NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 356

# TOXICOLOGY AND CARCINOGENESIS STUDIES OF FUROSEMIDE

## (CAS NO. 54-31-9)

## IN F344/N RATS AND B6C3F$_1$ MICE

## (FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NTP TECHNICAL REPORT

ON THE

# TOXICOLOGY AND CARCINOGENESIS STUDIES OF FUROSEMIDE

(CAS NO. 54-31-9)

# IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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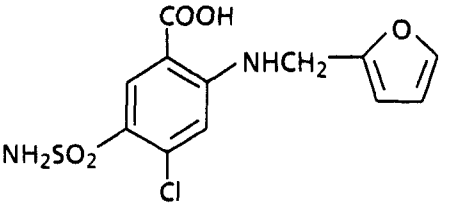


**FUROSEMIDE**

CAS No. 54-31-9

$C_{12}H_{11}ClN_2O_5S$ Molecular weight 330.8

Synonyms: 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid; frusemide; fursemide
Trade Names: Aisemide, Aluzine, Beronald, Desdemin, Diural, Dryptal, Errolon, Frusemin, Fulsix, Fuluvamide, Furosemide "Mita," Katlex, Lasilix, Lasix, Lowpstron, Rosemide, Transit, Urosemide

## ABSTRACT

Furosemide is a diuretic used in human and veterinary medicine. Toxicology and carcinogenesis studies were conducted by feeding diets containing furosemide (99% pure, USP grade) to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

*Fourteen-Day and Thirteen-Week Studies:* Dietary concentrations of furosemide used in the 14-day studies for rats and mice ranged up to 46,000 ppm. Two of five male and 3/5 female rats that received 46,000 ppm furosemide died before the end of the studies. Rats that received 15,300 or 46,000 ppm lost weight over the course of the studies. The final mean body weights of rats that received 1,700 or 5,100 ppm were 12% or 23% lower than that of controls for males and 8% or 16% lower for females. Nephrosis was dose related in rats. All five male and 1/5 female mice that received 46,000 ppm furosemide died before the end of the 14-day studies. Male mice that received 15,300 ppm and female mice that received 46,000 ppm lost weight. The final mean body weights of male mice that received 1,700 or 5,100 ppm were 16% or 14% lower than that of controls. The final mean body weight of females that received 15,300 ppm was 13% lower than that of controls. Slight dilatation of the renal cortical tubules and/or nephrosis were dose related in mice.

Dietary concentrations of furosemide used in the 13-week studies were 0 and 625-10,000 ppm for male rats and 0 and 938-15,000 ppm for female rats and male mice. Concentrations for female mice were 0 and 1,250-20,000 ppm. None of the rats died before the end of the studies. The final mean body weights of male rats that received 2,500, 5,000, or 10,000 ppm furosemide were 11%, 22%, or 44% lower than that of controls. The final mean body weights of female rats that received 3,750, 7,500, or 15,000 ppm were 18%, 26%, or 35% lower than that of controls. Minimal-to-mild nephrosis occurred in the two highest dose groups of male and female rats. Mineralization of minimal to mild severity was observed at the renal corticomedullary junction in dosed male rats receiving 625 ppm or more; the severity and incidence of the mineralization increased with increased dose. No compound-related deaths occurred in mice. The final mean body weights of male mice that received 3,750, 7,500, or 15,000 were 12%, 22%, or 17% lower than that of controls. Final mean body weights of dosed and control female mice were comparable. Compound-related lesions in mice included minimal-to-mild nephrosis.

Because of the lower body weights and the kidney lesions in the 13-week studies, doses selected for the 2-year studies were 0, 350, or 700 ppm furosemide in the diet for groups of 50 F344/N rats of each sex. Groups of 50 B6C3F$_1$ mice of each sex were fed diets containing 0, 700, or 1,400 ppm furosemide for 104 weeks.

*Body Weight and Survival in the Two-Year Studies:* Mean body weights of dosed and control rats were comparable throughout the studies. No significant differences in survival were observed between any groups of rats of either sex (final survival--male: control, 17/50; low dose, 17/50; high dose, 20/50; female: 35/50; 31/50; 34/50). The final survival of all groups of male rats was low, reflecting the large number of moribund animals killed after week 91. Survival at week 90 was 35/50, 28/50, and 34/50. Mean body weights of high dose male mice were up to 17% lower than those of controls, and mean body weights of low dose male mice were about 5%-10% lower than those of controls after week 31. Mean body weights of high dose female mice were up to 22% lower than those of controls. Mean body weights of low dose female mice were 5%-13% lower than those of controls after week 82. The survival of the high dose group of female mice was significantly lower than that of the controls after week 99 (final survival--male: 31/50; 24/50; 26/50; female: 36/50; 29/50; 18/50). Feed consumption by dosed rats was similar to that by controls. The estimated average amount of furosemide consumed per day was approximately 14-16 or 29-31 mg/kg for low dose or high dose rats. Feed consumption by dosed mice was approximately 5%-7% greater than that by controls. The average amount of furosemide consumed per day was approximately 91-99 or 191-214 mg/kg for low dose or high dose mice.

*Nonneoplastic and Neoplastic Effects in the Two-Year Studies:* Nephropathy occurred at similar incidences in all groups of rats, but the severity was greater in dosed male rats. Tubular cell hyperplasia was observed in 4/50 control, 2/50 low dose, and 4/50 high dose male rats. Tubular cell adenomas of the kidney occurred in 1/50 control, 3/50 low dose, and 1/50 high dose male rats. Tubular cell adenocarcinomas were seen in a fourth low dose male rat and in a second high dose male rat (adenomas or adenocarcinomas, combined: control, 1/50; low dose, 4/50; high dose, 2/50). The historical incidence of renal tubular cell adenomas or adenocarcinomas (combined) in untreated male F344/N rats is 9/1,928 (0.5%), and the highest incidence observed in controls is 3/50.

Malignant meningiomas of the brain occurred in 3/50 low dose male rats; none was observed in other groups. The historical incidence of meningiomas in untreated male F344/N rats is 2/1,928 (0.1%).

C-Cell adenomas of the thyroid gland in female rats occurred with a positive trend; the incidence in the high dose group was not statistically greater than that in the controls (4/50; 6/50; 11/50). A C-cell carcinoma occurred in another low dose female rat. The incidence of adenomas of the anterior pituitary gland in low dose male rats was marginally greater than that in controls (4/50; 11/50; 8/50). Neither of these marginal increases was considered to be chemically related.

Malignant mixed tumors (adenocarcinoma, type C) of the mammary gland occurred in dosed female mice (0/50; 1/50; 5/48). One mammary gland acinar cell carcinoma occurred in a second low dose female mouse. The historical incidence of all malignant mammary gland neoplasms in untreated female B6C3F$_1$ mice is 40/2,040 (2%).

Compound-related nonneoplastic lesions of the kidney in mice included nephropathy and dilatation of the renal pelvis for males and females and tubular cysts, suppurative inflammation, and epithelial hyperplasia of the renal pelvis for males. Kidney lesions may have contributed to the low survival of high dose female mice.

Mucosal epithelial hyperplasia and submucosal chronic focal inflammation of the urinary bladder were observed at increased incidences in dosed male mice. Suppurative inflammation of the prostate

was observed at an increased incidence in high dose male mice. Fighting may have contributed to urogenital lesions in male mice. Suppurative inflammation of the ovary or uterus was observed at an increased incidence in high dose female mice. Hematopoiesis was observed at increased incidences in the spleen and liver of dosed male and high dose female mice and in the adrenal cortex of high dose female mice.

*Genetic Toxicology:* Furosemide was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation. In the mouse lymphoma assay for trifluorothymidine (Tft) resistance, furosemide produced an equivocal response in the absence of metabolic activation and a positive response in the presence of activation. Furosemide induced sister chromatid exchanges and chromosomal aberrations in CHO cells in both the presence and absence of exogenous metabolic activation.

*Audit:* The data, documents, and pathology materials from the 2-year studies of furosemide have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

*Conclusions:* Under the conditions of these 2-year studies, there was *equivocal evidence of carcinogenic activity** of furosemide for male F344/N rats, as shown by marginal increases in uncommon tubular cell neoplasms of the kidney and meningiomas of the brain. There was *no evidence of carcinogenic activity* of furosemide for female F344/N rats fed diets containing 350 or 700 ppm furosemide for 2 years. There was *no evidence of carcinogenic activity* for male B6C3F$_1$ mice fed diets containing 700 or 1,400 ppm furosemide for 2 years. There was *some evidence of carcinogenic activity* of furosemide for female mice, as shown by an increase in malignant tumors of the mammary gland.

Nephropathy was more severe in the kidney of male rats and of male and female mice fed diets containing furosemide than in controls.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 7.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 10-11.

