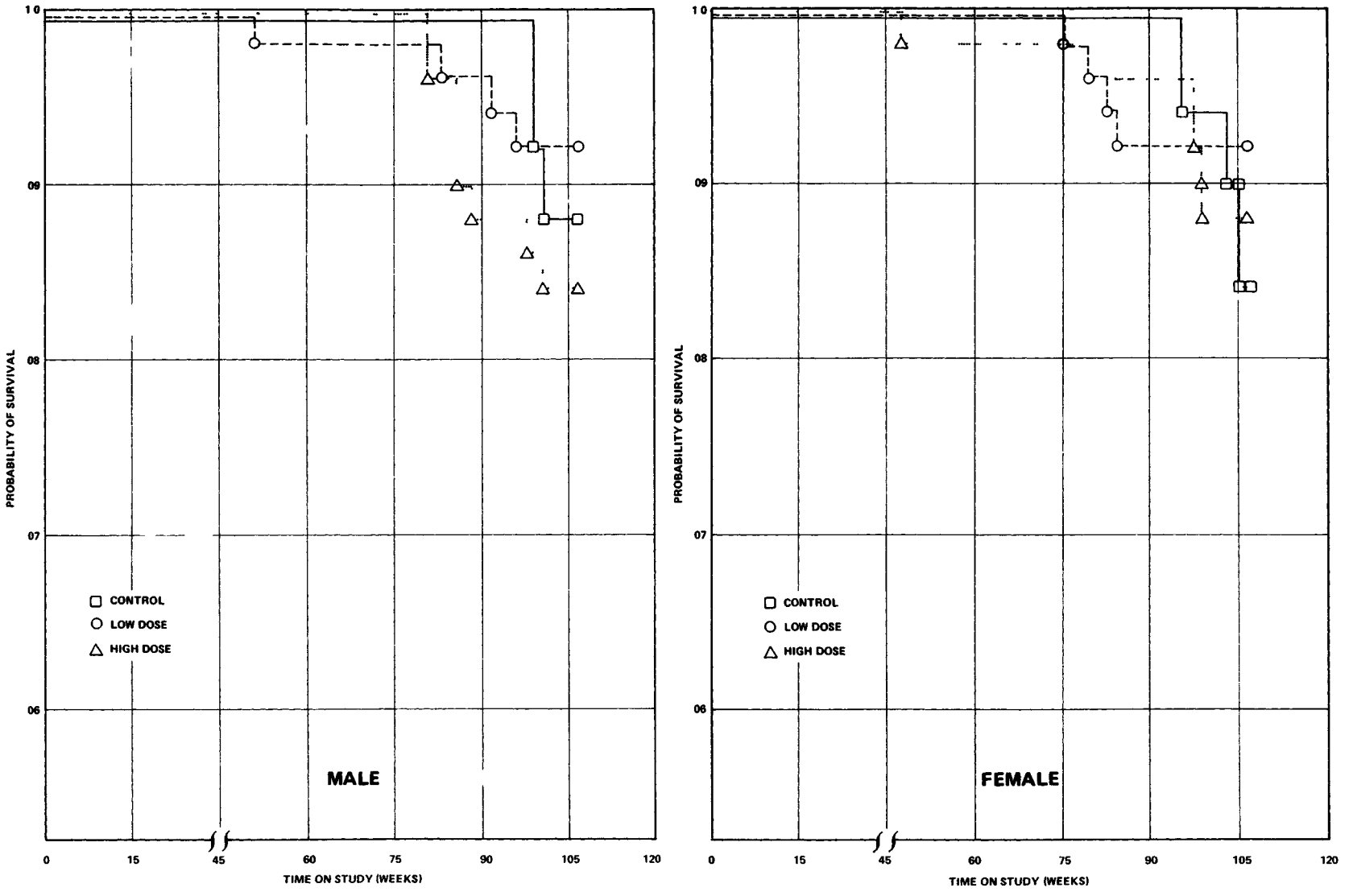


Figure 8. Survival Curves for Rats, $Na_3NTA \cdot H_2O$ - Litton Study

These animals were entered as censored observations in the Tarone test for dose-related survival, and they are included among the number of animals examined in the analyses of tumors.

3. Pathology

Histopathologic findings are tabulated in Appendix C, tables C1 and C2, covering neoplasms and other proliferative lesions, and in Appendix F, tables F1 and F2, covering nonneoplastic lesions.

Of the 200 treated and 40 control rats entered into this study, all were necropsied, and 238 were examined histopathologically. The tissues from one low-dose male and one high-dose female were lost. Thus, where the number of animals with lesions is delineated, comparisons should be made with the respective group size, i.e., 20 control male, 49 low-dose male, 50 high-dose male, 20 control female, 50 low-dose female, and 49 high-dose female animals.

As was noted in animals treated with NTA, a number of primary neoplasms of the urinary system occurred in both the high- and low-dose groups. No such neoplasm occurred in the controls. In the low-dose males, a tubular-cell adenocarcinoma of the kidney was found in one animal and a papilloma of the ureter in the other; similarly, in the high-dose males a tubular-cell carcinoma of the kidney was found in one animal and a papilloma of the

ureter in another. In female rats the tumors were confined to the bladder. One female bearing a transitional-cell carcinoma and a second bearing a papilloma were found in the high-dose group; a squamous-cell carcinoma in one female and transitional-cell carcinomas in three additional females were found in the low-dose group.

The tubular-cell adenocarcinomas seen in this study had many of the morphologic characteristics of those tumors seen in the rats dosed with NTA. One of the neoplasms in $Na_3NTA \cdot H_2O$-treated rats differed somewhat from those previously described. This carcinoma appeared as a discrete cortical multiloculated mass comprised of centers of necrosis surrounded by proliferating neoplastic cells. These varied in size from small cells resembling normal renal tubule epithelium to very large cells, many of which contained a single vacuole displacing the nucleus to the periphery of the cell.

In the respiratory system, nine males and six females had alveolar/bronchiolar carcinomas. This tumor is not infrequently encountered in the Fischer 344 rats in this laboratory. Other primary neoplasms of the lung appeared in about equal numbers in both treated and control animals. In contrast to the large number of neoplastic nodules of the liver found in rats receiving NTA, only one neoplastic nodule was found in each of one low-dose

and one high-dose female, one control and one high-dose male.

In the female rats, chromophobe adenoma of the pituitary occurred in three control, 15 low-dose, and 12 high-dose animals. Lesser numbers occurred in both treated and control males. This is a frequently encountered tumor in several strains of rats, particularly in females. Pheochromocytoma of the adrenal gland and isletcell adenomas of the pancreas were found in small numbers in both treated and control animals.

Interstitial-cell tumor of the testis occurred in nearly all male rats. This extremely high incidence is found only in Fischer 344 rats.

Various benign and malignant tumors of the mammary gland were scattered throughout both treated and control groups.

As is usually seen in long-term chronic studies, a large number of inflammatory, degenerative, and proliferative lesions occurred. The morphologic changes associated with senile rat kidneys were the most prominent nonneoplastic lesions occurring in the treated animals of both sexes. All the treated animals had moderate to severe chronic nephritis. Fewer control animals also exhibited this inflammation, but it was less severe.

Epithelial hyperplasia of the urinary tract occurred in the

treated animals only. Three high-dose males, four low-dose females, and five high-dose females had this proliferative change in the urinary bladder. Four low-dose males had urothelial proliferation in the ureter or renal pelvis. This lesion is considered noteworthy, since a substantial number of treated animals had urinary tract lesions.

The pulmonary lesions, consisting for the most part of morphologic changes associated with chronic murine pneumonia, were more prevalent in both high-dose males and females than in controls. However, the incidence of chronic murine pneumonia in controls was similar to that in low-dose males and females; thus, no compound-related effect appears to be present.

The treated females had a higher incidence of basophilic cytoplasmic alteration of hepatocytes than did the controls. This lesion was recognized in 2 controls, 15 low-dose, and 15 high-dose females. This change was observed in the males as well, but in nearly equal proportions in all groups, including the controls.

In the endocrine system, there were many types of lesions scattered through all the treated groups and controls. The only cluster of lesions that appears significant is in the 12 low-dose males with C-cell hyperplasia of the thyroid. This represents a 24% incidence, compared with 5% in the controls. However, C-cell

tumors of the thyroid appeared in approximately equal numbers in both treated and control groups.

4. Statistical Analyses of Results (Rats)

Tables 10 and 11 show the overall incidence of tumors as well as the incidence of site-specific tumors for male and female rats, respectively. In the males, the overall incidence of tumors in the treated animals did not differ statistically ($P > 0.10$) from that of the controls. Although the incidence of primary tumors in the lung in the low-dose group was higher than in the controls, the difference was not statistically significant.

In the female rats, no P values less than 0.10 occur.

B. MICE ($Na_3NTA \cdot H_2O$)

1. Body Weights and Clinical Signs

The average weights of male mice were depressed in a dose-related manner throughout most of the study, although the differences decreased toward the end of the test (figure 9). The average weights of female mice were depressed in a dose-related manner throughout the study.

No significant clinical signs were observed in the first year of treatment. In the second year an occasional tissue mass was observed, and a few animals appeared to lose weight.

Table 10. Incidence of Tumors in Male Rats (Litton - $Na_3NTA \cdot H_2O$)

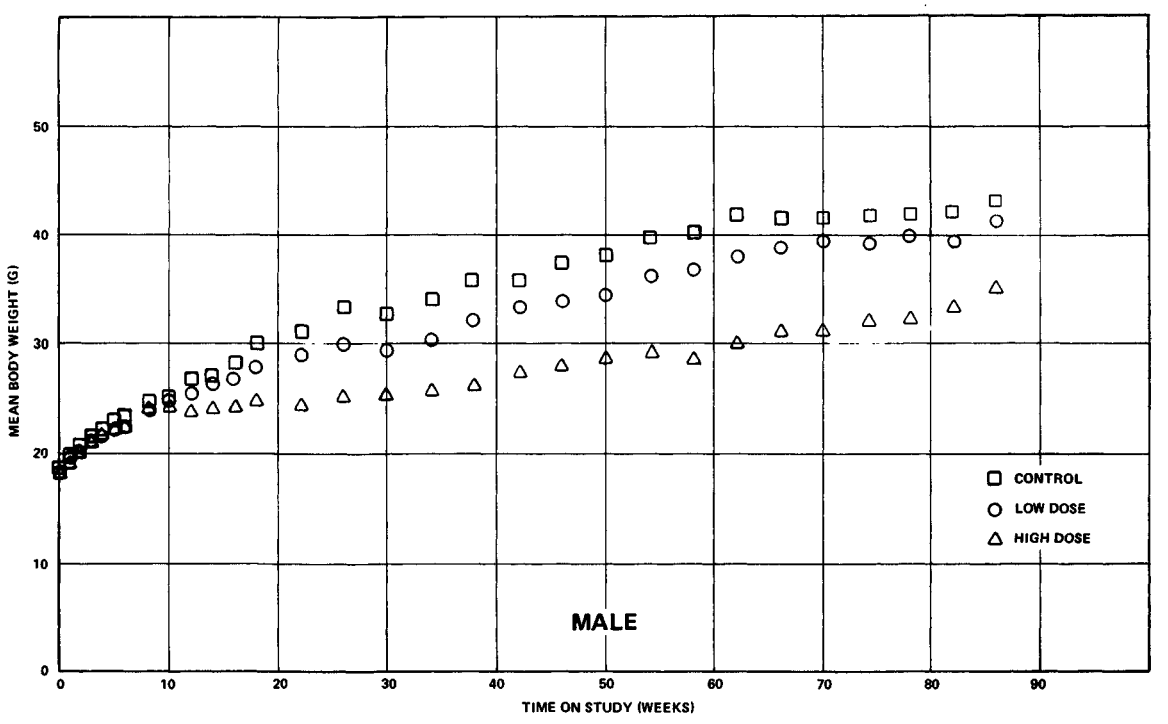
	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors (excluding testicular tumors)	10/20 (50%)	26/49 (53%)	24/50 (48%)
Statistical Tests[a]	N.S.,N.S.	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	99	51	81
Incidence of Tumors of the Lung (excluding metastatic)	2/19 (11%)	10/47 (21%)	3/48 (6%)
Statistical Tests[a]	P = 0.089 nonlinear	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	106	106	106

[a]Beneath the untreated-control incidence are two quantities: (a) the P value for a 2x3 contingency table using chisquare theory; (b) the P value for the test for linear trend if a linear trend fits the data, or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tailed test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.

Table 11. Incidence of Tumors in Female Rats (Litton - $Na_3NTA \cdot H_2O$)

	Controls 0 ppm	Low Dose 7,500 ppm	High Dose 15,000 ppm
Incidence of Tumors (all tumors)	13/20 (65%)	36/50 (72%)	26/49 (53%)
Statistical Tests[a]	N.S.,N.S.	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	96	75	84
Incidence of Tumors (excluding uterine tumors)	12/20 (60%)	33/50 (66%)	24/49 (49%)
Statistical Tests[a]	N.S.,N.S.	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	96	75	84

[a]Beneath the untreated-control incidence are two quantities: (a) the P value for a 2x3 contingency table using chisquare theory; (b) the P value for the test for linear trend if a linear trend fits the data, or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tailed test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.