SUMMARY OF THE TWO-YEAR FEED AND GENETIC TOXICOLOGY STUDIES OF FUROSEMIDE

| Male F344/N Rats | Female F344/N Rats | Male B6C3F$_1$ Mice | Female B6C3F$_1$ Mice |
|---|---|---|---|
| **Dietary concentration** | | | |
| 0, 350, or 700 ppm furosemide | 0, 350, or 700 ppm furosemide | 0, 700, or 1,400 ppm furosemide | 0, 700, or 1,400 ppm furosemide |
| **Body weights in the 2-year study** | | | |
| Dosed and controls similar | Dosed and controls similar | Dosed lower than controls | Dosed lower than controls |
| **Survival rates in the 2-year study** | | | |
| 17/50; 17/50; 20/50 | 35/50; 31/50; 34/50 | 31/50; 24/50; 26/50 | 36/50; 29/50; 18/50 |
| **Nonneoplastic effects** | | | |
| Increased severity of nephropathy | None | Nephropathy | Nephropathy |
| **Neoplastic effects** | | | |
| Renal tubular cell neoplasms (1/50; 4/50; 2/50); meningiomas of the brain (0/50; 3/50; 0/50) | None | None | Malignant tumors of the mammary gland (0/50; 2/50; 5/48) |
| **Level of evidence of carcinogenic activity** | | | |
| Equivocal evidence | No evidence | No evidence | Some evidence |
| **Genetic toxicology assays** | | | |

| Salmonella (gene mutation) | Mouse L5178Y/TK$^{+/-}$ (Tft resistance) | CHO cells in vitro | |
|---|---|---|---|
| | | SCE | Aberration |
| Negative with and without S9 | Equivocal without S9; positive with S9 | Positive with and without S9 | Positive with and without S9 |

## EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"); one category for uncertain findings ("Equivocal Evidence"); one category for no observable effects ("No Evidence"); and one category for experiments that because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.

- **Some Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

- **Equivocal Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.

- **Inadequate Study of Carcinogenic Activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct;
- Occurrence of common versus uncommon neoplasia;
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- Latency in tumor induction;
- Multiplicity in site-specific neoplasia;
- Metastases;
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- Survival-adjusted analyses and false positive or false negative concerns;
- Structure-activity correlations; and
- In some cases, genetic toxicology.

## CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Furosemide is based on 13-week studies that began in May 1980 and ended in August 1980 and on 2-year studies that began in May 1981 and ended in May 1983 at SRI International (Menlo Park, CA).

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## PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on furosemide on April 19, 1988, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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## SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF FUROSEMIDE

On April 19, 1988, the draft Technical Report on the toxicology and carcinogenesis studies of furosemide received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Dr. J.R. Bucher, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (equivocal evidence of carcinogenic activity for male and female rats, no evidence of carcinogenic activity for male mice, some evidence of carcinogenic activity for female mice). Nephropathy was more severe in the kidney of male rats and of male and female mice fed diets containing furosemide than in controls.

Dr. Ashby, a principal reviewer, agreed with the conclusions for male and female mice. Because of poor survival in all groups, he suggested that the conclusions for male rats be changed to inadequate study of carcinogenic activity. Dr. Bucher commented that survival was adequate in all groups at week 93, indicating that the majority of rats were at risk for developing tumors throughout most of the 2-year studies. Two of the three meningiomas occurred before 1 year, and kidney tumors are not known to increase dramatically late in 2-year studies. Dr. Ashby noted that furosemide was genotoxic and that there has been a report that it is a germ cell mutagen. He suggested in vivo bone marrow studies be conducted to confirm or refute this observation. Dr. Bucher said that furosemide would be considered for bone marrow studies. The link with other furans such as furfural, recently reported to be carcinogenic, might be explored.

Dr. Chinchilli, the second principal reviewer, agreed with the conclusions for female rats and male and female mice. For male rats, he proposed that a statistical test incorporating historical control data be used to evaluate the two rare tumors, tubular cell neoplasms of the kidney and meningiomas of the brain, on which the conclusion of equivocal evidence of carcinogenic activity was based. Dr. Bucher said such tests would be added to the Report [see page 66]. Dr. J. Haseman, NIEHS, added that although the NTP generally does not use historical data in a formal testing mode, he had fewer reservations with rare tumors. With the meningiomas and kidney tumors, the differences relative to the Program-wide historical control incidences were significant at the $P = 0.001$ level.

Dr. Capen, the third principal reviewer, agreed with the conclusions for male rats and male and female mice. He thought that the conclusion for female rats should be changed to no evidence of carcinogenic activity. Dr. Bucher said that the level of evidence chosen was based on the dose-related increases in thyroid gland C-cell adenomas, with the high dose incidence double the historical incidence, together with a statistically significant trend.

There was some discussion on the poor survival in rats and the possible impact on interpretation of carcinogenesis findings that would be borderline between two categories of evidence. Dr. J. Huff, NIEHS, said that over the last few years, there has been a trend across all laboratories toward decreased survival for male F344/N rats. Dr. E. McConnell, NIEHS, explained that this could be due, in part, to an increased emphasis on tissue accountability, leading to early kill, especially after week 91, to prevent losses due to autolysis or cannibalism. Dr. Huff added that more attention was being given to humane killing of moribund animals and pointed out that considerable numbers of control and dosed male rats were removed early in the studies [see Table 11, page 41]. Dr. Popp stated that the question of inadequacy of survival was causing more concern than necessary. Dr. Ashby proposed that this humane aggressive kill policy be discussed in the text when there is poor survival [see page 41].

Dr. Ashby moved that the conclusion for female rats be changed to no evidence of carcinogenic activity, based on the great variability of thyroid gland C-cell tumors in other studies, no increases in hyperplasia, and lack of effects on the thyroid gland in the other three study groups. Dr. Lijinsky seconded the motion, which was approved by six votes (Drs. Ashby, Capen, Gallo, Hughes, Lijinsky, and Sivak) to three (Drs. Chinchilli, Hooper, and Popp). Dr. Ashby moved that the conclusion for male rats be changed to inadequate study of carcinogenic activity, based primarily on the poor survival. The motion failed for lack of a second. Dr. Ashby then moved that the conclusion for male rats be accepted as written, equivocal evidence of carcinogenic activity. Dr. Hooper seconded the motion, which was approved unanimously with nine votes. Dr. Ashby moved that the conclusion for male mice, no evidence of carcinogenic activity, be accepted as written. Dr. Popp seconded the motion, which was approved unanimously with nine votes. Dr. Ashby moved that the conclusion for female mice, some evidence of carcinogenic activity, be accepted as written. Dr. Hooper seconded the motion, which was approved unanimously with nine votes.

**Further Pathology Findings on the Kidney of Male Rats (presented to the Peer Review Panel October 1988)**

The NTP toxicology and carcinogenesis studies of furosemide in F344/N rats and B6C3F$_1$ mice underwent peer review and were approved by the Panel on April 19, 1988. An important portion of the discussion focused on the marginally increased incidences in renal tubular cell neoplasms and the poor survival in male rats. The proposed conclusion for male rats, equivocal evidence of carcinogenic activity, was approved unanimously by the Panel with nine votes.

Dr. J.R. Bucher, NIEHS, reported in October 1988 that the furosemide studies were subsequently chosen as one of several studies in which the kidney in male rats would be reevaluated by a more extensive sampling procedure to determine if current NTP procedures give an accurate assessment of the "true" incidences of kidney tubular cell tumors. Furosemide was chosen because of apparently increased incidences in kidney tumors in dosed groups without a dose response.

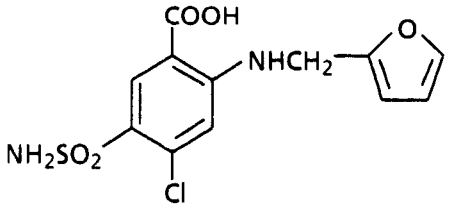
Dr. Bucher described additional pathology procedures and noted that the numbers of additional kidney sections reviewed were 300 for controls, 301 for low dose, and 299 for high dose. The incidences of tubular cell neoplasms originally reported were: control, 1/50; low dose, 4/50; high dose, 2/50. Of the seven neoplasms, five were adenomas, with one adenocarcinoma in the low dose group and one in the high dose group. The results of the additional tissue review were: control, 2; low dose, 1; high dose, 4 (all adenomas). When the diagnoses were combined, the incidences of tubular cell neoplasms of the kidney were: 3/50; 5/50; 6/50. Dr. Bucher stated that the presence of malignant tumors in dosed animals and the marginally increased incidences in combined tumors in a target organ for furosemide action still constituted equivocal evidence of carcinogenic activity. The Panel concurred. Information from the additional studies have been added to the Results in the Technical Report [see Tables 14 and 15].

Dr. S. Eustis, NIEHS, noted that the additional results will not be part of the historical control data base. Further, these reevaluations will not be a routine or common event but rather will be considered for studies with marginally increased incidences in rare tumors to aid in interpretation of the lesions. Dr. J. Huff, NIEHS, commented that in most cases, such additional reevaluations would be performed before the studies are brought to the Panel for review.

# I. INTRODUCTION

**Physical Properties, Production, and Use**
**Absorption, Distribution, and Metabolism**
**Pharmacology**
**Toxicity**
**Reproductive and Developmental Toxicity**
**Genetic Toxicology**
**Study Rationale**

# I. INTRODUCTION




**FUROSEMIDE**

CAS No. 54-31-9

$C_{12}H_{11}ClN_2O_5S$ Molecular weight 330.8

Synonyms: 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid; frusemide; fursemide

Trade Names: Aisemide, Aluzine, Beronald, Desdemin, Diural, Dryptal, Errolon, Frusemin, Fulsix, Fuluvamide, Furosemide "Mita," Katlex, Lasilix, Lasix, Lowpstron, Rosemide, Transit, Urosemide

**Physical Properties, Production, and Use**

Furosemide is a crystalline material with a melting point of 206° C. It is slightly soluble in water and chloroform but completely soluble in acetone, methanol, dimethylformamide, and aqueous solutions above pH 8 (Merck, 1983).

Specific production data for furosemide are not available, but the number of prescriptions for furosemide in the United States increased from 16 million in 1973 to 23 million in 1981 (Whelton, 1986). The oral form of Lasix alone was the eighth most frequently prescribed drug in the United States in 1985 (Pharmacy Times, 1986). Furosemide is a potent, short-acting sulfonamide diuretic chemically similar to the thiazides, and it is used in a variety of situations ranging from the control of hypertension to the reduction of edema of cardiac, hepatic, or renal origin (Gilman et al., 1985). It is particularly useful in the management of acute pulmonary edema and may be used in premature infants to promote the diuresis that usually follows birth (Green et al., 1983). Therapeutic doses in humans range from 40 to 200 mg/day (Gilman et al., 1985), and 600 mg per day is the maximum recommended dose (AMA Drug Evaluations, 1983). If given intravenously to adult humans, a 40-mg dose of furosemide results in a peak plasma concentration of about 10 µg/ml (Chennavasin et al., 1979). In Sprague Dawley rats, intravenous doses of 0.5-1.5 mg/kg will result in effective diuretic concentrations of about 1-25 µg/ml in plasma (Smith and Benet, 1979).

**Absorption, Distribution, and Metabolism**

In general, the absorption, distribution, and metabolism of furosemide appear to be similar in humans and most laboratory animals. Absorption from the gastrointestinal tract is usually rapid but incomplete. About 65% of the dose is absorbed after oral administration in humans (Beermann, 1984) compared with about 50% in dogs (Yakatan et al., 1979). In one study with male Sprague Dawley rats, the bioavailability of oral furosemide was estimated to be only 30%, but 61% of the dose disappeared from the gastrointestinal tract. The authors speculated that metabolic conversion occurred in the stomach wall, and they demonstrated formation of unidentified metabolites after incubation of furosemide with a 9,000 × *g* supernatant fraction of washed stomach homogenates. This apparent metabolism was greater per gram of tissue in the stomach than in the small intestine, large intestine, or liver (Lee and Chiou, 1983).

Once in the bloodstream, 90%-99% of the furosemide binds avidly to albumin, with the extent of binding inversely related to the concentration of furosemide (Hammarlund and Paalzow, 1982). This extensive protein binding leads to a low apparent volume of distribution and suggests that little of the drug is available for diffusion into tissues. Furosemide clearance can be higher

than expected in patients with nephrotic syndrome because of increased glomerular filtration of furosemide bound to serum proteins (Smith et al., 1985). The pharmacokinetics of disappearance of furosemide from the blood are described best by two- or three-compartment open models. With a two-compartment model, the initial fast phase of elimination in humans has a $t_{\frac{1}{2}}$ of 10-15 minutes, followed by a slower phase with a $t_{\frac{1}{2}}$ of 47-70 minutes (Beermann, 1984). In rats, generally similar pharmacokinetic data best fit a three-compartment model, with dose-dependent variations apparently due to differences in plasma protein binding (Hammarlund and Paalzow, 1982).

Approximately one-half of the total clearance of furosemide from the blood of humans is accounted for by renal excretion of unchanged drug; perhaps as much as 25% undergoes glucuronidation prior to urinary excretion, and the remainder is cleared by undetermined means (Branch, 1983). In rats, about 4% of intravenously administered furosemide can be recovered from the gut (Lee and Chiou, 1983); in humans, clearance through the gastrointestinal tract has been described as contributing only minimally to furosemide disappearance (Valentine et al., 1986). In contrast, biliary excretion of furosemide has been reported to be as high as 30% of doses of 50-100 mg/kg given to male Swiss mice (Spitznagle et al., 1977). Renal excretion includes a small amount of free drug filtered at the glomerulus and a major fraction secreted through the organic anion secretory mechanism in the proximal tubule. Glucuronidation of furosemide appears to take place in the kidney because removal of the liver does not affect clearance of furosemide in dogs (Branch, 1983). Andreasen et al. (1983) found that in healthy young men (average age, 27 years), about 14% of an 80-mg dose of furosemide was recovered from the urine as the glucuronide conjugate in 24 hours. In older men (average age, 64 years), the glucuronide accounted for only 7% of the dose. The ontogeny of rat liver UDP-glucuronyltransferase towards furosemide from late gestation through 22 days of age was described by Rachmel and Hazelton (1986). Activity on day 18 of gestation was 26%; at birth, 48%; and on day 22 postpartum, 250% of the adult rat liver UDP-glucuronyl transferase activity.

**Pharmacology**

The primary pharmacologic action of furosemide accounting for its diuretic effect is inhibition of the reabsorption of chloride ions in the thick ascending limb of the loop of Henle. Primarily through this action, a practical maximum of 20%-25% inhibition of chloride ion reabsorption (and concomitantly, sodium ion reabsorption) can be achieved (Williamson, 1977). With compensating fluid and electrolyte replacement, sodium ion loss can rise to nearly 70% of the filtered load (Middendorf and Grantham, 1985). When diuretics are not used, more than 99% of the filtered sodium ions is reabsorbed by the kidney (Valtin, 1973).

Furosemide is active only on the luminal side of the tubule, and it appears to be actively secreted from plasma to its site of action by one or more of the organic anion transport mechanisms (Hook and Williamson, 1965; Sandstrom, 1986; Bidiville and Roch-Ramel, 1986). Once inside the tubule, furosemide apparently binds to and inhibits the $Na^+$-$2Cl^-$-$K^+$ cotransport system (Schlatter et al., 1983; Feig, 1986), an activity that has been tentatively linked to the Tamm-Horsfall protein, a glycoprotein localized in the cell membrane of the thick ascending limb of the loop of Henle (Greven, 1983).

In addition to its potent natriuretic action, furosemide has several other renal and nonrenal actions. Hemodynamic effects include an increase in renal blood flow (Hook et al., 1966) and decreases in mesenteric (Gaffney et al., 1978), hepatic (Gaffney et al., 1979), and splenic (Gaffney and Williamson, 1979) blood flow. The increase in renal blood flow can be antagonized by indomethacin and other prostaglandin synthetase inhibitors (Williamson et al., 1975a,b; Gerber, 1983). It is known that prostaglandins $E_2$, $I_2$, and $D_2$ are direct renal vasodilators and that prostaglandins $E_2$ and $I_2$ are associated with renin release (Whorton et al., 1980; Datar et al., 1987), which is part of the autoregulatory response that acts to limit the effectiveness of the diuretic. (Renin is an enzyme released from the cells of the afferent arteriole of the glomerulus

which promotes production of angiotensin I and, ultimately, of angiotensin II. Angiotensin II promotes secretion of aldosterone from the adrenal cortex, which in turn acts to increase renal tubular absorption of sodium ions [Melby, 1986].) The decreases in blood flow to the extrarenal organ systems are largely a result of volume depletion brought on by diuresis and are compounded by the increased plasma levels of angiotensin II, a vasoconstrictor, and of antidiuretic hormone that, in addition to increasing the permeability to water of the renal collecting duct, has a direct vasoconstrictive effect on mesenteric blood circulation (Schmitt et al., 1981). An additional indomethacin-sensitive action of furosemide is to decrease the left atrial pressure, an action that is independent of its diuretic effect but that may account in part for its efficacy in the treatment of hypertension. This action is apparently secondary to the release of vasoactive substances from the kidney, since it is not seen in anephric animals (Bourland et al., 1977).

Furosemide may also affect sodium ion resorption in the proximal tubule (Christensen et al., 1986), the distal tubule (Velazquez and Wright, 1986), and the collecting duct (Wilson et al., 1983). The evidence for direct effects of furosemide on these renal structures and on the various sodium ion and potassium ion pumps is less well established than that for its action on the ascending limb of the loop of Henle. The involvement of prostaglandins in the natriuretic action of furosemide in the different segments of the kidney is an area of active investigation (Gerber, 1983; Kirchner et al., 1986).

Furosemide at high concentrations has been shown to inhibit rat kidney glycolysis and, in particular, the enzyme glyceraldehyde 3-phosphate dehydrogenase (Yoshida and Metcoff, 1970), but this inhibition does not appear to be involved in or account for the inhibition of ion transport seen at pharmacologically relevant doses (Bowman et al., 1973).

The consequences of furosemide therapy on fluid and electrolyte balance include increased excretion of sodium, chloride, potassium, calcium, and bicarbonate ions and of water. Continued use leads to a systemic alkalosis, resulting from reduced extracellular volume not completely offset by the increase in bicarbonate excretion (Williamson, 1977; Bushinsky et al., 1986). Hydrogen ion excretion has been reported to be lowered (Williamson, 1977) or enhanced (Hropot et al., 1985) by furosemide. An additional effect, apparently resulting from the reduced extracellular volume, is a reduction in excretion of uric acid (Iwaki and Yonetani, 1984).