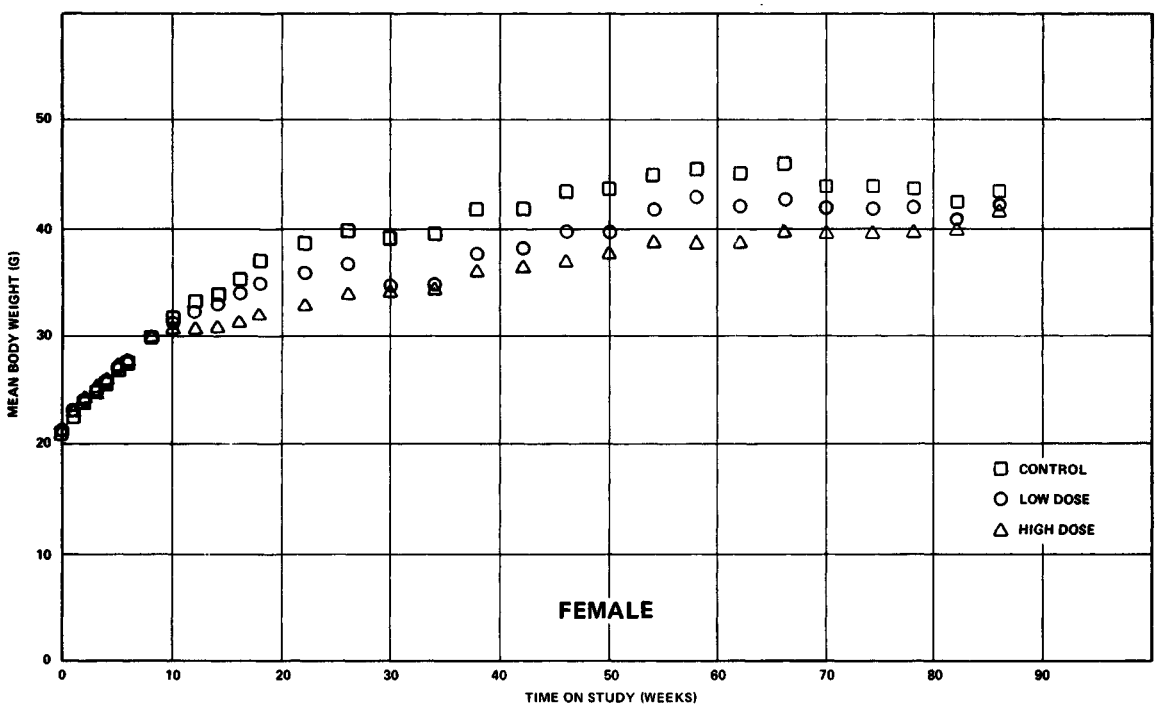


Figure 9. Growth Curves for Mice, $Na_3NTA \cdot H_2O$ - Litton Study

2. Survival

The survival curves for mice are shown in figure 10. Survival was comparable between all groups of both sexes. More than 85% of each group survived to the end of the study, and the results can be evaluated over the entire time period for both treated and control groups. There is no dose-related trend ($P > 0.10$).

3. Pathology

Histopathologic findings are tabulated in Appendix C, tables C3 and C4, covering neoplasms and other proliferative lesions, and in Appendix F, tables F3 and F4, covering nonneoplastic lesions.

Of the 200 treated and 40 control animals that were assigned to this study, all were necropsied and evaluated histopathologically with the exception of 11 that were autolyzed. Thus, where the number of animals with lesions is delineated, comparisons should be made with the respective group size, i.e., 20 control male, 48 low-dose male, 50 high-dose male, 18 control female, 46 low-dose female, and 47 high-dose female animals.

Hepatocellular carcinomas occurred in one control, five low-dose, and three high-dose males. In addition, three hepatocellular adenomas occurred in three male controls. In females the incidence of hepatocellular carcinomas was similar in both treated and control groups.

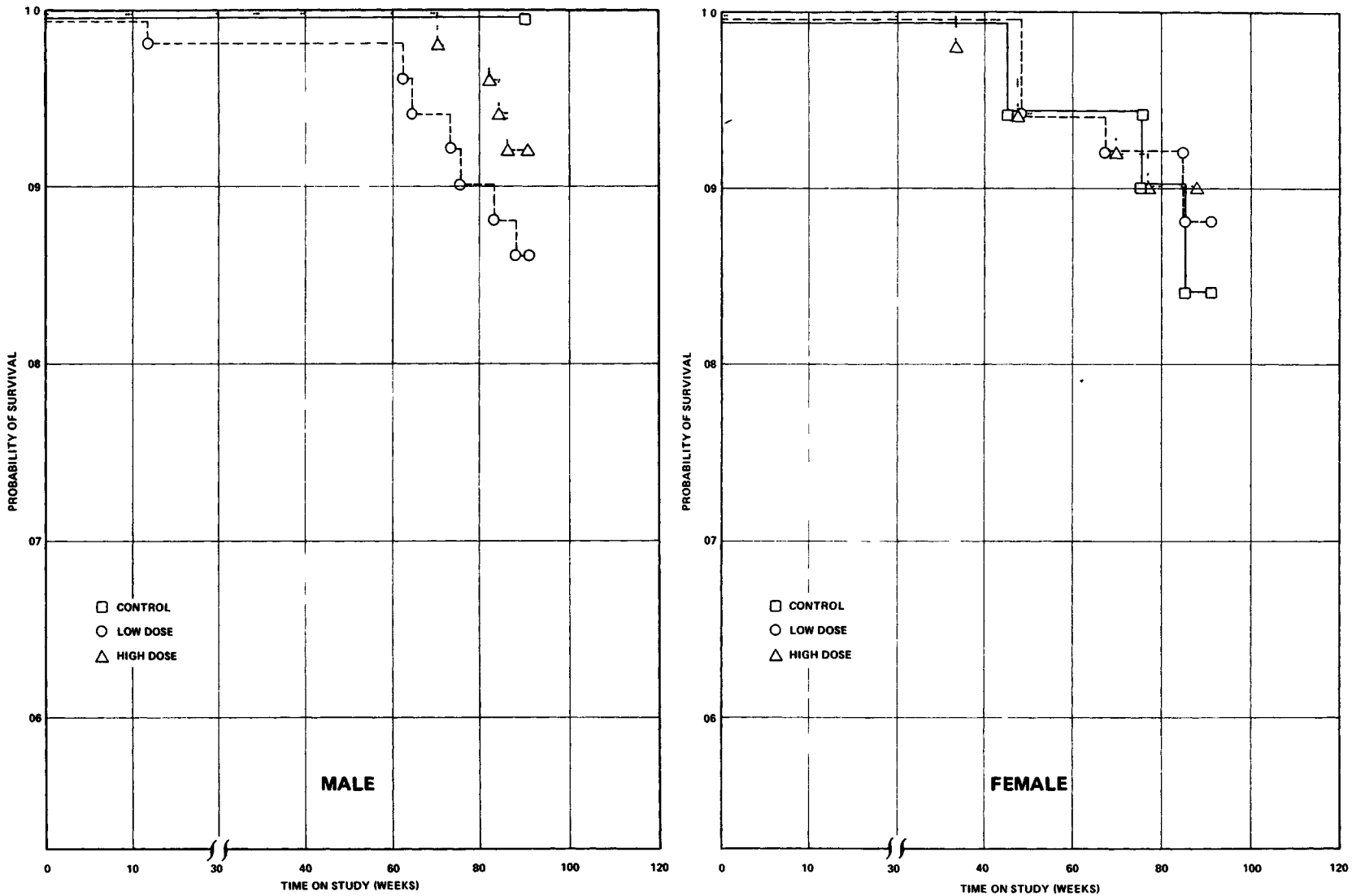


Figure 10. Survival Curves for Mice, $Na_3NTA \cdot H_2O$ - Litton Study

A leukemia appeared in four high-dose males and four high-dose females, seven low-dose females, and three female controls. Malignant lymphomas occurred only in treated animals; however, the lesions were randomly dispersed throughout the hematopoietic system as single entities. Their significance is not clear at this time.

Alveolar/bronchiolar carcinomas were observed in one control and six low-dose males. In addition, each of these groups displayed one adenoma, a frequently encountered neoplasm in this strain of mouse.

The only nonneoplastic lesion occurring in these mice was hydronephrosis, which was observed in 1 low-dose and 28 high-dose males. A similar picture was seen in the females: 1 low-dose and 30 high-dose animals had hydronephrosis in 1 or both kidneys. Hydronephrosis was not observed in any of the controls. No occlusive lesions were observed; however, no special procedures were used for their detection.

4. Statistical Analyses of Results (Mice)

In the male mice (table 12), the overall incidence of tumors in the treatment groups was comparable to that of the control group ($P > 0.10$). On the other hand, tumors of the hematopoietic system were statistically more frequent in the high-dose group

Table 12. Incidence of Tumors in Male Mice (Litton $Na_3NTA \cdot H_2O$)

	Controls 0 ppm	Low Dose 2,500 ppm	High Dose 5,000 ppm
Incidence of Tumors	7/20 (35%)	18/48 (38%)	14/50 (28%)
Statistical Tests[a]	N.S.,N.S.	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	91	75	72
Incidence of Tumors of the Lung	2/20 (10%)	8/48 (17%)	0/49 (0%)
Statistical Tests[a]	P = 0.013, nonlinear	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	91	76	---

Table 12. Incidence of Tumors in Male Mice (Litton $Na_3NTA \cdot H_2O$)

(continued)

	Controls 0 ppm	Low Dose 2,500 ppm	High Dose 5,000 ppm
Incidence of Tumors of the Hematopoietic System (all malignant)	0/20 (0%)	4/47 (9%)	9/50 (18%)
Statistical Tests[a]	P = 0.073, P = 0.015	N.S.	P = 0.038
95% Confidence Intervals	---	---	1.11 - ∞
Weeks to First Observed Tumor	---	75	86

[a]Beneath the untreated-control incidence are two quantities: (a) the P value for a 2x3 contingency table using chisquare theory; (b) the P value for the test for linear trend if a linear trend fits the data, or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tailed test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.

(P = 0.038) than in the controls; also, a significant linear positive dose-response relation was found (P = 0.015).

In the females, no significant findings were obtained (table 13).

Table 13. Incidence of Tumors in Female Mice (Litton $Na_3NTA \cdot H_2O$)

	Controls 0 ppm	Low Dose 2,500 ppm	High Dose 5,000 ppm
Incidence of Tumors	6/18 (33%)	11/46 (24%)	16/47 (34%)
Statistical Tests[a]	N.S.,N.S.	N.S.	N.S.
95% Confidence Intervals	---	---	---
Weeks to First Observed Tumor	85	68	70

[a]Beneath the untreated-control incidence are two quantities: (a) the P value for a 2x3 contingency table using chisquare theory: (b) the P value for the test for linear trend if a linear trend fits the data, or the word "nonlinear," which means that a linear trend on a logistic scale does not fit the data.
Beneath the dosed-group incidence is the Fisher-Irwin one-tailed test for comparison of that group with the control group when it is below 0.10; or, otherwise N.S. - not significant.

VII. DISCUSSION

The bioassays of NTA and $Na_3NTA \cdot H_2O$ showed that a variety of neoplastic and nonneoplastic lesions of the urinary tract developed in rats as a result of the administration of 7,500 to 20,000 ppm of either one of the test compounds in the diet. These same lesions were detected in mice as a result of the administration of 7,500 or 15,000 ppm of the acid, but not of 2,500 or 5,000 ppm of the salt form of the test compound. Almost all of the tumors were primary epithelial in origin. Their occurrence was particularly significant because they were not found in any control animals and only rarely develop spontaneously among animals of the strains on test. More tumors were found in the higher- than the lower-dose groups for each chemical and species (tables 14 and 15). When comparing results between compounds and between laboratories, it must be recognized that given concentrations of $Na_3NTA \cdot H_2O$ represent 30% less NTA than do equal concentrations of the free acid and that the SRI animals were treated for a total of 24 months while LBI animals were treated for only 18. Changing the units of concentration from parts per million to millimoles made such comparisons easier, and the results became more consistent. In all cases there was a rapid drop from the number of tumors observed at high doses to those at lower doses. This finding is consistent with

TABLE 14: SUMMARY OF PRIMARY EPITHELIAL NEOPLASMS OF THE URINARY TRACT – RATS

	All Controls		Stanford $Na_3NTA \cdot H_2O$ 20,000 ppm (73 mM/Kg)		Litton Bionetics $Na_3NTA \cdot H_2O$ 15,000 ppm (55 mM/Kg)		Litton Bionetics $Na_3NTA \cdot H_2O$ 7,500 ppm (27 mM/Kg)		Stanford $Na_3NTA \cdot H_2O$ 200-2,000 ppm (0.7-7 mM/Kg)	Litton Bionetics NTA 15,000 ppm (78 mM/Kg)		Litton Bionetics NTA 7,500 ppm (39 mM/Kg)	
SPECIFIC ORGAN/ TUMOR TYPE	M	F	M	F	M	F	M	F	M/F	M	F	M	F
KIDNEY													
Transitional-cell Carcinoma	0/63	0/64[a]	4/24(17%)	-	-	-	-	-	-	-	-	-	-
Transitional-cell Papilloma	0/63	0/64	-	-	1/49(2%)	-	-	-	-	-	1/49(2%)	-	-
Tubular-cell Adenocarcinoma	0/63	0/64	1/24(4%)	1/24(4%)	1/49(2%)	-	1/48(2%)	-	-	2/49(4%)	-	-	-
Tubular-cell Adenoma	0/63	0/64	3/24(13%)	3/24(13%)	-	-	-	-	-	3/49(6%)	1/49(2%)	1/49(2%)	-
URETER													
Transitional-cell Carcinoma	0/64	0/64	8/24(33%)	6/24(25%)	-	-	-	-	-	-	-	-	-
Papilloma	0/64	0/64	-	-	-	-	1/48(2%)	-	-	2/49(4%)	-	-	-
Papillary Adenoma	0/64	0/64	-	-	-	-	-	-	-	1/49(2%)	-	-	-
URINARY BLADDER													
Transitional-cell Carcinoma	0/57	0/56	1/24(4%)	5/24(21%)	-	1/42(2%)	-	3/43(7%)	-	-	11/48(23%)	-	2/45(4%)
Squamous-cell Carcinoma	0/57	0/56	-	-	-	-	-	1/43(2%)	-	-	1/48(2%)	-	-
Papilloma	0/57	0/56	-	-	-	1/42(2%)	-	-	1/23(4%)[b]	-	-	-	-
Total Number of Animals with Tumors of the Urinary Tract	0	0	13	13	2	2	2	4	1	7	14	1	2
Percent			(54)	(54)	(4)	(4)	(4)	(9)	(4)	(14)	(28)	(2)	(4)

[a]Denominators equal number of tissues examined histopathologically.

[b]In female rat receiving 2,000 ppm

TABLE 15: SUMMARY OF PRIMARY EPITHELIAL NEOPLASMS OF THE URINARY TRACT – MICE

	Litton Bionetics									
	All Controls		Na_3 NTA H_2O 5,000 ppm (18 mM/Kg)		Na_3 NTA·H_2O 2,500 ppm (9 mM/Kg		NTA 15,000 ppm (78 mM/Kg)		NTA 7,500 ppm (39 mM/Kg)	
SPECIFIC ORGAN/TUMOR TYPE	M	F	M	F	M	F	M	F	M	F
KIDNEY										
Tubular-cell Adenoma	0/40[a]	0/39	-	-	-	-	1/43(2%)	-	-	-
Tubular-cell Adenocarcinoma	0/40	0/39	-	-	-	-	22/43(50%)	4/50(8%)	5/48(10%)	-
Papilloma	0/40	0/39	-	-	-	-	1/43(2%)	-	-	-
Total Number of Animals with Tumors of the Urinary Tract	0	0	0	0	0	0	24	4	5	0
Percent							(56)	(8)	(10)	

[a]Denominators equal number of tissues examined histopathologically.

that of Nixon et al., (1972) who reported an absence of tumors in rats receiving 5,000 ppm $Na_3NTA \cdot H_2O$ in the diet for 2 years.

Most of the tumors of the urinary tract in rats were of transitional-cell origin and were found mainly in the kidneys of males, bladders of females, and ureters of both sexes. In addition to the transitional-cell tumors at the different sites, tubular-cell adenomas or carcinomas developed in highly significant frequencies in the kidney of the rat (table 14). The various lesions of the urinary tract may have been even more significant had a greater number of kidney and bladder sections been examined and had the ureters been routinely saved at necropsy; the tumors of the ureter that were actually examined were those recognized grossly or fortuitously sectioned with the kidneys. In mice, the tumors of the urinary tract consisted primarily of tubular-cell carcinomas of the kidney.

Transitional epithelial dysplasia and hyerplasia occurred in many of the treated animals that displayed no urinary neoplams, but not in untreated animals (Appendixes D, E, and F). Hydronephrosis and/or nephritis was observed in rats given high doses of the test compounds. In mice hydronephrosis was induced by low, nontumorigenic doses (5,000 ppm $Na_3NTA \cdot H_2O$) as well as by high, tumorigenic doses (15,000 ppm NTA).

Numerous other neoplastic lesions occurred, some of which

appeared statistically significant in certain tests. For example, hematopoietic tumors showed a dose-related trend in male rats given $Na_3NTA \cdot H_2O$ at SRI (P = 0.044), tumors of the liver in female rats given NTA at LBI (P = 0.003), and endocrine tumors in both male rats given $Na_3NTA \cdot H_2O$ at SRI (P = 0.037) and female rats given NTA at LBI (P = 0.036). However, in other instances the levels of incidence or of statistical significance were comparatively low, the data were not reproducible in the different tests, or the tumors were known to be variable in rate of spontaneous occurrence. Therefore, no conclusions are drawn as to the meaning of occurrence of tumors other than those of the urinary tract.

In all tests body weights of high-dose rats and mice were lower during the greater part of the test period than were respective controls. The lower-dose groups generally showed lesser changes. Decrease in survival also was related to the size of the dose, but only in rats, for which levels of significance of the trend were P = 0.001 in males given $Na_3NTA \cdot H_2O$ and P = 0.057 in females given NTA.

The pathologic findings in the kidney were not unexpected, since nephrotoxicity has been noted in several previous studies. Renal adenomas were reported in treated rats that died before termination of 2-year tests in which Na_3NTA was administered in

the drinking water (Chernoff and Courtney, 1970). Rats receiving diets containing up to 20,000 ppm Na_3NTA for 90 days developed hydronephrosis and renal tubule-cell damage at concentrations of 7,500 ppm and over (Nixon, 1971). The incidence and severity of nephritis and nephrosis increased in rats receiving 1,500 or 5,000 ppm of the compound in the diet for 2 years, but no compound-related increase in tumors was reported at these levels (Nixon et al., 1972). Rats administered 0.01, 0.1, or 1.0% Na_3NTA in the drinking water for 10 weeks showed marked vacuolization of renal tubules and high mortality at the 1% level, but no effect on weight gain and no histopathologic changes in the kidney, brain, liver, or pancreas at the two lower concentrations (Mahaffey and Goyer, 1972). When 20 ml of a 5% NTA solution, disodium salt, was administered to rats in place of drinking water 5 nights/week for 84 weeks, no significant increase in the incidence of tumors was noted (Lijinski et al., 1973); similarly treated mice also showed no significant increase in the incidence of tumors (Greenblatt and Lijinsky, 1974).

Metabolic studies with several species indicate that NTA and $Na_3NTA \cdot H_2O$ are either poorly absorbed, or if absorbed are rapidly excreted and that metabolic transformation does not occur. Michael and Wakim (1971) reported that NTA was readily absorbed from the gastrointestinal tract in rats and dogs, but was poorly absorbed in rabbits and monkeys. In either case, most of the

dose that remained in the body after 72 hours was found in the skeleton, or, in the case of rabbits, still in the gastro-intestinal tract. Absorption studies have not been reported with mice; however, Tjalve (1972) observed that radioactivity accumulated in the skeleton, kidney, and urinary bladder of mice given ^{14}C-labeled NTA. Metabolic data for NTA in humans resembled data for rats and dogs except that the extent of absorption for rats and dogs is four times greater than in humans (Budney and Arnold, 1973). Little or no biotransformation was observed in humans, judging by the finding that the radioactivity present in urine of humans given the labeled NTA was represented by unchanged NTA.

Since NTA and $Na_3NTA \cdot H_2O$ are eliminated rapidly through the kidneys, lesions of the urinary tract which arise as a result of administration of the compounds may be due to a local effect which can be brought about only by high concentrations. The occurrence of treatment-related neoplasms at high doses among animals of this bioassay should be taken into account in evaluations of hazards posed by use of the compounds.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS AND OTHER PROLIFERATIVE LESIONS IN RATS FED $NA_3NTA \cdot H_2O$ IN THE DIET (SRI)

Table A1. Male Rats

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): SRI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(VEH)	LOW DOSE	MID DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	24(100%)	24(100%)	24(100%)	24(100%)
ANIMALS WITH TUMORS	22 (92%)	23 (96%)	24 (100%)	24 (100%)
INTEGUMENTARY SYSTEM*	1 (4%)	4 (17%)		
SKIN	1	2		
PAPILLOMA		1		
SQUAMOUS CELL CARCINOMA	1	1		
SUBCUT TISSUE		2		
FIBROMA		1		
LIPOMA		1		
RESPIRATORY SYSTEM	1 (4%)		1 (4%)	5 (21%)
LUNG	1		1	5
TRANSIT-CELL CARCINOMA METASTAT				4
ALVEOLAR/BRONCHIOLAR ADENOMA	1			1
ALVEOLAR/BRONCHIOLAR CARCINOMA			1	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM	2 (8%)		1 (4%)	5 (21%)
LIVER	2		1	3
NEOPLASTIC NODULE	2		1	3
PANCREAS				2
TRANSIT-CELL CARCINOMA METASTAT				2
URINARY SYSTEM				14 (58%)
KIDNEY				9
TRANSITIONAL-CELL CARCINOMA				4
HEMANGIOMA				1
TUBULAR-CELL ADENOMA				3
TUBULAR-CELL ADENOCARCINOMA				1
URETER				8
TRANSITIONAL-CELL CARCINOMA				8
URINARY BLADDER				1
TRANSITIONAL-CELL CARCINOMA				1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table A1 Male Rats (Continued)

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O); SRI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(VEH)	LOW DOSE	MID DOSE	HIGH DOSE
ENDOCRINE SYSTEM*	2 (8%)	8 (33%)	7 (29%)	3 (13%)
PITUITARY		2	1	
CHROMOPHOBE ADENOMA		2	1	
ADRENAL	2	1	2	3
TRANSIT-CELL CARCINOMA METASTAT				1
PHEOCHROMOCYTOMA	1	1	2	2
CORTICAL CARCINOMA	1			
THYROID		4	4	
C-CELL CARCINOMA		3		
C-CELL ADENOMA		1	4	
PANCREATIC ISLETS		1	2	
ISLET-CELL ADENOMA		1	2	
HEMATOPOIETIC SYSTEM	2 (8%)	6 (25%)	1 (4%)	3 (13%)
MULTIPLE ORGANS	1	6	1	1
LEUKEMIA	1		1	1
LEUKEMIA GRANULOCYTIC		6		
MESENTERIC LYMPHNODE				2
TRANSIT-CELL CARCINOMA METASTAT				2
THYMUS	1			
MESOTHELIOMA MALIGNANT	1			
REPRODUCTIVE SYSTEM	22 (92%)	23 (96%)	24 (100%)	23 (96%)
MAMMARY GLAND				1
FIBROADENOMA				1
SEMINAL VESICLE				1
TRANSIT-CELL CARCINOMA METASTAT				1
TESTIS	22	23	24	23
INTERSTITIAL-CELL TUMOR	22	23	24	23
MESOTHELIOMA			1	

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table A1. Male Rats (Continued)

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): SRI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(VEH)	LOW DOSE	MID DOSE	HIGH DOSE
NERVOUS SYSTEM				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
ALL OTHER SYSTEMS*	2 (8%)			
DIAPHRAGM	1			
MESOTHELIOMA MALIGNANT	1			
PLEURA	1			
MESOTHELIOMA	1			
TUMOR SUMMARY				
TOTAL ANIMALS WITH BENIGN TUMORS	22 (92%)	23 (96%)	24 (100%)	23 (96%)
TOTAL BENIGN TUMORS	24	31	33	31
TOTAL ANIMALS WITH MALIGNANT TUMORS	4 (17%)	8 (33%)	2 (8%)	11 (46%)
TOTAL MALIGNANT TUMORS	5	10	2	15
TOTAL ANIMALS WITH METASTATIC TUMORS				5 (21%)
TOTAL METASTATIC TUMORS				10

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table A2. Female Rats

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O):

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(VEH)	LOW DOSE	MID DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	24 (100%)	24 (100%)	24 (100%)	24 (100%)
ANIMALS WITH TUMORS	18 (75%)	19 (79%)	17 (71%)	20 (83%)
INTEGUMENTARY SYSTEM*		2 (8%)	1 (4%)	1 (4%)
SKIN		2	1	1
SQUAMOUS CELL CARCINOMA		2		
SEBACEOUS ADENOCARCINOMA			1	
SEBACEOUS ADENOMA				1
RESPIRATORY SYSTEM	1 (4%)	1 (4%)	3 (13%)	8 (33%)
NASAL CAVITY			1	
UNDIFFERENTIATED CARCINOMA			1	
LUNG	1	1	3	8
UNDIFFERENTIATED CARCINOMA METAS			2	
TRANSIT-CELL CARCINOMA METASTAT				5
ALVEOLAR/BRONCHIOLAR ADENOMA			1	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1		3
ADENOCARCINOMA METASTATIC	1			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM	2 (8%)	3 (13%)	2 (8%)	2 (8%)
SALIVARY GLAND			1	
UNDIFFERENTIATED CARCINOMA METAS			1	
LIVER	1	3	1	1
NEOPLASTIC NODULE	1	3	1	1
SMALL INTESTINE	1			1
ADENOCARCINOMA	1			1
CECUM			1	
UNDIFFERENTIATED CARCINOMA			1	

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table A2. Female Rats (Continued)

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): SRI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL (VEH)	LOW DOSE	MID DOSE	HIGH DOSE
URINARY SYSTEM*	1 (4%)		2 (8%)	13 (54%)
KIDNEY	1		1	4
UNDIFFERENTIATED CARCINOMA METAS			1	
ADENOCARCINOMA METASTATIC	1			
TUBULAR-CELL ADENOCARCINOMA				1
TUBULAR-CELL ADENOMA				3
URETER				6
TRANSITIONAL-CELL CARCINOMA				6
URINARY BLADDER			1	5
PAPILLOMA			1	
TRANSITIONAL-CELL CARCINOMA				5
ENDOCRINE SYSTEM	3 (13%)	8 (33%)	5 (21%)	4 (17%)
PITUITARY	2	4	3	2
CHROMOPHOBE ADENOMA	2	4	3	2
ADRENAL	1	1	2	1
PHEOCHROMOCYTOMA	1	1	2	1
THYROID		3		
C-CELL CARCINOMA		2		
C-CELL ADENOMA		1		
PANCREATIC ISLETS				1
ISLET-CELL ADENOMA				1
HEMATOPOIETIC SYSTEM	5 (21%)	4 (17%)	7 (29%)	4 (17%)
MULTIPLE ORGANS	4	4	7	3
MALIGNANT LYMPHOMA	3	1	4	3
MALIG.LYMPHOMA LYMPHOCYTIC TYPE		1	1	
LEUKEMIA GRANULOCYTIC	1		2	
LEUKEMIA		2		
LYMPH NODE				1
TRANSIT-CELL CARCINOMA METASTAT				1
LIVER	1			
MALIGNANT LYMPHOMA	1			

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table A2. Female Rats (Continued)

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): SRI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(VEH)	LOW DOSE	MID DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM*	11 (46%)	11 (46%)	7 (29%)	3 (13%)
MAMMARY GLAND	3	2	2	1
ADENOMA	1		1	
FIBROADENOMA	2	2	1	1
CLITORAL GLAND	1			
SEBACEOUS ADENOCARCINOMA	1			
UTERUS	8	11	6	2
ADENOCARCINOMA	2	2		
ENDOMETRIAL STROMAL POLYP	6	10	6	2
LEIOMYOMA		1		
CERVIX UTERI	1			
ENDOMETRIAL STROMAL POLYP	1			
NERVOUS SYSTEM	1 (4%)	1 (4%)		1 (4%)
BRAIN	1	1		1
ASTROCYTOMA	1			1
MENINGIOMA		1		
MUSCULOSKELETAL SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
ALL OTHER SYSTEMS				
NONE				
TUMOR SUMMARY				
TOTAL ANIMALS WITH BENIGN TUMORS	11 (46%)	13 (54%)	12 (50%)	9 (33%)
TOTAL BENIGN TUMORS	13	19	15	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	9 (38%)	8 (33%)	10 (42%)	16 (67%)
TOTAL MALIGNANT TUMORS	10	12	10	20
TOTAL ANIMALS WITH METASTATIC TUMORS	1 (4%)		2 (8%)	5 (21%)
TOTAL METASTATIC TUMORS	2		4	6

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS AND OTHER PROLIFERATIVE LESIONS IN RATS AND MICE FED NTA IN THE DIET (LBI)