### Toxicity

In clinical studies, the incidence of adverse reactions in patients receiving furosemide has been estimated at about 6% (Tuzel, 1981). Most common were impaired hearing and vertigo, followed by rashes, pruritus, hives and sweating, muscle weakness, hypotension, and cramps. Allergic reactions have been reported to result in chronic aortitis (Sommers et al., 1984) and interstitial nephritis (Jennings et al., 1986; Magil, 1983). Severe diuresis can lead to dehydration and electrolyte imbalance (Council on Drugs, 1967). Potassium loss can be life threatening to patients in cardiac failure receiving diuretic therapy with furosemide (Lawson et al., 1982). Attempts to produce potassium loss from the myocardium of rats fed diets containing 1,500 ppm furosemide for 4 weeks were not successful, although a pronounced reduction of potassium and magnesium ion concentration in plasma and bone was observed (Borchgrevink et al., 1987). Calcium excretion remains elevated during long-term furosemide therapy in humans (Yu et al., 1981) and has resulted in several reports of renal calcification in infants (Hufnagle et al., 1982). Secondary hyperparathyroidism with bone demineralization has also been linked to long-term furosemide therapy in infants (Venkataraman et al., 1983). Attempts to recreate this effect in newborn rats resulted in a somewhat different effect. Subcutaneous doses of furosemide (5 or 15 mg/kg per day) were given to Sprague Dawley rat pups from day 4 to day 28 after birth (Koo et al., 1986). Increased urinary calcium and magnesium excretion was observed, and the total concentration of calcium and magnesium in bone was lower; the growth of the pups was inhibited in a dose-dependent manner, and bone mineral content was appropriate for the smaller bone mass.

Temporary hearing loss has been linked to blood concentrations of furosemide higher than 85-90 µg/ml (Rybak, 1985). This has been related to reduced endolymph potassium concentrations and a reduced action potential amplitude in the eighth (vestibulocochlear) cranial nerve. The stria vascularis is a portion of the outer wall of the cochlear duct and is active in endolymph production. It has been proposed that furosemide inhibits certain enzymes in this area, including carbonic anhydrase, $Na^+$-$K^+$ ATPase, and adenylate cyclase (Brown et al., 1985). In in vitro studies, glucose oxidation by the cochlea was inhibited by furosemide, and this inhibition was attributed to the specific inhibition of glyceraldehyde 3-phosphate dehydrogenase. The $I_{50}$ for inhibition of glucose oxidation was 1 µM. Similar inhibitory effects were seen in companion studies with kidney and liver, so the relevance of glucose oxidation inhibition by furosemide to the ototoxic effects of furosemide is unclear (Tachibana et al., 1985).

Some patients receiving prolonged furosemide therapy develop abnormal glucose tolerance (Jung and Mookerjee, 1976). This apparently is not due to impaired pancreatic function secondary to reduced blood flow from volume depletion (Holland and Williamson, 1984), nor is insulin secretion decreased in response to elevated blood glucose (Senft et al., 1966). Carrier-mediated glucose transport in erythrocytes is inhibited by furosemide at high concentrations, but the significance of this finding for the apparent glucose intolerance in humans is unclear (Jung and Mookerjee, 1976).

The oral $LD_{50}$ value for furosemide for 60-day-old Charles River CD® rats is approximately 2,700 mg/kg (NIOSH, 1987). Furosemide at doses of 400 mg/kg given to male Swiss albino mice by intraperitoneal injection produced massive necrosis in both the midzonal and centrilobular areas of the liver, damage that was prevented by prior administration of cytochrome P450 enzyme inhibitors (Mitchell et al., 1974). Covalent binding of furosemide to mouse liver proteins has been shown, and it was enhanced by administration of an inhibitor of epoxide hydrase, suggesting an arene oxide intermediate involving the furan ring (Wirth et al., 1976).

Furosemide at 0.5 or 1.0 mM is toxic to isolated mouse hepatocytes (Massey et al., 1987). Cellular sulfhydryl groups appear to be involved in protecting liver cells from furosemide toxicity at high concentrations. Furosemide is not known to be hepatotoxic in humans at normal therapeutic doses, but in vitro studies have shown that human liver microsomes are capable of converting furosemide to metabolites that bind irreversibly to microsomal proteins (Dybing, 1977a).

### Reproductive and Developmental Toxicity

Furosemide crosses the placental barrier in humans (Beermann et al., 1978). Although no adequate epidemiologic studies of pregnant women have been conducted, furosemide is not recommended for use during pregnancy (PDR, 1986). Administration of furosemide to pregnant CRCD rats on days 6 through 17 of gestation resulted in increased resorption rates and decreased live fetal weights at doses of 300 or 600 mg/kg; these doses also resulted in maternal deaths. There was no evidence of a teratogenic effect, but wavy ribs were noted in all dosed groups. In studies with other diuretics, skeletal malformations were shown to result from the diuretic effect on the mother (Robertson et al., 1981).

### Genetic Toxicology

Furosemide demonstrated no mutagenic activity when tested in the presence or absence of exogenous metabolic activation in a variety of *Salmonella typhimurium* strains (Minnich et al., 1976; Dybing, 1977b; Ishidate and Yoshikawa, 1980; Zeiger et al., 1987). In contrast to the negative results obtained in bacterial mutagenicity tests, tests for chromosomal effects have yielded some evidence of activity. When cultured Chinese hamster lung cells were exposed to furosemide at 0.5 and 2.0 mg/ml and harvested 24 hours later, chromosomal aberrations were reported to occur in 5% and 14% of the cells, respectively (Ishidate et al., 1978; Matsuoka et al., 1979); 11% of the cells treated with 2.0 mg/ml furosemide exhibited chromosomal breaks. A significant increase in chromosomal aberrations was reported in human lymphocytes exposed in vitro at 0.2-0.8 mg/ml furosemide for 24-72 hours; at the highest dose tested in the 24-hour protocol,

49% of the cells observed were classified as abnormal (Jameela et al., 1979). Chromosomal translocations, polyploidy, and pairing abnormalities were observed in the meiotic cells of C3H/HE male mice 1-5 weeks after a single injection of 0.0078-1.25 mg/kg furosemide (Subramanyam and Jameela, 1977).

**Study Rationale**

Furosemide was nominated for study by the Food and Drug Administration, Bureau of Drugs, based on a review of long-term toxicity and carcinogenicity data available on antihypertensive agents. There are no adequate epidemiologic studies that have examined the use of furosemide and the occurrence of cancer in humans. The feed route of exposure was chosen for these studies to mimic the oral route of administration of the drug in humans. Another diuretic, hydrochlorothiazide, has also been studied by the NTP by the feed route, and the results of these evaluations are presented in Technical Report No. 357 (NTP, 1989a).

# II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF FUROSEMIDE

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

- Study Design
- Source and Specifications of Animals
- Animal Maintenance
- Clinical Examinations and Pathology
- Statistical Methods

GENETIC TOXICOLOGY

## PROCUREMENT AND CHARACTERIZATION OF FUROSEMIDE

Furosemide was obtained from Hoechst-Roussel Pharmaceuticals in three lots (Table 1). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on analyses performed in support of the furosemide studies are on file at the National Institute of Environmental Health Sciences.

The identity of each lot was confirmed by spectroscopic analysis. The infrared (see representative Figure 1) and ultraviolet/visible spectra agreed with the literature spectra (Salim et al., 1968; Isolation and Identification of Drugs, 1969). The nuclear magnetic resonance spectra (see representative Figure 2) were consistent with that expected for the structure of furosemide. Purity was determined by elemental analyses, Karl Fischer water analysis, titration of the carboxylic acid group, thin-layer chromatography on silica gel plates with a mobile phase of either toluene:*p*-dioxane:isopropanol:ammonium hydroxide (20:30:30:15) (system 1) or methylethylketone:glacial acetic acid (98:2) (system 2) and visualization by ultraviolet light with a 1% *p*-dimethylaminobenzaldehyde spray, and high-performance liquid chromatography on a μBondapak $C_{18}$ column with a mobile phase of aqueous 2% acetic acid:2% acetic acid in methanol and ultraviolet detection at 280 nm. Results of elemental analyses of all lots were in agreement with the theoretical values.

Lot no. Y-3285 (USP grade) was obtained as a colorless powder with a melting point of 212.2° C. Cumulative data indicated that this lot contained 0.27% water and was approximately 99% pure. Titration of the carboxylic acid group with 0.1 N sodium hydroxide and with 0.1 N *t*-butylammonium hydroxide indicated purities of 99.2% and 99.5%, respectively. Thin-layer chromatography by system 1 indicated two trace impurity spots; no impurities were detected by system 2. High-performance liquid chromatography with a 50:50 solvent ratio at a flow rate of 1 ml/minute indicated four impurity peaks with a cumulative area 0.19% of the major peak; two impurity peaks with a total relative area of 0.12% were detected with a 60:40 solvent ratio at a flow rate of 2 ml/minute.

Lot no. H052880 was obtained as a white, microcrystalline powder. Cumulative data indicated that this lot contained 0.07% water and was approximately 99% pure. Titration with 0.1 N sodium hydroxide and with 0.1 N *t*-butylammonium hydroxide indicated purities of 98.8% and 99.2%, respectively. No impurities were detected by thin-layer chromatography with either system. High-performance liquid chromatography with a 68:32 solvent ratio at a flow rate of 1 ml/minute or with a 66:34 solvent ratio at a flow rate of 2 ml/minute detected no impurities with peak areas greater than or equal to 0.1% of the major peak area.