Table B1. Male Rats

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL (UNTP)	LOW DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	20(100%)	50(100%)	50(100%)
ANIMALS WITH TUMORS	19 (95%)	43 (86%)	47 (94%)
INTEGUMENTARY SYSTEM*	2 (10%)	4 (8%)	4 (8%)
SKIN	1	2	3
PAPILLOMA		1	
BASAL CELL CARCINOMA	1		
ADNEXAL ADENOMA			1
ADNEXAL CARCINOMA		1	
KERATOACANTHOMA			1
SARCOMA,NOS			1
SUBCUT TISSUE	1	2	1
FIBROMA		1	
FIBROSARCOMA	1		
HEMANGIOMA			1
KERATOACANTHOMA		1	
RESPIRATORY SYSTEM	1 (5%)	5 (10%)	6 (12%)
LUNG	1	5	6
ALVEOLAR/BRONCHIOLAR ADENOMA		1	1
ALVEOLAR/BRONCHIOLAR CARCINOMA	1	4	4
SARCOMA METASTATIC			1
CIRCULATORY SYSTEM			1 (2%)
MYOCARDIUM			1
SARCOMA METASTATIC			1
DIGESTIVE SYSTEM	3 (15%)	5 (10%)	3 (6%)
LIVER	3	5	2
NEOPLASTIC NODULE	3	2	2
HEPATOCELLULAR CARCINOMA		3	
STOMACH			1
PAPILLOMA			1
URINARY SYSTEM		1 (2%)	7 (14%)
KIDNEY		1	5
TUBULAR-CELL ADENOMA		1	3
TUBULAR-CELL ADENOCARCINOMA			2
URETER			3
PAPILLOMA			2
PAPILLARY ADENOMA			1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table B1. Male Rats (Continued)

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM*	2 (10%)	16 (32%)	8 (16%)
PITUITARY	1	6	2
CHROMOPHOBE ADENOMA	1	5	2
BASOPHIL ADENOMA		1	
ADRENAL	1	9	5
PHEOCHROMOCYTOMA	1	9	5
THYROID		2	1
FOLLICULAR-CELL ADENOMA		1	1
FOLLICULAR-CELL CARCINOMA		1	
PANCREATIC ISLETS			1
ISLET-CELL ADENOMA			1
HEMATOPOIETIC SYSTEM	1 (5%)	3 (6%)	2 (4%)
MULTIPLE ORGANS	1	3	1
LEUKEMIA	1	3	
ERYTHROCYTIC LEUKEMIA			1
LIVER			
ERYTHROCYTIC LEUKEMIA			1
REPRODUCTIVE SYSTEM	17 (85%)	42 (84%)	45 (90%)
PREPUTIAL GLAND		2	4
ADENOCARCINOMA		2	4
TESTIS	16	42	45
INTERSTITIAL-CELL TUMOR	16	42	45
EPIDIDYMIS	1		
HEMANGIOMA	1		
NERVOUS SYSTEM			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS	1 (5%)		
EYELID	1		
SARCOMA,NOS	1		

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table B1. Male Rats (Continued)

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS*			1 (2%)
MULTIPLE ORGANS			1
TUBULAR-CELL ADENOCARCINOMA META			1
TUMOR SUMMARY			
TOTAL ANIMALS WITH BENIGN TUMORS	18 (90%)	43 (86%)	46 (92%)
TOTAL BENIGN TUMORS	19	63	65
TOTAL ANIMALS WITH MALIGNANT TUMORS	5 (25%)	13 (26%)	12 (24%)
TOTAL MALIGNANT TUMORS	5	14	13
TOTAL ANIMALS WITH METASTATIC TUMORS			2 (4%)
TOTAL METASTATIC TUMORS			3

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table B2. Female Rats

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	20(100%)	50(100%)	50(100%)
ANIMALS WITH TUMORS	14 (70%)	33 (66%)	47 (94%)
INTEGUMENTARY SYSTEM*	2 (10%)	1 (2%)	
SKIN	2		
BASAL CELL CARCINOMA	1		
CARCINOSARCOMA	1		
SUBCUT TISSUE	1	1	
SQUAMOUS CELL CARCINOMA		1	
CARCINOSARCOMA	1		
RESPIRATORY SYSTEM	1 (5%)	4 (8%)	7 (14%)
LUNG	1	4	7
SQUAMOUS CELL CARCINOMA METASTAT		1	
ALVEOLAR/BRONCHIOLAR CARCINOMA		3	7
CARCINOSARCOMA METASTAT	1		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM	2 (10%)	8 (16%)	22 (44%)
LIVER	2	8	22
NEOPLASTIC NODULE	2	8	22
PANCREAS			1
ACINAR-CELL ADENOMA			1
URINARY SYSTEM		2 (4%)	14 (28%)
KIDNEY			2
TRANSIT-CELL PAPILLOMA			1
TUBULAR-CELL ADENOMA			1
URINARY BLADDER		2	12
SQUAMOUS CELL CARCINOMA			1
TRANSITIONAL-CELL CARCINOMA		2	11
ENDOCRINE SYSTEM	6 (30%)	13 (26%)	25 (50%)
PITUITARY	6	10	12
CHROMOPHOBE ADENOMA	6	9	11
BASOPHIL ADENOMA		1	1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table B2. Female Rats (Continued)

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
ADRENAL	1		14
PHEOCHROMOCYTOMA	1		14
THYROID	3	3	3
C-CELL ADENOMA	2	2	2
C-CELL CARCINOMA	1	1	1
HEMATOPOIETIC SYSTEM*	3 (15%)	6 (12%)	6 (12%)
MULTIPLE ORGANS	3	4	6
MALIGNANT LYMPHOMA		1	
MALIG.LYMPHOMA LYMPHOCYTIC TYPE			1
MALIG.LYMPHOMA HISTIOCYTIC TYPE		1	
LEUKEMIA	3	2	3
ERYTHROCYTIC LEUKEMIA			2
SUBMANDIBULAR L.NODE		1	
SQUAMOUS CELL CARCINOMA METASTAT		1	
PANCREATIC L.NODE		1	
MALIG.LYMPHOMA HISTIOCYTIC TYPE		1	
LUNG		1	
MALIG.LYMPHOMA HISTIOCYTIC TYPE		1	
REPRODUCTIVE SYSTEM	7 (35%)	15 (30%)	7 (14%)
MAMMARY GLAND	6	4	2
ADENOCARCINOMA		1	
CYSTADENOMA	3		1
FIBROADENOMA	3	3	1
MAMMARY DUCT		3	
CARCINOMA		1	
CYSTADENOCARCINOMA		1	
FIBROADENOMA		1	
CLITORAL GLAND	1	2	
ADENOCARCINOMA	1	2	
UTERUS	2	7	4
ADENOCARCINOMA			2
MULTIPLE POLYPOSIS		2	
ENDOMETRIAL STROMAL POLYP	2	4	2
ENDOMETRIAL STROMAL SARCOMA		1	
OVARY			1
FIBROSARCOMA			1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table B2. Female Rats (Continued)

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM*		1 (2%)	2 (4%)
CEREBRUM			1
ASTROCYTOMA			1
BRAIN		1	1
GLIOMA		1	1
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS	1 (5%)		
EAR CANAL	1		
SQUAMOUS CELL CARCINOMA	1		
ALL OTHER SYSTEMS			
NONE			
TUMOR SUMMARY			
TOTAL ANIMALS WITH BENIGN TUMORS	9 (45%)	18 (36%)	27 (54%)
TOTAL BENIGN TUMORS	17	23	35
TOTAL ANIMALS WITH MALIGNANT TUMORS	7 (35%)	16 (32%)	25 (50%)
TOTAL MALIGNANT TUMORS	8	20	31
TOTAL ANIMALS WITH METASTATIC TUMORS	1 (5%)	1 (2%)	
TOTAL METASTATIC TUMORS	1	2	

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table B3. Male Mice

NUMBER OF MALE MICE WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	20(100%)	49(100%)	44(100%)
ANIMALS WITH TUMORS	10 (50%)	14 (29%)	31 (70%)
INTEGUMENTARY SYSTEM*			2 (5%)
SUBCUT TISSUE			2
HEMANGIOMA			2
RESPIRATORY SYSTEM	4 (20%)	5 (10%)	6 (14%)
LUNG	4	5	6
ALVEOLAR/BRONCHIOLAR ADENOMA		3	2
ALVEOLAR/BRONCHIOLAR CARCINOMA	4	2	4
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM	3 (15%)	4 (8%)	2 (5%)
LIVER	3	4	2
HEPATOCELLULAR ADENOMA	3	1	
HEPATOCELLULAR CARCINOMA		2	2
HAMARTOMA		1	
URINARY SYSTEM		5 (10%)	25 (55%)
KIDNEY		5	23
TUBULAR-CELL ADENOMA			1
TUBULAR-CELL ADENOCARCINOMA		5	22
KIDNEY/PELVIS			1
PAPILLOMA			1
ENDOCRINE SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM	2 (10%)	4 (8%)	6 (14%)
MULTIPLE ORGANS	1	4	4
MALIGNANT LYMPHOMA			1
LEUKEMIA	1	2	3
LEUKEMIA LYMPHOCYTIC		2	
SPLEEN	1		
HEMANGIOMA	1		

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

NUMBER OF MALE MICE WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
PANCREATIC L.NODE			1
MALIGNANT LYMPHOMA			1
MESENTERIC LYMPHNODE			1
MALIGNANT LYMPHOMA			1
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS*	1 (5%)		
PELVIS	1		
HEMANGIOMA	1		
TUMOR SUMMARY			
TOTAL ANIMALS WITH BENIGN TUMORS	5 (25%)	5 (10%)	5 (11%)
TOTAL BENIGN TUMORS	5	5	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	5 (25%)	11 (22%)	28 (64%)
TOTAL MALIGNANT TUMORS	5	13	34
TOTAL ANIMALS WITH METASTATIC TUMORS			
TOTAL METASTATIC TUMORS			

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table B4. Female Mice

NUMBER OF FEMALE MICE WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	20(100%)	39(100%)	50(100%)
ANIMALS WITH TUMORS	4 (20%)	13 (33%)	19 (38%)
INTEGUMENTARY SYSTEM*			1 (2%)
SKIN			1
HEMANGIOMA			1
RESPIRATORY SYSTEM		2 (5%)	4 (8%)
LUNG		2	4
ALVEOLAR/BRONCHIOLAR ADENOMA			2
ALVEOLAR/BRONCHIOLAR CARCINOMA		2	2
CIRCULATORY SYSTEM		1 (3%)	
HEART		1	
NONCHROMAFFIN PARAGANGLIOMA		1	
DIGESTIVE SYSTEM		2 (5%)	1 (2%)
LIVER		2	
HEPATOCELLULAR CARCINOMA		1	
HEMANGIOMA		1	
ESOPHAGUS			1
SQUAMOUS CELL CARCINOMA			1
URINARY SYSTEM			4 (8%)
KIDNEY			4
TUBULAR-CELL ADENOCARCINOMA			4
ENDOCRINE SYSTEM		1 (3%)	
PITUITARY		1	
CHROMOPHOBE ADENOMA		1	
HEMATOPOIETIC SYSTEM	4 (20%)	7 (18%)	10 (20%)
MULTIPLE ORGANS	3	7	7
MALIGNANT LYMPHOMA	1	3	5
LEUKEMIA	2	3	1
LEUKEMIA LYMPHOCYTIC		1	1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table B4. Female Mice (Continued)

NUMBER OF FEMALE MICE WITH TUMORS BY ANATOMIC SITE(NTA): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
SPLEEN	1		
MALIGNANT LYMPHOMA	1		
MEDIASTINAL L.NODE			1
MALIGNANT LYMPHOMA			1
MESENTERIC LYMPHNODE			2
MALIGNANT LYMPHOMA			2
REPRODUCTIVE SYSTEM*		1 (3%)	1 (2%)
UTERUS		1	1
ADENOMA		1	
LEIOMYOMA			1
NERVOUS SYSTEM			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS		1 (3%)	
UTERINE LIGAMENT		1	
HEMANGIOMA		1	
TUMOR SUMMARY			
TOTAL ANIMALS WITH BENIGN TUMORS		4 (10%)	4 (8%)
TOTAL BENIGN TUMORS		5	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	4 (20%)	10 (26%)	16 (32%)
TOTAL MALIGNANT TUMORS	4	10	17
TOTAL ANIMALS WITH METASTATIC TUMORS			
TOTAL METASTATIC TUMORS			

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

APPENDIX C

SUMMARY OF THE INCIDENCE OF NEOPLASMS AND OTHER PROLIFERATIVE LESIONS IN RATS AND MICE FED $Na_3NTA \cdot H_2O$ IN THE DIET (LBI)

Table C1. Male Rats

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	20(100%)	49(100%)	50(100%)
ANIMALS WITH TUMORS	20(100%)	48 (98%)	44 (88%)
INTEGUMENTARY SYSTEM *		2 (4%)	2 (4%)
SKIN		1	
SQUAMOUS CELL CARCINOMA		1	
SUBCUT TISSUE		1	2
FIBROMA		1	1
SARCOMA,NOS			1
RESPIRATORY SYSTEM	2 (10%)	12 (24%)	3 (6%)
LUNG	2	12	3
SQUAMOUS CELL CARCINOMA METASTAT		1	
ALVEOLAR/BRONCHIOLAR ADENOMA	1	2	2
ALVEOLAR/BRONCHIOLAR CARCINOMA	1	8	1
SARCOMA METASTATIC		1	
CIRCULATORY SYSTEM			1 (2%)
HEART			1
SARCOMA,NOS			1
DIGESTIVE SYSTEM	2 (10%)	1 (2%)	3 (6%)
SALIVARY GLAND			1
MIXED TUMOR MALIGNANT			1
LIVER	1		1
NEOPLASTIC NODULE	1		1
STOMACH	1		
PAPILLOMA	1		
SMALL INTESTINE		1	1
ADENOCARCINOMA		1	1
URINARY SYSTEM		4 (8%)	2 (4%)
KIDNEY		3	1
TUBULAR-CELL ADENOCARCINOMA		1	1
SARCOMA METASTATIC		1	
HAMARTOMA		1	
KIDNEY/PELVIS			1
TRANSIT-CELL PAPILLOMA			1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL (UNTF)	LOW DOSE	HIGH DOSE
URETER		1	
PAPILLOMA		1	
ENDOCRINE SYSTEM*	9 (40%)	11 (22%)	11 (22%)
PITUITARY	2	3	1
CHROMOPHOBE ADENOMA	2	3	1
ADRENAL	4	3	5
PHEOCHROMOCYTOMA	4	3	5
THYROID	2	2	5
C-CELL ADENOMA	2	1	2
C-CELL CARCINOMA		1	2
FIBROSARCOMA			1
PANCREATIC ISLETS		3	1
ISLET-CELL ADENOMA		3	1
HEMATOPOIETIC SYSTEM	3 (15%)	1 (2%)	6 (12%)
MULTIPLE ORGANS	2		5
LEUKEMIA	2		4
ERYTHROCYTIC LEUKEMIA			1
MALIG.LYMPHOMA HISTIOCYTIC TYPE		1	
SPLEEN	1		1
SARCOMA			1
MALIG.LYMPHOMA HISTIOCYTIC TYPE	1		
REPRODUCTIVE SYSTEM	20 (100%)	45 (92%)	41 (92%)
MAMMARY GLAND			1
FIBROADENOMA			1
PREPUTIAL GLAND	1	1	
ADENOCARCINOMA	1	1	
TESTIS	20	45	41
INTERSTITIAL-CELL TUMOR	20	45	41
EPIDIDYMIS		1	
HEMANGIOMA		1	
NERVOUS SYSTEM		1 (2%)	1 (2%)
BRAIN		1	1
ASTROCYTOMA		1	
OLIGODENDROGLIOMA			1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table C1. Male Rats (Continued)

NUMBER OF MALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS*		2 (4%)	1 (2%)
MULTIPLE ORGANS		1	1
MESOTHELIOMA		1	1
PERITONEUM		1	
MESOTHELIOMA		1	
TUMOR SUMMARY			
TOTAL ANIMALS WITH BENIGN TUMORS	20 (100%)	47 (96%)	42 (84%)
TOTAL BENIGN TUMORS	30	61	55
TOTAL ANIMALS WITH MALIGNANT TUMORS	5 (25%)	15 (31%) **	13 (26%)
TOTAL MALIGNANT TUMORS	5	16	16
TOTAL ANIMALS WITH METASTATIC TUMORS		2 (4%)	
TOTAL METASTATIC TUMORS		3	

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE
** ANIMAL #108 OF GROUP 11-1203 HAS A PRIMARY SARCOMA WHICH THE LITTON BIONETICS LABORATORY WAS UNABLE TO FIND.

Table C2. Female Rats

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	20(100%)	50(100%)	49(100%)
ANIMALS WITH TUMORS	13 (65%)	36 (72%)	26 (53%)
INTEGUMENTARY SYSTEM *		2 (4%)	
SUBCUT TISSUE		2	
FIBROMA		1	
LIPOMA		1	
RESPIRATORY SYSTEM	1 (5%)	5 (10%)	3 (6%)
LUNG	1	5	3
ALVEOLAR/BRONCHIOLAR ADENOMA	1	1	1
ALVEOLAR/BRONCHIOLAR CARCINOMA		4	2
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM		2 (4%)	2 (4%)
LIVER		1	1
NEOPLASTIC NODULE		1	1
ESOPHAGUS			1
SQUAMOUS CELL CARCINOMA			1
COLON		1	
ADENOMATOUS POLYP		1	
URINARY SYSTEM		4 (8%)	2 (4%)
URINARY BLADDER		4	2
PAPILLOMA			1
SQUAMOUS CELL CARCINOMA		1	
TRANSITIONAL-CELL CARCINOMA		3	1
ENDOCRINE SYSTEM	7 (35%)	19 (38%)	13 (27%)
PITUITARY	3	16	13
ADENOMA			1
CHROMOPHOBE ADENOMA	3	15	12
BASOPHIL ADENOMA		1	
ADRENAL	1		
PHEOCHROMOCYTOMA	1		

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table C2. Female Rats (Continued)

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
THYROID	2	4	2
FOLLICULAR-CELL ADENOMA		1	
C-CELL ADENOMA	1	4	1
C-CELL CARCINOMA	1		1
PANCREATIC ISLETS	1		
ISLET-CELL ADENOMA	1		
HEMATOPOIETIC SYSTEM*	2 (10%)	1 (2%)	5 (10%)
MULTIPLE ORGANS	2	1	5
MALIGNANT LYMPHOMA			1
LEUKEMIA	2	1	4
REPRODUCTIVE SYSTEM	8 (40%)	18 (36%)	8 (16%)
MAMMARY GLAND	4	10	3
SQUAMOUS CELL CARCINOMA			1
ADENOMA		1	
ADENOCARCINOMA			1
CYSTADENOMA		1	
FIBROMA		1	
FIBROADENOMA	4	7	1
CLITORAL GLAND	1	2	1
ADENOCARCINOMA	1	2	1
UTERUS	4	7	4
ENDOMETRIAL STROMAL POLYP	4	7	4
NERVOUS SYSTEM			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS			
NONE			

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

NUMBER OF FEMALE RATS WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH BENIGN TUMORS	10 (50%)	29 (58%)	17 (35%)
TOTAL BENIGN TUMORS	15	42	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	4 (20%)	11 (22%)	12 (24%)
TOTAL MALIGNANT TUMORS	4	11	13
TOTAL ANIMALS WITH METASTATIC TUMORS			
TOTAL METASTATIC TUMORS			

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table C3. Male Mice

NUMBER OF MALE MICE WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	20(100%)	48(100%)	50(100%)
ANIMALS WITH TUMORS	7 (35%)	18 (38%)	14 (28%)
INTEGUMENTARY SYSTEM *		1 (2%)	1 (2%)
SKIN		1	1
SEBACEOUS ADENOMA			1
FIBROSARCOMA		1	
RESPIRATORY SYSTEM	3 (15%)	9 (19%)	
LUNG	3	9	
HEPATOCELLULAR CARCINOMA METASTA	1		
ALVEOLAR/BRONCHIOLAR ADENOMA	1	1	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1	6	
NONCHROMAFFIN PARAGANGLIOMA		1	
PHEOCHROMOCYTOMA METASTATIC		1	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM	5 (25%)	6 (13%)	4 (8%)
LIVER	4	6	4
HEPATOCELLULAR ADENOMA	3		
HEPATOCELLULAR CARCINOMA	1	5	3
NONCHROMAFFIN PARAGANGLIOMA		1	
SARCOMA,UNDIFFERENTIATED			1
SMALL INTESTINE	1		
ADENOCARCINOMA	1		
URINARY SYSTEM		1 (2%)	
KIDNEY		1	
NONCHROMAFFIN PARAGANGLIOMA		1	
ENDOCRINE SYSTEM	1 (5%)	4 (8%)	
ADRENAL		3	
CORTICAL CARCINOMA		1	
NONCHROMAFFIN PARAGANGLIOMA		1	
PHEOCHROMOCYTOMA MALIGNANT		1	
THYROID	1	1	
FOLLICULAR-CELL ADENOMA	1	1	

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table C3. Male Mice (Continued)

NUMBER OF MALE MICE WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM*		4 (8%)	9 (18%)
MULTIPLE ORGANS		1	5
MALIGNANT LYMPHOMA		1	1
LEUKEMIA			4
SPLEEN		1	1
HEMANGIOMA		1	1
MESENTERIC LYMPHNODE		1	3
MALIGNANT LYMPHOMA		1	3
LUNG			1
MALIGNANT LYMPHOMA			1
KIDNEY		1	
MALIGNANT LYMPHOMA		1	
REPRODUCTIVE SYSTEM			1 (2%)
TESTIS			1
INTERSTITIAL-CELL TUMOR			1
NERVOUS SYSTEM			1 (2%)
BRAIN STEM			1
SARCOMA,UNDIFFERENTIATED			1
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS			1 (2%)
MESENTERY			1
HEMANGIOMA			1
AUTOLYSIS/NO NECROPSY PERFORMED		2	

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table C3. Male Mice (Continued)

NUMBER OF MALE MICE WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH BENIGN TUMORS	4 (20%)	4 (8%)	4 (8%)
TOTAL BENIGN TUMORS	5	4	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	3 (15%)	15 (31%)	12 (24%)
TOTAL MALIGNANT TUMORS	3	17	13
TOTAL ANIMALS WITH METASTATIC TUMORS	1 (5%)	1 (2%)	
TOTAL METASTATIC TUMORS	1	1	

Table C4. Female Mice

NUMBER OF FEMALE MICE WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
EFFECTIVE NUMBER OF ANIMALS	18(100%)	46(100%)	47(100%)
ANIMALS WITH TUMORS	6 (33%)	11 (24%)	16 (34%)
INTEGUMENTARY SYSTEM *		1 (2%)	
SKIN		1	
FIBROSARCOMA		1	
RESPIRATORY SYSTEM	1 (6%)		2 (4%)
LUNG	1		2
ALVEOLAR/BRONCHIOLAR ADENOMA			1
ALVEOLAR/BRONCHIOLAR CARCINOMA	1		1
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM	2 (11%)	3 (6%)	3 (6%)
LIVER	2	3	3
HEPATOCELLULAR ADENOMA		1	
HEPATOCELLULAR CARCINOMA	2	2	2
SARCOMA METASTATIC			1
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM		1 (2%)	3 (6%)
PITUITARY		1	1
CHROMOPHOBE ADENOMA		1	1
ADRENAL			1
PHEOCHROMOCYTOMA			1
THYROID			1
FOLLICULAR-CELL CARCINOMA			1
HEMATOPOIETIC SYSTEM	3 (17%)	8 (17%)	9 (19%)
MULTIPLE ORGANS	3	8	8
MALIGNANT LYMPHOMA		1	4
LEUKEMIA	3	7	4
SPLEEN			1
MALIGNANT LYMPHOMA			1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

Table C4. Female Mice (Continued)

NUMBER OF FEMALE MICE WITH TUMORS BY ANATOMIC SITE(NTA.NA3.H2O): LBI

(PERCENTAGES BY SYSTEM ARE BASED ON THE EFFECTIVE NUMBER OF ANIMALS)

	CONTROL(UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM*			1 (2%)
UTERUS			1
SARCOMA,NOS			1
NERVOUS SYSTEM			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS			
NONE			
TUMOR SUMMARY			
TOTAL ANIMALS WITH BENIGN TUMORS		2 (4%)	3 (6%)
TOTAL BENIGN TUMORS		2	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	6 (33%)	11 (24%)	13 (28%)
TOTAL MALIGNANT TUMORS	6	11	14
TOTAL ANIMALS WITH METASTATIC TUMORS			1 (2%)
TOTAL METASTATIC TUMORS			1

* COLUMNS ARE OFFSET ACCORDING TO ORGAN SYSTEM, SPECIFIC ORGAN AND TUMOR TYPE

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS FED $NA_3NTA \cdot H_2O$ IN THE DIET (SRI)

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED $NA_3NTA \cdot H_2O$ IN THE DIET

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	24	24	24	24
ANIMALS NECROPSIED	24 (100%)	24 (100%)	24 (100%)	24 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	24	23	24	24
ANIMALS WITH NON-TUMOR PATHOLOGY	24 (100%)	23 (96%)	24 (100%)	24 (100%)
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM *	15 (63%)	17 (74%)	15 (63%)	18 (75%)
TRACHEA	7	5	8	4
INFLAMMATION	7	5	8	4
LUNG/BRONCHUS	4	10	7	1
BRONCHIECTASIS	4	2	3	
LYMPHOID HYPERPLASIA	1	9	5	1
LUNG	11	8	10	15
MINERALIZATION	1			
EMPHYSEMA	2	1	2	
ATELECTASIS	1	1	1	
CONGESTION	2			
EDEMA		1		
HEMORRHAGE		1		
INFLAMMATION				13
INFLAMMATION INTERSTITIAL	4	3	5	1
ABSCESS		1	2	
HYPERPLASIA ADENOMATOUS				4
HYPERPLASIA ALVEOLAR-CELL	2			
CIRCULATORY SYSTEM	24 (100%)	23 (100%)	24 (100%)	22 (92%)
EPICARDIUM	1			
INFLAMMATION	1			
HEART				3
FIBROSIS				3

TABLE D1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
MYOCARDIUM	24	23	24	19
INFLAMMATION	2			3
FIBROSIS DIFFUSE	23	23	24	17
PULMONARY ARTERY		1		
THROMBOSIS		1		
DIGESTIVE SYSTEM	17 (71%)	18 (78%)	22 (92%)	10 (42%)
LIVER	5	8	8	
GRANULOMA	4	6	6	
FIBROSIS FOCAL			1	
PELIOSIS HEPATIS	1			
NECROSIS FOCAL		1		
BASOPHILIC CYTOPLASM ALTERATION			1	
HEMATOPOIESIS		1		
LIVER/HEPATOCYTES			1	
CYTOPLASMIC VACUOLIZATION			1	
BILE DUCT	12	12	19	5
INFLAMMATION	3	6	7	5
HYPERPLASIA	11	11	15	
PANCREAS	3	4	4	6
INFLAMMATION			1	
INFLAMMATION CHRONIC	3	4	3	
FIBROSIS				3
FIBROSIS DIFFUSE				3
STOMACH	4	1	3	1
INFLAMMATION	2		1	1
ULCER	1	1	3	
INFLAMMATION CHRONIC	1			
GASTRIC MUCOSA			2	
EROSIVE INFLAMMATION			2	

TABLE D1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
URINARY SYSTEM	17 (71%)	19 (83%)	24 (100%)	24 (100%)
KIDNEY	17	19	24	24
HYDRONEPHROSIS				18
INFLAMMATION		1	2	4
INFLAMMATION CHRONIC	17	18	22	
NEPHROSIS				2
HYPERPLASIA				1
HYPERPLASIA TUBULAR-CELL				20
KIDNEY/PELVIS	1		3	7
HYPERPLASIA EPITHELIAL	1		3	7
URETER		1	4	3
HYPERPLASIA EPITHELIAL		1	4	3
URINARY BLADDER		3	5	8
HYPERPLASIA EPITHELIAL		3	3	8
DYSPLASIA EPITHELIAL		1	4	
ENDOCRINE SYSTEM	4 (17%)	5 (22%)	6 (25%)	1 (4%)
ADRENAL CORTEX	2	5	6	
CYTOPLASMIC VACUOLIZATION	2	4	6	
HYPERPLASTIC NODULE		1		
ADRENAL MEDULLA	1			
HYPERPLASIA FOCAL	1			
THYROID	1	1		1
COLLOID CYST				1
HYPERPLASIA C-CELL		1		
HYPERPLASIA FOLLICULAR-CELL	1			
HEMATOPOIETIC SYSTEM	22 (92%)	17 (71%)	23 (96%)	23 (96%)
SPLEEN	22	17	23	23
HEMOSIDEROSIS	20	17	23	16

TABLE D1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
SPLEEN (CONT.)				
LYMPHOID HYPERPLASIA				1
HEMATOPOIESIS	21	15	22	20
MANDIBULAR L. NODE	1			
GRANULOMA	1			
MESENTERIC LYMPHNODE	1			
ATROPHY	1			
REPRODUCTIVE SYSTEM	3 (13%)	2 (9%)	3 (13%)	
MAMMARY GLAND	1			
HYPERPLASIA	1			
PROSTATE	1	2	3	
INFLAMMATION	1	2	3	
SEMINAL VESICLE	1			
DISTENTION	1			
NERVOUS SYSTEM		1 (4%)		
BRAIN		1		
HEMORRHAGE		1		
MUSCULOSKELETAL SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

TABLE D1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ALL OTHER SYSTEMS	10 (42%)	4 (17%)	10 (42%)	8 (33%)
ADIPOSE TISSUE	10	4	10	8
NECROSIS FAT	10	4	10	8
AUTOLYSIS/NECROPSY PERF/NO HISTO PERF		1		
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	24	24	24	24
NATURAL DEATH*		5		
MORIBUND SACRIFICE	6	3		18
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	18	16	24	6
ANIMAL MISSING				

* INCLUDES AUTOLYZED ANIMALS

* SYSTEM PERCENTAGES ARE BASED ON NUMBER OF ANIMALS NECROPSIED.