Lot no. Y-4050 was obtained as a white, microcrystalline solid. Cumulative data indicated that this lot was approximately 99% pure and contained less than 0.06% water. Titration with 0.1 N sodium hydroxide and with 0.1 N *t*-butylammonium hydroxide indicated purities of 99.7% and 99.6%, respectively. No impurities were detected by thin-layer chromatographic

TABLE 1. IDENTITY AND SOURCE OF FUROSEMIDE USED IN THE FEED STUDIES

| Fourteen-Day Studies | Thirteen-Week Studies | Two-Year Studies |
|---|---|---|
| **Lot Numbers** | | |
| Y-3285 | Y-3285; H052880 | Y-4050 |
| **Date of Initial Use** | | |
| 7/11/79 | Y-3285--5/20/80; H052880--7/80 | 5/1/81 |
| **Supplier** | | |
| Hoechst-Roussel Pharmaceuticals (Somerville, NJ) | Hoechst-Roussel Pharmaceuticals (Somerville, NJ) | Hoechst-Roussel Pharmaceuticals (Somerville, NJ) |

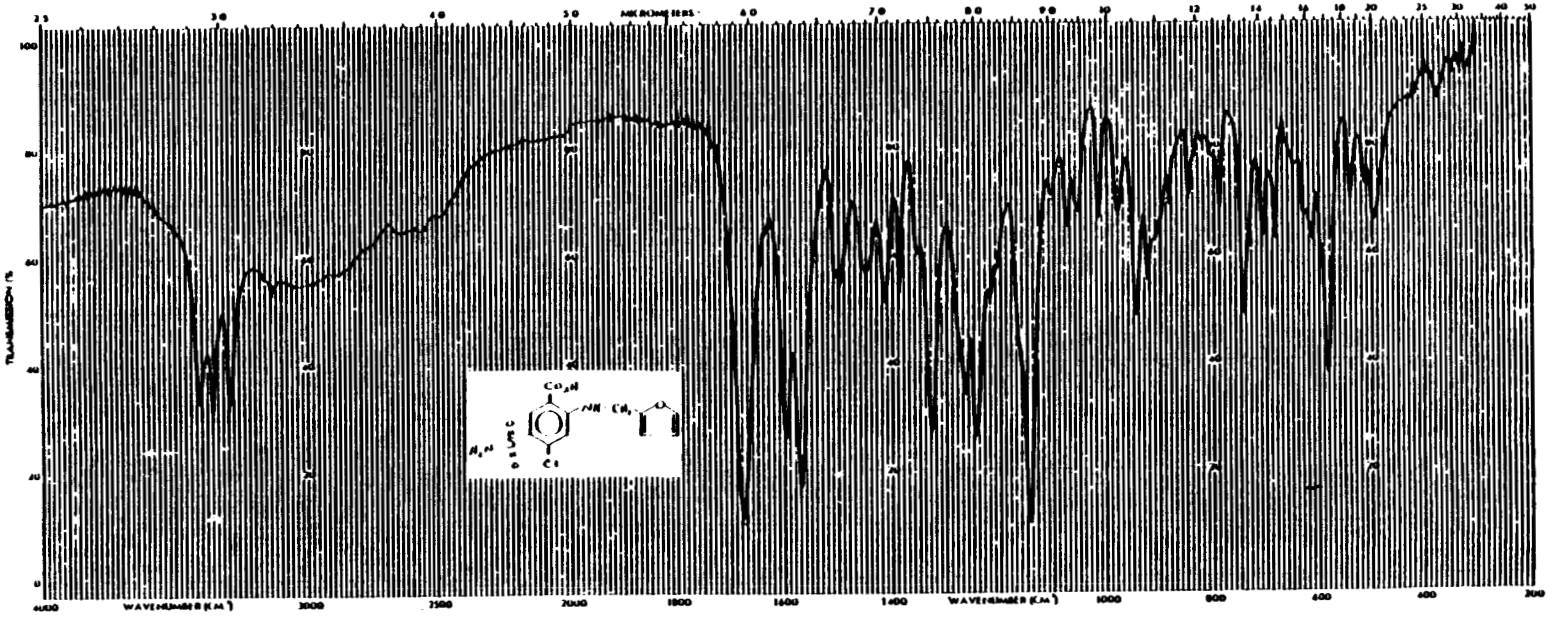


**FIGURE 1. INFRARED ABSORPTION SPECTRUM OF FUROSEMIDE (LOT NO. Y-4050)**

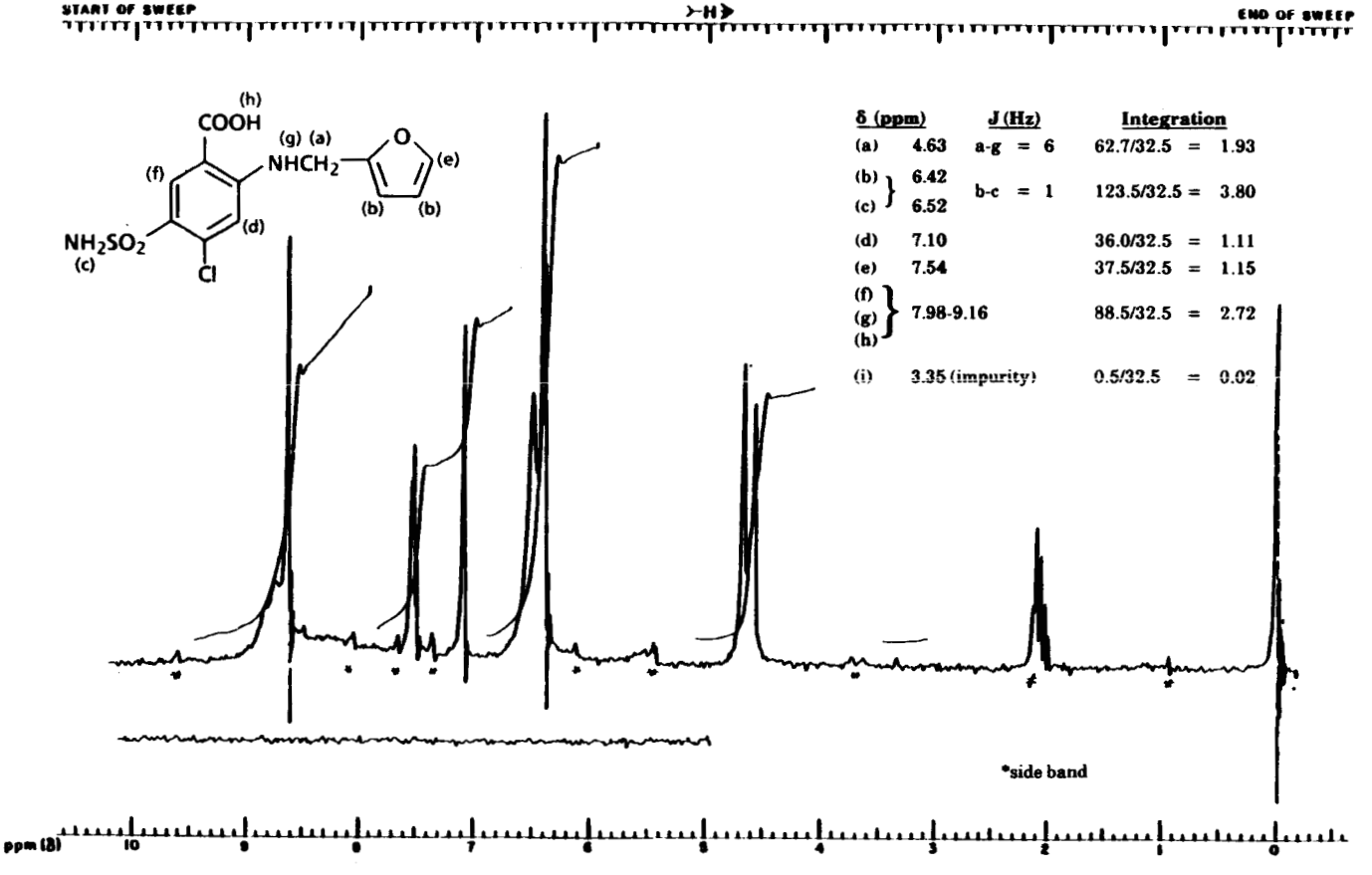



**FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF FUROSEMIDE (LOT NO. Y-4050)**

system 1. The second thin-layer chromatographic system detected a trace impurity. High-performance liquid chromatography with a 55:45 solvent ratio at a flow rate of 1 or 2 ml/minute detected no impurities with peak areas greater than or equal to 0.1% of the major peak area.

Stability studies performed by the high-performance liquid chromatographic system described above with a 50:50 solvent ratio at a flow rate of 2 ml/minute indicated that furosemide was stable as a bulk chemical when kept for 2 weeks in the dark at temperatures up to 60° C. Further confirmation of the stability of the bulk chemical during the toxicity studies (storage at room temperature) was obtained by titration with 0.1 N sodium hydroxide, and high-performance liquid chromatography on a Waters Radial Pak A column with a mobile phase of aqueous 1% acetic acid:methanol with 1% acetic acid (40:60) at a flow rate of 0.4-2 ml/minute and ultraviolet detection at 280 nm. Results of these analyses indicated that no degradation of the stored chemical occurred during the studies. The identity of the chemical at the study laboratory was confirmed by infrared spectroscopy.

## PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

Formulated diets were prepared by adding a dry premix of furosemide and feed to the appropriate amount of feed (Table 2). The mixture then was blended in a V-blender for 15 minutes. A study to determine the homogeneity of a 1,500-g, 10,000-ppm formulated diet mixture indicated less than 1% deviation in the concentration of samples taken from three locations in the blender. Stability studies showed that a blended diet containing 700 ppm furosemide when stored in the dark at 5° C exhibited losses of 2%, 7%, 9%, or 11% after 7, 14, 21, or 24 days; losses were approximately 13% when this formulated diet was stored under simulated animal room conditions for 3 or 7 days.