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED $NA_3NTA \cdot H_2O$ IN THE DIET

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	24	24	24	24
ANIMALS NECROPSIED	24 (100%)	24 (100%)	24 (100%)	24 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	24	24	24	24
ANIMALS WITH NON-TUMOR PATHOLOGY	23 (96%)	23 (96%)	24 (100%)	23 (96%)
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM *	11 (46%)	11 (46%)	7 (29%)	13 (54%)
TRACHEA	6	7	5	9
INFLAMMATION	6	7	5	9
LUNG/BRONCHUS	4	1	1	10
BRONCHIECTASIS	1	1		
LYMPHOID HYPERPLASIA	3		1	10
LUNG	4	4	2	1
EDEMA			1	
INFLAMMATION				1
INFLAMMATION INTERSTITIAL	3	3	1	
ABSCESS	1	1		
LUNG/ALVEOLI				1
PHAGOCYTIC CELL				1
CIRCULATORY SYSTEM	23 (96%)	23 (96%)	24 (100%)	6 (25%)
HEART			1	4
MINERALIZATION				1
FIBROSIS				3
FIBROSIS FOCAL			1	
MYOCARDIUM	23	23	23	2
INFLAMMATION	1	1		
FIBROSIS DIFFUSE	23	23	23	2

TABLE D2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
DIGESTIVE SYSTEM	19 (79%)	17 (71%)	19 (79%)	17 (71%)
LIVER	11	8	12	8
INFLAMMATION GRANULOMATOUS			1	1
GRANULOMA	9	7	8	6
FIBROSIS FOCAL			1	
NECROSIS FOCAL			1	1
BASOPHILIC CYTOPLASM ALTERATION	2	1	3	
BILE DUCT	16	11	11	15
INFLAMMATION	13	9	6	14
HYPERPLASIA	10	6	6	11
PANCREAS	8	3	1	1
INFLAMMATION	2			
INFLAMMATION CHRONIC	6	3	1	1
STOMACH	1			
INFLAMMATION	1			
COLON	2	1		
INFLAMMATION	2	1		
CECUM		1		
INFLAMMATION		1		
URINARY SYSTEM	5 (21%)	5 (21%)	16 (67%)	24 (100%)
KIDNEY	3	3	5	21
HYDRONEPHROSIS				20
INFLAMMATION		2	1	
INFLAMMATION CHRONIC	3	1	1	
HYPERPLASIA TUBULAR-CELL				11
HEMOSIDEROSIS			3	
KIDNEY/CORTEX		1		
FIBROSIS		1		
RENAL TUBULE				11
HYPERPLASIA				11
KIDNEY/PELVIS	1	2		10
HYPERPLASIA EPITHELIAL	1	2		10
URETER				5
HYPERPLASIA EPITHELIAL				5

TABLE D2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
URETER (CONT.)				
DYSPLASIA				2
URINARY BLADDER	1	1	15	18
EDEMA				1
HYPERPLASIA EPITHELIAL	1	1	13	14
HYPERPLASIA FOCAL			1	
DYSPLASIA EPITHELIAL		1	3	8
ENDOCRINE SYSTEM	3 (13%)	4 (17%)	6 (25%)	6 (25%)
PITUITARY		1		
CYST		1		
ADRENAL	1		3	
FIBROSIS FOCAL			2	
ANGIECTASIS	1		1	
ADRENAL CORTEX	2	3	3	
CYST		1		
FIBROSIS FOCAL		1	2	
NECROSIS FOCAL			1	
CYTOPLASMIC VACUOLIZATION	1			
HYPERPLASIA FOCAL	1	1		
THYROID			1	5
HYPERPLASIA C-CELL			1	5
THYROID FOLLICLE				1
HYPERPLASIA				1
HEMATOPOIETIC SYSTEM	18 (75%)	19 (79%)	16 (67%)	18 (75%)
SPLEEN	17	18	16	18
HEMOSIDEROSIS	17	17	16	12
HEMATOPOIESIS	17	18	13	17
MANDIBULAR L. NODE	1			
ATROPHY	1			

TABLE D2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
MESENTERIC LYMPHNODE		1		
LYMPHANGIECTASIS		1		
REPRODUCTIVE SYSTEM	7 (29%)	7 (29%)	4 (17%)	7 (29%)
MAMMARY GLAND	1	1		
INFLAMMATION CYSTIC		1		
HYPERPLASIA	1			
MAMMARY GLAND/LOBULE	2		1	
HYPERPLASIA	2		1	
UTERUS			1	3
HYDROMETRA				3
CYST			1	
INFLAMMATION	1			
METAPLASIA SQUAMOUS				1
UTERUS/ENDOMETRIUM	5	4	2	4
INFLAMMATION	4	4	1	
INFLAMMATION SUPPURATIVE				4
INFLAMMATION CYSTIC	1			
HYPERPLASIA			1	
FALLOPIAN TUBE				1
INFLAMMATION				1
OVARY		2		1
CYST				1
INFLAMMATION		1		
INFLAMMATION GRANULOMATOUS		1		
NERVOUS SYSTEM				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

TABLE D2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ALL OTHER SYSTEMS	2 (8%)			
ADIPOSE TISSUE	1			
NECROSIS FAT	1			
OMENTUM	1			
CYST	1			
NO LESION REPORTED		1		
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	24	24	24	24
NATURAL DEATH*	3	1	2	1
MORIBUND SACRIFICE	3	4	6	6
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	18	19	16	17
ANIMAL MISSING				

* INCLUDES AUTOLYZED ANIMALS

* SYSTEM PERCENTAGES ARE BASED ON NUMBER OF ANIMALS NECROPSIED.

APPENDIX E

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS AND MICE FED NTA IN THE DIET (LBI)

TABLE E1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED NTA IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20 (100%)	50 (100%)	50 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
ANIMALS WITH NON-TUMOR PATHOLOGY	19 (95%)	49 (98%)	49 (98%)
INTEGUMENTARY SYSTEM	1 (5%)		
SKIN	1		
EPIDERMAL INCLUSION CYST	1		
RESPIRATORY SYSTEM	6 (30%)	34 (68%)	34 (68%)
TRACHEA	1	1	1
INFLAMMATION		1	1
INFLAMMATION CHRONIC	1		
LUNG/BRONCHUS			1
INFLAMMATION			1

TABLE E1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
LUNG/BRONCHIOLE		1	
HYPERPLASIA ADENOMATOUS		1	
LUNG	5	33	32
BRONCHOPNEUMONIA SUPPURATIVE		1	1
ABSCESS			2
PNEUMONIA CHRONIC MURINE	4	31	29
INFLAMMATION CHRONIC	1	1	1
CIRCULATORY SYSTEM	5 (25%)	18 (36%)	14 (28%)
MYOCARDIUM	5	18	14
INFLAMMATION FOCAL	1		
FIBROSIS	2	13	9
FIBROSIS FOCAL			1
FIBROSIS DIFFUSE	1	1	
DEGENERATION	1	4	4
DIGESTIVE SYSTEM	13 (65%)	13 (26%)	13 (26%)

TABLE E1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SALIVARY GLAND			2
FIBROSIS DIFFUSE			1
HYPERPLASIA			1
LIVER	4	1	2
METAMORPHOSIS FATTY	3	1	1
BASOPHILIC CYTOPLASM ALTERATION	1		
LEUKEMOID REACTION			1
PANCREAS	4	6	2
FIBROSIS		2	
FIBROSIS FOCAL	1		
FIBROSIS DIFFUSE	2	2	1
HYPERPLASIA INTRADUCTAL	1	2	1
PANCREATIC ACINUS	1	1	
ATROPHY	1	1	
SMALL INTESTINE	7	4	7
INFLAMMATION			1
ULCER			1
INFLAMMATION CHRONIC	1		
LYMPHOID HYPERPLASIA	6	4	6

TABLE E1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
COLON	2	1	1
LYMPHOID HYPERPLASIA	2	1	1
URINARY SYSTEM	10 (50%)	46 (92%)	43 (86%)
KIDNEY	10	46	43
INFLAMMATION CHRONIC	9	46	43
TOXIC NEPHROPATHY	1		
HYPERPLASIA RENAL-CELL			1
HYPERPLASIA FOCAL		1	
URETER			1
HYPERPLASIA EPITHELIAL			1
URINARY BLADDER			1
HYPERPLASIA EPITHELIAL			1

TABLE E1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM		2 (4%)	4 (8%)
PITUITARY		1	
HEMATOCYST		1	
ADRENAL			1
LYMPHANGIECTASIS			1
ADRENAL CORTEX			1
HYPERPLASIA NODULAR			1
THYROID		1	2
HYPERPLASIA C-CELL		1	2
HEMATOPOIETIC SYSTEM	8 (40%)	6 (12%)	17 (34%)
BONE MARROW		1	
HYPERPLASIA		1	
SPLEEN	4	2	4
CONGESTION			1
LEUKEMOID REACTION			1
HYPERPLASIA RETICULUM-CELL	2	2	

TABLE E1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
LYMPHOID HYPERPLASIA	2		2
LYMPH NODE	3	3	3
HYPERPLASIA RETICULUM-CELL	1	1	3
LYMPHOID HYPERPLASIA	2	2	
SUBMANDIBULAR LYMPH		2	4
INFLAMMATION HEMORRHAGIC		1	
INFLAMMATION CHRONIC			1
INFLAMMATION CHRONIC SUPPURATIVE			1
LYMPHOID HYPERPLASIA		1	2
CERVICAL LYMPH NODE			1
HYPERPLASIA RETICULUM-CELL			1
MESENTERIC LYMPHNODE	1	1	6
EDEMA			1
INFLAMMATION HEMORRHAGIC			1
INFLAMMATION CHRONIC			1
HYPERPLASIA RETICULUM-CELL		1	3
LYMPHOID HYPERPLASIA	1		

TABLE E1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM	3 (15%)	3 (6%)	2 (4%)
PREPUTIAL GLAND			1
HYPERPLASIA EPITHELIAL			2
PROSTATE	2	2	2
INFLAMMATION CYSTIC		1	
HYPERPLASIA	1		1
HYPERPLASIA EPITHELIAL			1
HYPERPLASIA CYSTIC	1		
HYPERPLASIA INTRADUCTAL		1	
SEMINAL VESICLE	1	1	
INFLAMMATION SUPPURATIVE		1	
HYPERPLASIA CYSTIC	1		
TESTIS	1		
ATROPHY	1		
NERVOUS SYSTEM		1 (2%)	
BRAIN		1	
HYDROCEPHALUS INTERNAL		1	

TABLE E1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS			2 (4%)
NO ASSOCIATED ORGAN			1
AUTOLYSIS/NECROPSY PERF/NO HISTO			1
MESENTERY			1
NECROSIS FAT			1

TABLE E1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH*			1
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			6
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	18	39	41
ANIMAL MISSING			

* INCLUDES AUTOLYZED ANIMALS

TABLE E2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED NTA IN THE DIET

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20 (100%)	50 (100%)	50 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
ANIMALS WITH NON-TUMOR PATHOLOGY	19 (95%)	46 (92%)	48 (96%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM	12 (60%)	18 (36%)	24 (48%)
TRACHEA	1		1
INFLAMMATION NECROTIZING	1		
INFLAMMATION CHRONIC SUPPURATIVE			1
LUNG	11	18	23
PNEUMONIA CHRONIC MURINE	10	18	22
INFLAMMATION CHRONIC			1
INFARCT	1		

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM	3 (15%)	9 (18%)	13 (26%)
EPICARDIUM			1
INFLAMMATION			1
HEART		1	
PERIARTERITIS		1	
MYOCARDIUM	3	8	13
FIBROSIS	2	3	8
FIBROSIS DIFFUSE		1	
DEGENERATION	1	4	5
DIGESTIVE SYSTEM	6 (30%)	11 (22%)	7 (14%)
LIVER	3	5	1
NECROSIS FOCAL	1		
METAMORPHOSIS FATTY		2	
HEMOSIDEROSIS			1