In the 13-week studies, formulated diets were stored in the dark at 5° C for no longer than 2 weeks. The formulated diets were analyzed two times over the course of the 13-week studies. The method consisted of extraction with acetone, a methanol dilution step, and addition of propiophenone as an internal standard. Analysis was

TABLE 2. PREPARATION AND STORAGE OF FORMULATED DIETS IN THE FEED STUDIES OF FUROSEMIDE

| Fourteen-Day Studies | Thirteen-Week Studies | Two-Year Studies |
|---|---|---|
| **Preparation** | | |
| Weighed chemical was mixed with equal weight of feed with spatula in glass beaker. Volume was doubled with more feed and the mixture was stirred; this procedure was repeated until approximately one-third of meal was mixed. Final mixing was in an 8-qt twin-shell blender with the premix layered between equal portions of feed for 5 min with the intensifier bar on and 10 min with the intensifier bar off | Same as 14-d studies; yellow fluorescent light used during preparation | Weighed amount of chemical and approximately 30 g of feed ground in a mortar and then handmixed with a spatula with one-third total feed required; premix and remaining feed mixed in an 8-qt, 16-qt, or 1-ft$^3$ twin-shell V-blender. Yellow fluorescent light used during mixing procedure |
| **Maximum Storage Time** | | |
| 1 wk | Same as 14-d studies | 24 d |
| **Storage Conditions** | | |
| 4° C or 5° C in the dark | 5° C in the dark | 5° C in the dark |

performed by high-performance liquid chromatography with a Waters RCM-100 $C_{18}$ column and with a mobile phase of 1% (v/v) aqueous acetic acid:1% (v/v) acetic acid in methanol (55:45). The formulated diets were tested for homogeneity at the highest and lowest dietary concentrations (Table 3). All concentrations of furosemide in feed were within ±10% of the target concentrations.

In the 2-year studies, formulated diets were stored in the dark at 5° C for up to 24 days. Analyses for furosemide in feed mixtures were conducted by the study and analytical chemistry laboratories to confirm that the desired concentrations were administered to the animals. During the initial part of the studies, the study laboratory used two methods of analysis: the high-performance liquid chromatographic method previously described for analyzing formulated feed samples, and a spectrophotometric method at 273 nm with an acetonitrile extraction. However, a major problem with dose analysis was observed at the start of the 2-year studies; of 56 formulated diets analyzed, 41 differed from the target concentrations by more than ±10%. Studies conducted at the analytical chemistry and study laboratories indicated that extraction of furosemide from the feed and stability of furosemide in the extracting solvent were the source of the problem. Methanolic extracts of the formulated diets were found to deteriorate rapidly, with a loss of approximately 17% after storage for 2 days at 5° C in the dark. From May 1982 to the end of the studies, the study laboratory used an extraction with 0.1 N potassium hydroxide in methanol followed by high-performance liquid chromatographic quantitation. This procedure provided satisfactory results.

TABLE 3. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF FUROSEMIDE

| Date Mixed | Concentration of Furosemide in Feed (ppm) | | Determined as a Percent of Target |
|---|---|---|---|
| | Target | Determined | |
| 05/23/80 | 625 | 614 | 98.2 |
| | 938 | 968 | 103.2 |
| | 1,250 | 1,262 | 101.0 |
| | 1,875 | 1,863 | 99.4 |
| | 2,500 | 2,412 | 96.5 |
| | 3,750 | 3,516 | 93.8 |
| | 5,000 | 4,913 | 98.3 |
| | 7,500 | 7,358 | 98.1 |
| | 10,000 | 9,634 | 96.3 |
| | 15,000 | 15,663 | 104.4 |
| | 20,000 | 20,333 | 101.7 |
| 06/12/80 | 625 | (a) 520 | (b) 83.2 |
| (homogeneity study) | 625 | (c) 514 | (b) 82.2 |
| | 625 | (d) 576 | 92.2 |
| | 20,000 | (a) 20,734 | 103.7 |
| | 20,000 | (c) 21,852 | 109.3 |
| | 20,000 | (d) 20,858 | 104.3 |
| 07/17/80 | 1,250 | 1,164 | 93.0 |
| | 1,875 | 1,731 | 92.3 |
| | 2,500 | 2,325 | 93.0 |
| | 3,750 | 3,719 | 99.2 |
| | 5,000 | 5,086 | 101.7 |
| | 7,500 | 7,301 | 97.3 |
| | 10,000 | 10,197 | 102.0 |
| | 15,000 | 15,657 | 104.4 |
| | 20,000 | 21,070 | 105.4 |

(a) Sample taken from top right of blender
(b) Out of specifications
(c) Sample taken from top left of blender
(d) Sample taken from bottom of blender

During the 2-year studies, the formulated diets were analyzed at approximately 8-week intervals, but after December 16, 1982, every eighth blend was analyzed. All mixtures were formulated within ±10% of the target concentrations after the basic methanol extraction procedure was used beginning in May 1982 (Table 4). Referee analyses were periodically performed by the analytical chemistry laboratory. Agreement was found between laboratories after the analytical methods were modified (Table 5).

TABLE 4. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE

| Date Mixed | Concentration of Furosemide in Feed for Target Concentration (ppm) (a) | | |
|---|---|---|---|
| | 350 | 700 | 1,400 |
| 05/12/82 | 320 | 653 | 1,310 |
| 06/09/82 | 321 | 654 | 1,292 |
| 08/04/82 | 360 | 702 | 1,360 |
| 09/29/82 | 318 | 658 | 1,430 |
| 11/24/82 | 336 | 708 | 1,400 |
| 01/05/83 | 347 | 697 | 1,390 |
| 02/16/83 | 352 | 675 | 1,340 |
| 03/30/83 | (b) 330 | 672 | (b) 1,347 |
| Mean (ppm) | 336 | 677 | 1,359 |
| Standard deviation | 16.0 | 22.3 | 46.4 |
| Coefficient of variation (percent) | 4.8 | 3.3 | 3.4 |
| Range (ppm) | 318-360 | 653-708 | 1,292-1,430 |
| Number of samples | 8 | 8 | 8 |

(a) Results of duplicate analysis unless otherwise specified; analytical procedures used before 5/12/82 gave low results and are excluded from this table.
(b) Results of five analyses

TABLE 5. RESULTS OF REFEREE ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE

| Date Mixed | Target Concentration (ppm) | Determined Concentration (ppm) | |
|---|---|---|---|
| | | Study Laboratory (a) | Referee Laboratory (b) |
| 08/04/82 | 350 | 360 | 324 |
| 02/16/83 | 1,400 | 1,340 | 1,440 |

(a) Results of duplicate analysis
(b) Results of triplicate analysis

## FOURTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and held for 20 days before the studies began. Rats were 7-8 weeks old when placed on study, and mice were 7-9 weeks old. Groups of five rats and five mice of each sex were fed diets containing 0, 570, 1,700, 5,100, 15,300, or 46,000 ppm furosemide for 14 consecutive days. The rats and mice were observed two times per day and weighed on days 0, 7, and 14. A necropsy was performed on all animals. Details of animal maintenance are presented in Table 6.

## THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to furosemide and to determine the concentrations to be used in the 2-year studies.

Six-week-old male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries, observed for 13 or 14 days, distributed to weight classes, and then assigned to dosed or control groups according to a table of random numbers.

Groups of 9 or 10 male rats were given diets containing 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm furosemide for 13 weeks. Groups of 10 female rats and 10 male mice were given diets containing 0, 938, 1,875, 3,750, 7,500, or 15,000 ppm on the same schedule, and groups of 10 female mice were fed diets containing 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm. Control diets consisted of NIH 07 Rat and Mouse Ration. Formulated or control diets and water were available ad libitum.

Animals were observed one time per day; moribund animals were killed. Feed consumption was measured one time per week by cage. Individual animal weights were recorded one time per week. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 6.

## TWO-YEAR STUDIES

### Study Design

Groups of 50 rats of each sex were administered diets containing 0, 350, or 700 ppm furosemide for 103 weeks (rats) or 104 weeks (mice). Groups of 50 mice of each sex were administered diets containing 0, 700, or 1,400 ppm furosemide.

### Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female $\times$ C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories under a contract to the Carcinogenesis Program. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats were shipped to the study laboratory at 5 weeks of age and mice at 6 weeks of age. The rats were quarantined at the study facility for 15 days and the mice for 18 days. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rats were placed on study at 7 weeks of age and the mice at 8 weeks of age. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix E).

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid B6C3F$_1$ study animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci.