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
LIVER (CONT.)			
BASOPHILIC CYTOPLASM ALTERATION	2	3	
BILE DUCT			1
LYMPHOCYTIC INFLAM INFILTRATE			1
PANCREAS	2	1	4
INFLAMMATION FOCAL			1
LYMPHOCYTIC INFLAM INFILTRATE			1
FIBROSIS DIFFUSE	2	1	1
HYPERPLASIA INTRADUCTAL			3
SMALL INTESTINE	1	6	1
INFLAMMATION		1	
LYMPHOID HYPERPLASIA	1	6	1
URINARY SYSTEM	4 (20%)	26 (52%)	40 (80%)
KIDNEY	4	25	36
HYDRONEPHROSIS			1
PYELONEPHRITIS			1
INFLAMMATION SUBACUTE		2	1
INFLAMMATION CHRONIC	2	24	33
NEPHROPATHY	2		

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
GLOMERULOSCLEROSIS			1
RENAL TUBULE			1
REGENERATION			1
URINARY BLADDER		2	11
HYPERPLASIA EPITHELIAL		2	11
ENDOCRINE SYSTEM	6 (30%)	8 (16%)	12 (24%)
PITUITARY	4	3	4
CYST	2	2	2
HEMATOCYST	1		
INFLAMMATION CYSTIC			1

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
PITUITARY (CONT.)			
PIGMENTATION	1		
HEMOSIDEROSIS			1
HYPERPLASIA FOCAL		1	
ADRENAL CORTEX	2	2	1
HEMATOCYST		1	
INFLAMMATION SUPPURATIVE	1		
LIPOIDOSIS	1		
HYPERPLASIA NODULAR			1
HYPERPLASIA FOCAL		1	
ADRENAL MEDULLA		1	
HEMATOCYST		1	
THYROID		2	8
CYSTIC FOLLICLES			1
HYPERPLASIA C-CELL		2	7
HEMATOPOIETIC SYSTEM	2 (10%)	8 (16%)	8 (16%)
BONE MARROW		2	
FIBROSIS FOCAL		1	
HYPERPLASIA HEMATOPOIETIC		1	

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
SPLEEN	1	1	4
INFLAMMATION CHRONIC			1
INFLAMMATION CHRONIC NECROTIZING	1		
NECROSIS DIFFUSE			1
HEMOSIDEROSIS			1
ERYTHROPHAGOCYTOSIS			1
HYPERPLASIA ERYTHROID			1
HYPERPLASIA RETICULUM-CELL		1	
LYMPH NODE		3	2
HYPERPLASIA RETICULUM-CELL		2	3
LYMPHOID HYPERPLASIA		1	
SUBMANDIBULAR LYMPH		2	1
INFLAMMATION SUPPURATIVE			1

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SUBMANDIBULAR LYMPH NODE (CONT.)			
HYPERPLASIA RETICULUM-CELL		3	
MESENTERIC LYMPHNODE	1	1	3
HYPERPLASIA RETICULUM-CELL	1	1	2
LYMPHOID HYPERPLASIA			1
REPRODUCTIVE SYSTEM	2 (10%)	18 (36%)	11 (22%)
MAMMARY GLAND			1
CYST			1
UTERUS		8	2
HEMATOMA		1	
INFLAMMATION SUPPURATIVE		1	
PYOMETRA		3	2
ABSCESS		2	
FIBROSIS		1	
HYPERPLASIA CYSTIC		1	
UTERUS/ENDOMETRIUM	1	7	6
INFLAMMATION		1	
INFLAMMATION SUPPURATIVE	1	5	2
INFLAMMATION ACUTE			1

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION ACUTE SUPPURATIVE			1
HYPERPLASIA		2	
HYPERPLASIA CYSTIC		2	3
FALLOPIAN TUBE		1	
INFLAMMATION SUPPURATIVE		1	
OVARY	2	4	5
CYST		4	4
INFLAMMATION CYSTIC			1
INFLAMMATION CHRONIC	1		
NECROSIS FAT	1		
NERVOUS SYSTEM	1 (5%)		

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BRAIN	1		
HYDROCEPHALUS INTERNAL	1		
MUSCULOSKELETAL SYSTEM		1 (2%)	
SPLENIUS MUSCLE		1	
INFLAMMATION		1	
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS	1 (5%)	2 (4%)	1 (2%)
PERITONEUM			1
INFLAMMATION			1
NO ASSOCIATED ORGAN	1	2	
NO LESION REPORTED	1	2	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH*			

TABLE E2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	1	1	
TERMINAL SACRIFICE	6	39	39
ANIMAL MISSING			

* INCLUDES AUTOLYZED ANIMALS

TABLE E3
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED NTA IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	6
ANIMALS NECROPSIED	20 (100%)	48 (100%)	44 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	48	44
ANIMALS WITH NON-TUMOR PATHOLOGY	12 (60%)	30 (63%)	33 (75%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM	1 (5%)	1 (2%)	3 (7%)
LUNG	1	1	3
CONGESTION			1
PNEUMONIA CHRONIC MURINE	1	1	2
CIRCULATORY SYSTEM			
NONE			

TABLE E3 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM	2 (10%)	9 (19%)	2 (5%)
LIVER	1	4	
METAMORPHOSIS FATTY	1	2	
HYPERPLASIA NODULAR		1	
ANGIECTASIS		1	
BILE DUCT		1	
INFLAMMATION		1	
SMALL INTESTINE		3	1
LYMPHOID HYPERPLASIA		3	1
PEYERS PATCH	1		1
LYMPHOID HYPERPLASIA	1		1
LARGE INTESTINE		1	
LYMPHOID HYPERPLASIA		1	

TABLE E3 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM	2 (10%)	5 (10%)	16 (36%)
KIDNEY	1	3	13
HYDRONEPHROSIS		3	8
HEMATOCYST			1
INFLAMMATION			1
INFLAMMATION CHRONIC			4
INFARCT			1
CALCINOSIS	1		
METAPLASIA OSSEOUS			1
KIDNEY/CORTEX		1	1
CYST		1	
DEGENERATION			1
RENAL TUBULE			3
DEGENERATION			1
NEPHROSIS			2
URETER		1	1
INFLAMMATION CHRONIC			1
HYPERPLASIA EPITHELIAL		1	
URETHRA	1		

TABLE E3 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA EPITHELIAL	1		
ENDOCRINE SYSTEM		3 (6%)	4 (9%)
ADRENAL MEDULLA		1	
HYPERPLASIA		1	
THYROID		2	4
CYSTIC FOLLICLES			1
GOITER COLLOID		1	1
HYPERPLASIA C-CELL		1	3
HEMATOPOIETIC SYSTEM	2 (10%)	4 (8%)	9 (20%)
SPLEEN	1	1	3
LYMPHOID HYPERPLASIA	1	1	3
LYMPH NODE			2
LYMPHOID HYPERPLASIA			2
MANDIBULAR L. NODE			1
LYMPHOID HYPERPLASIA			1

TABLE MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MESENTERIC LYMPHNODE	1	3	6
HYPERPLASIA			1
LYMPHOID HYPERPLASIA	1	3	5
HEMATOPOIESIS		1	
REPRODUCTIVE SYSTEM	3 (15%)	10 (21%)	19 (43%)
PROSTATE	3	10	19
INFLAMMATION CHRONIC		1	
HYPERPLASIA CYSTIC	3	9	19
NERVOUS SYSTEM	7 (35%)	7 (15%)	5 (11%)
BRAIN	7	7	5
CORPORA AMYLACEA	7	7	5
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			

TABLE E3 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NONE			
ALL OTHER SYSTEMS	5 (25%)	12 (25%)	8 (18%)
PERITONEUM		1	
INFLAMMATION NECROTIZING		1	
NO ASSOCIATED ORGAN	4	10	8
NO LESION REPORTED	4	9	2
ANIMAL MISSING		1	6
MESENTERY	1	1	
NECROSIS FAT	1	1	

TABLE E3 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH*	1	4	2
MORIBUND SACRIFICE			2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	19	44	39
ANIMAL MISSING		1	6

* INCLUDES AUTOLYZED ANIMALS

TABLE E4
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED NTA IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		11	
ANIMALS NECROPSIED	20 (100%)	38 (100%)	49 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	38	49
ANIMALS WITH NON-TUMOR PATHOLOGY	15 (75%)	31 (82%)	44 (90%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM	4 (20%)	2 (5%)	3 (6%)
LUNG	4	2	2
PNEUMONIA CHRONIC MURINE	4	1	2
HYPERPLASIA ALVEOLAR-CELL		1	
LUNG/ALVEOLI			1
EMPHYSEMA			1

TABLE E4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			1 (2%)
MYOCARDIUM			1
INFLAMMATION NECROTIZING			1
DIGESTIVE SYSTEM	6 (30%)	4 (11%)	
SALIVARY GLAND	1		
LYMPHOCYTIC INFLAM INFILTRATE	1		
LIVER	2	2	
NECROSIS FOCAL		1	
INFARCT		1	
HYPERPLASIA NODULAR	1		
HEMATOPOIESIS	1		
GALLBLADDER	1		
CALCULUS	1		
INFLAMMATION CHRONIC	1		
BILE DUCT	1		
INFLAMMATION	1		

TABLE E4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
PEYERS PATCH		2	
LYMPHOID HYPERPLASIA		2	
COLON	1		
CYST	1		
URINARY SYSTEM			22 (45%)
KIDNEY			19
HYDRONEPHROSIS			12
INFLAMMATION			2
INFLAMMATION CHRONIC			5
NEPHROSIS			1
INFARCT			4
KIDNEY/CORTEX			1
NEPHROSIS			1
RENAL TUBULE			4
DEGENERATION			4
KIDNEY/PELVIS			1
HYPERPLASIA EPITHELIAL			1

TABLE E4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM		5 (13%)	4 (8%)
THYROID		5	4
GOITER COLLOID		2	1
HYPERPLASIA C-CELL		3	3
PARATHYROID		1	
HYPERPLASIA		1	
HEMATOPOIETIC SYSTEM	1 (5%)	5 (13%)	7 (14%)
SPLEEN		5	5
LYMPHOID HYPERPLASIA		5	5

TABLE E4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
LYMPH NODE			1
LYMPHOID HYPERPLASIA			1
MANDIBULAR L. NODE		1	
LYMPHOID HYPERPLASIA		1	
LYMPH NODE OF THORAX		1	
LYMPHOID HYPERPLASIA		1	
MESENTERIC LYMPHNODE	1	2	4
EDEMA		1	
INFLAMMATION CYSTIC	1		
LYMPHOID HYPERPLASIA		2	4
RENAL LYMPH NODE		1	
LYMPHOID HYPERPLASIA		1	
REPRODUCTIVE SYSTEM	11 (55%)	23 (61%)	30 (61%)
UTERUS		4	6
INFLAMMATION			1
PYOMETRA			1
INFLAMMATION CYSTIC		1	3

TABLE E4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA CYSTIC		3	1
CERVIX UTERI		3	2
INFLAMMATION		2	1
INFLAMMATION SUPPURATIVE		1	
INFLAMMATION ACUTE			1
UTERUS/ENDOMETRIUM	9	15	19
INFLAMMATION		1	1
INFLAMMATION CYSTIC		1	
HYPERPLASIA CYSTIC	9	14	19
OVARY	3	6	6
CYST		5	3
HEMORRHAGE		1	
INFLAMMATION CYSTIC	3	1	3

TABLE E4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM	3 (15%)	4 (11%)	5 (10%)
BRAIN	3	4	5
CORPORA AMYLACEA	3	4	5
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS	3 (15%)	13 (34%)	4 (8%)
TAIL			1
INFLAMMATION FOCAL GRANULOMATOUS			1
NO ASSOCIATED ORGAN	2	13	2
NO LESION REPORTED	2	2	2
ANIMAL MISSING		11	
MESENTERY	1		1

TABLE E4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CYST			1
NECROSIS FAT	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH*	1	2	1
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	35	47
ANIMAL MISSING		11	

* INCLUDES AUTOLYZED ANIMALS

APPENDIX F

SUMMARY OF THE INCIDENCE OF NONNEOPASTIC LESIONS

IN RATS AND MICE FED $NA_3NTA \cdot H_2O$ IN THE DIET (LBI)

TABLE F1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED $NA_3NTA \cdot H_2O$ IN THEDIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20 (100%)	49 (100%)	50 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
ANIMALS WITH NON-TUMOR PATHOLOGY	16 (80%)	43 (88%)	50 (100%)
INTEGUMENTARY SYSTEM *		1 (2%)	1 (2%)
SUBCUT TISSUE		1	1
EPIDERMAL INCLUSION CYST		1	1
RESPIRATORY SYSTEM	2 (10%)	4 (8%)	26 (52%)
TRACHEA			3
INFLAMMATION			2
INFLAMMATION CHRONIC			1
LUNG	2	4	25
HEMORRHAGE			6
ABSCESS			1
PNEUMONIA CHRONIC MURINE	2	4	20
HYPERPLASIA ALVEOLAR-CELL			1
LUNG/ALVEOLI			1
CALCIFICATION			1
CIRCULATORY SYSTEM	5 (25%)	18 (37%)	19 (38%)
MYOCARDIUM	5	18	19
INFLAMMATION FOCAL			1
FIBROSIS	1	6	2
DEGENERATION	4	12	16

TABLE F1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM	7 (35%)	13 (27%)	13 (26%)
LIVER	4	10	10
NECROSIS			1
NECROSIS FOCAL			1
METAMORPHOSIS FATTY		3	2
CYTOPLASMIC ALTERATION	1		
CYTOPLASMIC VACUOLIZATION			2
BASOPHILIC CYTOPLASM ALTERATION	4	7	4
HEMATOPOIESIS		1	
PANCREAS		2	4
FIBROSIS		1	2
FIBROSIS DIFFUSE		1	2
PERIARTERITIS			1
HYPERPLASIA INTRADUCTAL		1	
PANCREATIC ACINUS		1	
HYPERPLASIA NODULAR		1	
STOMACH			1
HYPERPLASIA EPITHELIAL			1
SMALL INTESTINE	3	1	1
LYMPHOID HYPERPLASIA	3	1	1
COLON	1	1	
NEMATODIASIS	1	1	
URINARY SYSTEM	9 (45%)	36 (73%)	49 (98%)
KIDNEY	9	36	47
HEMORRHAGE			5
INFLAMMATION CHRONIC	9	36	39
NEPHROSIS CHOLEMIC			2
PIGMENTATION		1	1
HYPERPLASIA TUBULAR-CELL			1
KIDNEY/PELVIS			3
HYPERPLASIA EPITHELIAL			3
URETER			1
HYPERPLASIA EPITHELIAL			1
URINARY BLADDER			3
HYPERPLASIA EPITHELIAL			3

TABLE F1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM	2 (10%)	16 (33%)	5 (10%)
PITUITARY		3	
CYST		1	
HYPERPLASIA FOCAL		1	
HYPERPLASIA BASOPHILIC		1	
ADRENAL CORTEX		2	
HYPERPLASIA NODULAR		2	
ADRENAL MEDULLA	1		
CYST	1		
THYROID	1	12	5
HYPERPLASIA C-CELL	1	12	4
HYPERPLASIA FOLLICULAR-CELL			1
PARATHYROID		1	
HYPERPLASIA		1	
HEMATOPOIETIC SYSTEM	5 (25%)	8 (16%)	5 (10%)
SPLEEN	1	1	3
FIBROSIS FOCAL			1
HEMOSIDEROSIS			1
LYMPHOID DEPLETION			1
LYMPHOID HYPERPLASIA	1	1	
LYMPH NODE	2	5	
INFLAMMATION		1	
HYPERPLASIA RETICULUM-CELL		3	
LYMPHOID HYPERPLASIA	2	1	
SUBMANDIBULAR L.NODE	1	1	
INFLAMMATION SUPPURATIVE		1	
HYPERPLASIA CYSTIC	1		
MESENTERIC LYMPHNODE	1	2	2
CYST			1
INFLAMMATION		2	
HYPERPLASIA RETICULUM-CELL	1		1

TABLE F1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM		3 (6%)	2 (4%)
PREPUTIAL GLAND		1	1
ABSCESS		1	
HYPERPLASIA		1	1
PROSTATE			1
METAPLASIA SQUAMOUS			1
SEMINAL VESICLE		1	
INFLAMMATION SUPPURATIVE		1	
EPIDIDYMIS		1	
NECROSIS FAT		1	
NERVOUS SYSTEM	1 (5%)		5 (10%)
BRAIN/MENINGES			5
HEMORRHAGE			5
BRAIN	1		
HYDROCEPHALUS INTERNAL	1		
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS		2 (4%)	
MESENTERY		2	
PERIARTERITIS		1	
NECROSIS FAT		1	

TABLE F1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH*	1	3	4
MORIBUND SACRIFICE	1	1	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			7
TERMINAL SACRIFICE	18	45	36
ANIMAL MISSING		1	

* INCLUDES AUTOLYZED ANIMALS

* SYSTEM PERCENTAGES ARE BASED ON NUMBER OF ANIMALS NECROPSIED.

TABLE F2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED $NA_3NTA \cdot H_2O$ IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20 (100%)	50 (100%)	49 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
ANIMALS WITH NON-TUMOR PATHOLOGY	12 (60%)	46 (92%)	46 (94%)
INTEGUMENTARY SYSTEM *			1 (2%)
SKIN			1
HYPERKERATOSIS			1
PARAKERATOSIS			1
RESPIRATORY SYSTEM	3 (15%)	3 (6%)	14 (29%)
LUNG	3	3	14
HEMORRHAGE			5
PNEUMONIA CHRONIC MURINE	3	2	8
NECROSIS FOCAL			1
HYPERPLASIA ALVEOLAR-CELL		1	
CIRCULATORY SYSTEM	2 (10%)	9 (18%)	4 (8%)
MYOCARDIUM	2	9	4
INFLAMMATION FOCAL			1
FIBROSIS	1	4	2
DEGENERATION	1	5	1
DIGESTIVE SYSTEM	5 (25%)	22 (44%)	19 (39%)
LIVER	4	17	17
METAMORPHOSIS FATTY	2		2
CYTOPLASMIC VACUOLIZATION		2	
BASOPHILIC CYTOPLASM ALTERATION	2	15	15
HEMATOPOIESIS		1	
PANCREAS		4	1
FIBROSIS			1
FIBROSIS FOCAL		1	
FIBROSIS DIFFUSE		3	

TABLE F2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
PANCREATIC ACINUS			1
ATROPHY			1
SMALL INTESTINE	1	2	1
LYMPHOID HYPERPLASIA	1	2	1
URINARY SYSTEM	2 (10%)	32 (64%)	39 (80%)
KIDNEY	2	29	37
HYDRONEPHROSIS		1	
HEMORRHAGE			2
PYELONEPHRITIS		1	
INFLAMMATION			1
INFLAMMATION CHRONIC	2	27	34
TOXIC NEPHROPATHY			2
NEPHROSIS CHOLEMIC		1	
HYPERPLASIA TUBULAR-CELL			1
KIDNEY/PELVIS		1	1
INFLAMMATION SUPPURATIVE			1
NECROSIS		1	
URINARY BLADDER		4	5
HYPERPLASIA EPITHELIAL		4	5
ENDOCRINE SYSTEM	3 (15%)	13 (26%)	13 (27%)
PITUITARY	1	4	6
CYST	1	4	3
HEMATOCYST			2
HYPERPLASIA BASOPHILIC			1

TABLE F2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ADRENAL		4	3
CYST		1	
HEMATOCYST		2	2
LIPOIDOSIS		1	1
ADRENAL CORTEX	1	1	1
HEMATOCYST		1	
METAMORPHOSIS FATTY	1		
HYPERPLASIA FOCAL			1
ADRENAL MEDULLA		1	
CYST		1	
THYROID	2	5	5
HYPERPLASIA C-CELL	2	5	5
PARATHYROID		1	
HEMATOCYST		1	
HEMATOPOIETIC SYSTEM	2 (10%)	6 (12%)	5 (10%)
SPLEEN	2	2	2
INFARCT	1		
LYMPHOID HYPERPLASIA	1	2	2
LYMPH NODE		1	1
HYPERPLASIA RETICULUM-CELL		1	1
SUBMANDIBULAR L.NODE		2	
INFLAMMATION		1	
LYMPHOID HYPERPLASIA		1	
CERVICAL LYMPH NODE			1
HEMORRHAGE			1
MESENTERIC LYMPHNODE		1	1
EDEMA			1
HYPERPLASIA RETICULUM-CELL		1	

TABLE F2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM	1 (5%)	3 (6%)	5 (10%)
MAMMARY GLAND			2
CYST			2
UTERUS		1	
HEMATOMETRA		1	
UTERUS/ENDOMETRIUM	1	1	2
INFLAMMATION CYSTIC			1
HYPERPLASIA		1	1
HYPERPLASIA CYSTIC	1		
OVARY		1	1
CYST		1	1
NERVOUS SYSTEM	1 (5%)	3 (6%)	6 (12%)
BRAIN/MENINGES		1	1
HEMORRHAGE			1
INFLAMMATION		1	
CHOROID PLEXUS	1		
CORPORA AMYLACEA	1		
CEREBRUM			1
HEMORRHAGE			1
BRAIN			4
HYDROCEPHALUS			1
HYDROCEPHALUS INTERNAL			2
HEMORRHAGE			1
HYPOTHALAMUS		1	1
ATROPHY		1	1
MIDBRAIN		1	
ATROPHY		1	

TABLE F2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS	3 (15%)		2 (4%)
PELVIS			1
HEMORRHAGE			1
NECROSIS FAT			1
ADIPOSE TISSUE	2		1
INFLAMMATION	1		1
NECROSIS FAT	1		
MESENTERY	1		
NECROSIS FAT	1		
NO LESION REPORTED	2	1	2
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH*		3	
MORIBUND SACRIFICE	2		5
SCHEDULED SACRIFICE		1	
ACCIDENTALLY KILLED			5
TERMINAL SACRIFICE	18	46	39
ANIMAL MISSING			

* INCLUDES AUTOLYZED ANIMALS

* SYSTEM PERCENTAGES ARE BASED ON NUMBER OF ANIMALS NECROPSIED.

TABLE F3

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED $NA_3NTA \cdot H_2O$ IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20 (100%)	48 (100%)	50 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	48	50
ANIMALS WITH NON-TUMOR PATHOLOGY	11 (55%)	18 (38%)	34 (68%)
INTEGUMENTARY SYSTEM *		2 (4%)	2 (4%)
SKIN		1	2
CYST		1	1
SEBACEOUS CYST			1
SUBCUT TISSUE		1	
ABSCESS		1	
RESPIRATORY SYSTEM		2 (4%)	1 (2%)
LUNG		2	1
PNEUMONIA CHRONIC MURINE		2	1
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM	4 (20%)	5 (10%)	4 (8%)
LIVER	3	4	2
HEMATOMA	1		
INFLAMMATION GRANULOMATOUS			1
NECROSIS		1	
METAMORPHOSIS FATTY	1	1	
LIPOIDOSIS		1	
HYPERPLASIA NODULAR			1
HYPERPLASTIC NODULE	1		
ANGIECTASIS		1	
LIVER/PERIPORTAL			1
METAMORPHOSIS FATTY			1
LIVER/CENTRILOBULAR		1	
NECROSIS		1	
BILE DUCT	1		
INFLAMMATION FOCAL	1		

TABLE F3 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SMALL INTESTINE		1	1
LYMPHOID HYPERPLASIA		1	1
URINARY SYSTEM		1 (2%)	28 (56%)
KIDNEY		1	28
HYDRONEPHROSIS		1	28
ENDOCRINE SYSTEM		1 (2%)	2 (4%)
THYROID			1
CYSTIC FOLLICLES			1
PANCREATIC ISLETS		1	1
HYPERPLASIA			1
HYPERPLASIA FOCAL		1	
HEMATOPOIETIC SYSTEM	2 (10%)	4 (8%)	5 (10%)
BONE MARROW	1		
HYPERPLASIA	1		
SPLEEN	1	2	3
HYPERPLASIA NODULAR			2
LYMPHOID HYPERPLASIA	1	2	1
MANDIBULAR L. NODE	1		
HYPERPLASIA RETICULUM-CELL	1		
LYMPHOID HYPERPLASIA	1		
MESENTERIC LYMPHNODE	1	2	2
CYST		1	
HEMORRHAGE		1	
INFLAMMATION GRANULOMATOUS	1		
HYPERPLASIA			1
HEMATOPOIESIS			1
REPRODUCTIVE SYSTEM	1 (5%)		1 (2%)
PROSTATE	1		1
HYPERPLASIA CYSTIC	1		
LYMPHOID HYPERPLASIA			1

TABLE F3 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM	1 (5%)	5 (10%)	2 (4%)
BRAIN	1	5	2
COPPORA AMYLACEA	1	5	2
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS	4 (20%)	1 (2%)	
ADIPOSE TISSUE	2		
INFLAMMATION	2		
MESENTERY	2	1	
INFLAMMATION	2	1	
NO LESION REPORTED	7	18	11
AUTOLYSIS/NO NECROPSY PERFORMED		2	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH*		6	4
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	20	43	46
ANIMAL MISSING			

* INCLUDES AUTOLYZED ANIMALS

* SYSTEM PERCENTAGES ARE BASED ON NUMBER OF ANIMALS NECROPSIED.

TABLE F4

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED $NA_3NTA \cdot H_2O$ IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	18 (100%)	46 (100%)	47 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	18	46	47
ANIMALS WITH NON-TUMOR PATHOLOGY	12 (67%)	35 (76%)	41 (87%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM *		3 (7%)	
LUNG		3	
PNEUMONIA CHRONIC MURINE		3	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM		4 (9%)	1 (2%)
LIVER		2	
NECROSIS FOCAL		1	
LYMPHOID HYPERPLASIA		1	
GALLBLADDER		1	
CALCULUS		1	
BILE DUCT		1	
LYMPHOCYTIC INFLAM INFILTRATE		1	
LARGE INTESTINE			1
ABSCESS			1
URINARY SYSTEM	1 (6%)	1 (2%)	31 (66%)
KIDNEY		1	30
HYDRONEPHROSIS		1	30

TABLE F4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
KIDNEY (CONT.)			
INFLAMMATION CHRONIC			1
LYMPHOID HYPERPLASIA			1
URETER			1
INFLAMMATION			1
URINARY BLADDER	1		
INFLAMMATION	1		
ENDOCRINE SYSTEM		2 (4%)	
THYROID		2	
HYPERPLASIA C-CELL		2	
HEMATOPOIETIC SYSTEM	4 (22%)	5 (11%)	3 (6%)
SPLEEN	3	4	3
HYPERPLASIA		1	
HYPERPLASIA RETICULUM-CELL			1
LYMPHOID HYPERPLASIA	3	3	2
MANDIBULAR L. NODE		1	
LYMPHOID HYPERPLASIA		1	
BRONCHIAL LYMPH NODE			1
LYMPHOID HYPERPLASIA			1
PANCREATIC L.NODE	1		
CYST	1		
MESENTERIC LYMPHNODE		2	1
EDEMA		1	
INFLAMMATION		1	
HYPERPLASIA RETICULUM-CELL		1	

TABLE F4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MESENTERIC LYMPHNODE (CONT.)			
LYMPHOID HYPERPLASIA			1
THYMUS			1
LYMPHOID HYPERPLASIA			1
REPRODUCTIVE SYSTEM	11 (61%)	28 (61%)	23 (49%)
UTERUS		3	
HEMORRHAGE		1	
PYOMETRA		2	
CERVIX UTERI	1	1	
INFLAMMATION	1	1	
UTERUS/ENDOMETRIUM	5	22	20
INFLAMMATION SUPPURATIVE		1	
HYPERPLASIA	1	1	
HYPERPLASIA CYSTIC	4	20	20
OVARY	6	8	5
CYST	6	7	4
HEMATOMA		1	
ABSCESS			1
NERVOUS SYSTEM	2 (11%)	3 (7%)	5 (11%)
BRAIN	2	3	5
ARTERIOSCLEROSIS	1		
NECROSIS FOCAL	1		
CORPORA AMYLACEA	1	3	5
CHOLESTEATOMA	1		
MUSCULOSKELETAL SYSTEM			
NONE			

TABLE F4 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS	1 (5%)	12 (26%)	1 (2%)
MESENTERY	1		1
ABSCESS			1
GRANULOMA	1		
NO LESION REPORTED	4	8	3
AUTOLYSIS/NO NECROPSY PERFORMED	2	4	3
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH*	3	6	6
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	43	44
ANIMAL MISSING			

* INCLUDES AUTOLYZED ANIMALS

* SYSTEM PERCENTAGES ARE BASED ON NUMBER OF ANIMALS NECROPSIED.

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