The C57BL/6N mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF FUROSEMIDE

| Fourteen-Day Studies | Thirteen-Week Studies | Two-Year Studies |
|---|---|---|
| **EXPERIMENTAL DESIGN** | | |
| **Size of Study Groups** | | |
| 5 males and 5 females of each species | 10 males and 10 females of each species (9 male rats in the 1,250- and 10,000-ppm groups) | 50 males and 50 females of each species |
| **Doses** | | |
| 0, 570, 1,700, 5,100, 15,300, or 46,000 ppm furosemide in feed | Rats--male: 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm furosemide in feed; female: 0, 938, 1,875, 3,750, 7,500, or 15,000 ppm; mice--male: 0, 938, 1,875, 3,750, 7,500, or 15,000 ppm; female: 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm | Rats--0, 350, or 700 ppm furosemide in feed; mice--0, 700, or 1,400 ppm |
| **Date of First Dose** | | |
| 7/11/79 | Rats--5/21/80; mice--5/20/80 | Rats--5/1/81; mice--5/4/81 |
| **Date of Last Dose** | | |
| 7/24/79 | Rats--8/20/80; mice--8/19/80 | Rats--350-ppm groups, 4/26/83; 700-ppm groups, 4/22/83; mice--700-ppm groups, 5/10/83; 1,400-ppm groups, 5/5/83 |
| **Duration of Dosing** | | |
| 14 consecutive d | 13 wk | Rats--103 wk; mice--104 wk |
| **Type and Frequency of Observation** | | |
| Observed 2 × d; weighed initially and 1 × wk thereafter | Observed 1 × d for deaths and 1 × wk for clinical signs; weighed initially and 1 × wk thereafter; feed consumption measured 1 × wk | Observed 2 × d; weighed initially, 1 × wk for 13 wk, and then 1 × mo; feed consumption measured 1 × mo |
| **Necropsy and Histologic Examinations** | | |
| Necropsy performed on all animals; histologic exams performed on kidney and liver of all animals | Necropsy performed on all animals; the following tissues examined histologically for control and high dose groups: adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur or sternebrae or vertebrae including marrow, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, skin, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Tissues examined from lower dose groups in rats include liver and kidneys from the 7,500-ppm male and 10,000-ppm female groups; tissues examined from lower dose groups in mice include liver and kidneys from 7,500-ppm male and 10,000-ppm female groups and kidneys from the remaining dose groups. Liver weights recorded at necropsy | Necropsy performed on all animals; histologic exams performed on low dose female mice, all controls, all high dose animals, and all animals that died before the end of the studies; tissues examined include adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, skin, spinal cord, spleen, sternebrae and vertebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. Tissues examined in the low dose groups include brain, gross lesions, kidneys, pituitary gland, and thyroid gland for male rats; clitoral gland, gross lesions, liver, pituitary gland, and thyroid gland for female rats; adrenal glands, bone marrow, brain, gross lesions, kidneys, liver, pituitary gland, prostate, spleen, thyroid gland, and urinary bladder for male mice |

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF FUROSEMIDE (Continued)

| Fourteen-Day Studies | Thirteen-Week Studies | Two-Year Studies |
|---|---|---|
| **ANIMALS AND ANIMAL MAINTENANCE** | | |
| **Strain and Species**<br>F344/N rats; B6C3F$_1$ mice | F344/N rats; B6C3F$_1$ mice | F344/N rats; B6C3F$_1$ mice |
| **Animal Source**<br>Charles River Breeding Laboratories (Portage, MI) | Harlan Industries (Indianapolis, IN) | Charles River Breeding Laboratories (Portage, MI) |
| **Study Laboratory**<br>SRI International | SRI International | SRI International |
| **Method of Animal Identification**<br>Ear clip | Ear clip | Ear punch |
| **Time Held Before Study**<br>20 d | Rats--14 d; mice--13 d | Rats--15 d; mice--18 d |
| **Age When Placed on Study**<br>Rats--7-8 wk; mice--7-9 wk | 8 wk | Rats--7 wk; mice--8 wk |
| **Age When Killed**<br>Rats--9-10 wk; mice--9-11 wk | 21 wk | Rats--111-113 wk; mice--113-115 wk |
| **Necropsy Dates**<br>7/26/79 | Rats--8/21/80-8/22/80; mice--8/20/80-8/21/80 | Rats--4/29/83-5/10/83; mice--5/11/83-5/20/83 |
| **Method of Animal Distribution**<br>Distributed to weight classes and then assigned to groups according to a table of random numbers | Distributed to weight classes and then assigned to cages by one table of random numbers and to groups by another table of random numbers | Same as 13-wk studies |
| **Feed**<br>Rodent Laboratory Chow 5001® (Ralston Purina Co., St. Louis, MO); available ad libitum | NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum | Same as 13-wk studies |
| **Bedding**<br>Hardwood chips (Pressed Wood, Inc.) | Absorb-Dri (Lab Products, Inc., Maywood, NY) | Same as 13-wk studies |
| **Water**<br>Automatic watering system (Systems Engineering, Napa, CA); deionized water sterilized by ultraviolet radiation; available ad libitum | Automatic watering system (Systems Engineering, Napa, CA); deionized and filtered water sterilized by ultraviolet radiation; available ad libitum | Same as 13-wk studies |
| **Cages**<br>Polycarbonate (Lab Products, Inc., Rochelle Park, NJ) | Same as 14-d studies | Same as 14-d studies |
| **Cage Filters**<br>Nonwoven polyester fiber (Lab Products, Rochelle Park, NJ, or Research Equipment Co., Bryon, TX) | Filter sheets (Lab Products, Inc., Rochelle Park, NJ) | Nonwoven fiber filters (Snow Filtration, Cincinnati, OH) |
| **Animals per Cage**<br>5 | 5 | 5 |

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF FUROSEMIDE (Continued)

| Fourteen-Day Studies | Thirteen-Week Studies | Two-Year Studies |
|---|---|---|
| **ANIMALS AND ANIMAL MAINTENANCE (Continued)** | | |
| **Other Chemicals on Study in the Same Room** | | |
| None | None | None |
| **Animal Room Environment** | | |
| Temp--64°-79° F; hum--45%-55%; fluorescent light 12 h/d; 12-15 room air changes/h | Temp--74°-78° F; hum--46%-76%; fluorescent light 12 h/d; 13 room air changes/h | Temp--67°-80° F ; hum--19%-88%; fluorescent light 12 h/d; 11-17 room air changes/h |

monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6N colony were used as parents for the hybrid B6C3F$_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

### Animal Maintenance

Rats and mice were housed five per cage. Feed and water were available ad libitum. Cages were not rotated during the studies. Further details on animal maintenance are given in Table 6.

### Clinical Examinations and Pathology

All animals were observed two times per day, and clinical signs were recorded at least once per month. Body weights were recorded once per week for the first 13 weeks of the study and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals including those found dead, unless they were excessively autolyzed or cannibalized, missexed, or missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examination of tissues was performed according to an "inverse pyramid" design (McConnell, 1983a,b). That is, complete histopathologic examinations (Table 6) were performed on all high dose and control animals and on low dose animals dying before the end of the study. In addition, histopathologic examinations were performed on all grossly visible lesions in all dose groups. Potential target organs for chemically related neoplastic and nonneoplastic effects were identified from the short-term studies or the literature and were determined by examination of the pathology data; these target organs/tissues in the lower dose group were examined histopathologically. If mortality in the highest dose group exceeded that in the control group by 15%, complete histopathologic examinations were performed on all animals in the second highest dose group in addition to those in the high dose group.

When the pathology evaluation was completed, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor

diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG without knowledge of previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

### Statistical Methods

*Data Recording:* Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathology results, as recommended by the International Union Against Cancer (Berenblum, 1969).

*Survival Analyses:* The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found to be missing or dead from other than natural causes; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

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

*Analysis of Tumor Incidence:* Three statistical methods are used to analyze tumor incidence data: life table tests, incidental tumor analysis, and Fisher exact/Cochran-Armitage trend analyses. Tests of significance include pairwise comparisons of high dose and low dose groups with controls and tests for overall dose-response trends. For studies in which administration of the study compound has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described below also were used to evaluate selected nonneoplastic lesions.

Life Table Analyses--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method (1959) to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.) A method for the analysis of incidental tumors based on logistic regression (Dinse and Lagakos, 1983) was also employed as a supplemental test in some instances. This method has the advantage of not requiring time intervals in the statistical evaluation.

Fisher Exact/Cochran-Armitage Trend Analyses--In addition to survival-adjusted methods, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendixes containing the analyses of tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

*Historical Control Data:* Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

## GENETIC TOXICOLOGY

*Salmonella Protocol:* Testing was performed as reported by Ames et al. (1975) with modifications listed below and described in greater detail by Mortelmans et al. (1986) and Zeiger et al. (1987). Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, Texas). The study chemical was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37° C before the addition of soft agar supplemented with L-histidine and D-biotin and subsequent plating on minimal glucose agar plates. Incubation was continued for an additional 48 hours.

Chemicals were tested in a series (four strains used) or in a hierarchy (initial testing in TA98 and TA100; if results were negative, then the chemical was tested further in additional strains). If all results were negative, the chemical was retested in all strains with a different concentration of S9.

Each test consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of the study chemical. The high dose was limited by toxicity or solubility but did not exceed 10 mg/plate. All negative assays were repeated, and all positive assays were

repeated under the conditions that elicited the positive response.

A positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants which was not dose related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A response was considered negative when no increase in revertant colonies was observed after chemical treatment.

*Mouse Lymphoma Protocol:* The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). All study chemicals were supplied as coded aliquots from Radian Corporation (Austin, Texas). The highest dose of the study compound was determined by solubility or toxicity and did not exceed 5 mg/ml. Mouse lymphoma L5178Y cells were maintained at 37° C as suspension cultures in Fischer's medium supplemented with 2 mM L-glutamine, 110 µg/ml sodium pyruvate, 0.05% pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was about 10 hours. To reduce the number of spontaneously occurring trifluorothymidine (Tft)-resistant cells, subcultures were exposed once to medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day, to thymidine, hypoxanthine, and glycine for 1 day, and to normal medium for 3-5 days. For cloning, horse serum content was increased and Noble agar was added. Freshly prepared S9 metabolic activation factors were obtained from the liver of either Aroclor 1254-induced or noninduced male F344 rats.

All doses within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained $6 \times 10^6$ cells in 10 ml of medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with the study chemical continued for 4 hours, after which time the medium plus chemical was removed and the cells were resuspended in 20 ml of fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, $3 \times 10^6$ cells were plated in medium and soft agar supplemented with Tft for selection of Tft-resistant cells ($TK^{+/+}$), and 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C under 5% carbon dioxide for 10-12 days. All data were evaluated statistically for both trend and peak response. Both responses had to be significant ($P<0.05$) for a chemical to be considered capable of inducing Tft resistance; a single significant response led to an "equivocal" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Myhr et al. (1985). This assay was initially performed without S9; if a clearly positive response was not obtained, the experiment was repeated with induced S9.

*Chinese Hamster Ovary Cytogenetics Assays:* Testing was performed as reported by Galloway et al. (1985, 1987) and is described briefly below. Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, Texas). Chemicals were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine (BrdU)-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of the study chemical; the high dose was limited by toxicity or solubility but did not exceed 5 mg/ml.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing the study chemical was removed and replaced with fresh medium plus BrdU and colcemid, and incubation was continued for 2 more hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258

and Giemsa. In the SCE test with S9, cells were incubated with the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no study chemical; incubation proceeded for an additional 26 hours, with colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the chromosomal aberration test without S9, cells were incubated in McCoy's 5A medium with the study chemical for 8 hours; colcemid was added, and incubation was continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the chromosomal aberration test with S9, cells were treated with the study chemical and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

For the SCE test, if significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable cells. The harvest time for the chromosomal aberration test was based on the cell cycle information obtained in the SCE test; if cell cycle delay was anticipated, the incubation period was extended approximately 5 hours.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype ($21 \pm 2$ chromosomes). All slides were scored blind, and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were usually scored for frequency of SCEs per cell from each dose; 100 (more recently, 200) first-division metaphase cells were scored at each dose for the chromosomal aberration test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Chromosomal aberration data are presented as percentage of cells with aberrations. As with SCEs, both the dose-response curve and individual dose points were statistically analyzed. A statistically significant ($P<0.003$) trend test or a significantly increased dose point ($P<0.05$) was sufficient to indicate a chemical effect.

# III. RESULTS

## RATS

### FOURTEEN-DAY STUDIES

### THIRTEEN-WEEK STUDIES

### TWO-YEAR STUDIES

#### Body Weights, Feed Consumption, and Clinical Signs

#### Survival

#### Pathology and Statistical Analyses of Results

## MICE

### FOURTEEN-DAY STUDIES

### THIRTEEN-WEEK STUDIES

### TWO-YEAR STUDIES

#### Body Weights, Feed Consumption, and Clinical Signs

#### Survival

#### Pathology and Statistical Analyses of Results

## GENETIC TOXICOLOGY

## FOURTEEN-DAY STUDIES

Two of five males and 3/5 females that received 46,000 ppm furosemide died before the end of the studies (Table 7). Rats that received 15,300 or 46,000 ppm lost weight over the course of the studies. The final mean body weights of rats that received 1,700 or 5,100 ppm were 12% or 23% lower than that of controls for males and 8% or 16% lower for females. Rough fur, hunched backs, and depression were observed from day 5 to the end of the studies for rats that received 15,300 or 46,000 ppm. A dark exudate from the nose was detected after day 6 for males and after day 8 for females in the 46,000-ppm groups. Minimal-to-mild nephrosis was found in all rats that received 15,300 or 46,000 ppm and in one male that received 5,100 ppm. Microscopically, the lesion was subcapsular or cortical and was characterized by tubular cell regeneration; mineralization was also present at the corticomedullary junction.

## THIRTEEN-WEEK STUDIES

None of the rats died before the end of the studies (Table 8). The final mean body weights of male rats that received 2,500, 5,000, or 10,000 ppm furosemide were 11%, 22%, or 44% lower than that of controls. The final mean body weights of females that received 3,750, 7,500, or 15,000 ppm were 18%, 26%, or 35% lower than that of controls. Feed consumption by male rats that received 10,000 ppm was lower than that by controls. The liver weight to body weight ratios for males that received 1,250 ppm or more furosemide and females that received 15,000 ppm were significantly greater than those for controls (Table 9). The fur was rough for males that received 10,000 ppm and for females that received 7,500 or 15,000 ppm. Diuresis was observed from week 4 to week 13 for all dosed groups. The severity of the diuresis appeared to increase with increased dose. A compound-related, minimal-to-moderate nephrosis occurred

TABLE 7. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY FEED STUDIES OF FUROSEMIDE

| Concentration (ppm) | Survival (a) | Mean Body Weights (grams) Initial (b) | Final | Change (c) | Final Weight Relative to Controls (percent) |
|---|---|---|---|---|---|
| **MALE** | | | | | |
| 0 | 5/5 | 150 ± 3 | 219 ± 3 | +69 ± 2 | |
| 570 | 4/4 | 144 ± 3 | 204 ± 4 | +60 ± 2 | 93 |
| 1,700 | 5/5 | 137 ± 6 | 192 ± 7 | +55 ± 2 | 88 |
| 5,100 | 5/5 | 147 ± 3 | 169 ± 3 | +22 ± 2 | 77 |
| 15,300 | 5/5 | 137 ± 2 | 100 ± 2 | −37 ± 3 | 46 |
| 46,000 | (d) 3/5 | 142 ± 4 | 86 ± 4 | −59 ± 3 | 39 |
| **FEMALE** | | | | | |
| 0 | 5/5 | 128 ± 5 | 153 ± 5 | +25 ± 1 | |
| 570 | 5/5 | (e) | 154 ± 2 | (e) | 101 |
| 1,700 | 5/5 | 126 ± 5 | 141 ± 4 | +15 ± 2 | 92 |
| 5,100 | 5/5 | 121 ± 3 | 129 ± 2 | +8 ± 2 | 84 |
| 15,300 | 5/5 | 123 ± 4 | 92 ± 2 | −31 ± 3 | 60 |
| 46,000 | (f) 2/5 | 121 ± 3 | 83 ± 4 | −46 ± 1 | 54 |

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean weight change of the survivors ± standard error of the mean
(d) Day of death: 8,11
(e) Initial body weight data not available
(f) Day of death: 7,11,13

TABLE 8. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF FUROSEMIDE

| Concentration (ppm) | Survival (a) | Mean Body Weights (grams) | | | Final Weight Relative to Controls (percent) | Feed Consumption (d) | |
|---|---|---|---|---|---|---|---|
| | | Initial (b) | Final | Change (c) | | Week 7 | Week 13 |
| **MALE** | | | | | | | |
| 0 | 10/10 | 118 ± 3 | 347 ± 5 | +229 ± 6 | | 17.3 | 20.9 |
| 625 | 10/10 | 114 ± 4 | 340 ± 4 | +226 ± 6 | 98 | 14.6 | 19.6 |
| 1,250 | 9/9 | 115 ± 3 | 337 ± 5 | +222 ± 6 | 97 | 17.3 | 19.1 |
| 2,500 | 10/10 | 115 ± 3 | 310 ± 7 | +195 ± 8 | 89 | 17.1 | 18.3 |
| 5,000 | 10/10 | 114 ± 3 | 272 ± 5 | +158 ± 5 | 78 | 16.3 | 17.7 |
| 10,000 | 9/9 | 117 ± 3 | 194 ± 3 | +77 ± 5 | 56 | 16.5 | 12.5 |
| **FEMALE** | | | | | | | |
| 0 | 10/10 | 98 ± 2 | 195 ± 3 | +97 ± 4 | | 12.0 | 10.9 |
| 938 | 10/10 | 97 ± 3 | 195 ± 3 | +98 ± 4 | 100 | 11.7 | 11.8 |
| 1,875 | 10/10 | 97 ± 2 | 184 ± 4 | +87 ± 3 | 94 | 11.3 | 11.6 |
| 3,750 | 10/10 | 98 ± 2 | 159 ± 3 | +61 ± 4 | 82 | 11.5 | 11.4 |
| 7,500 | 10/10 | 98 ± 3 | 144 ± 3 | +46 ± 2 | 74 | 12.1 | 13.3 |
| 15,000 | 10/10 | 89 ± 4 | 126 ± 2 | +37 ± 3 | 65 | 13.4 | 15.9 |

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.

TABLE 9. ANALYSIS OF LIVER WEIGHTS OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF FUROSEMIDE (a)

| Concentration (ppm) | No. of Animals Weighed | Necropsy Body Weight (grams) | Liver Weight (mg) | Liver Weight/ Body Weight (mg/g) |
|---|---|---|---|---|
| **MALE** | | | | |
| 0 | (b) 10 | 326 ± 5.1 | 10,333 ± 269 | 31.5 ± 0.6 |
| 625 | 10 | 321 ± 3.8 | 11,471 ± 514 | 35.7 ± 1.4 |
| 1,250 | 9 | 328 ± 6.3 | 12,632 ± 966 | (c) 38.4 ± 2.5 |
| 2,500 | 10 | 307 ± 8.4 | 12,068 ± 765 | (c) 38.9 ± 1.6 |
| 5,000 | 10 | (d) 264 ± 7.3 | 11,039 ± 581 | (d) 41.7 ± 1.8 |
| 10,000 | 9 | (d) 194 ± 5.1 | 9,367 ± 520 | (d) 48.0 ± 1.6 |
| **FEMALE** | | | | |
| 0 | 10 | 193 ± 2.9 | 7,050 ± 656 | 36.3 ± 3.0 |
| 938 | 10 | 187 ± 2.6 | 6,042 ± 298 | 32.4 ± 1.6 |
| 1,875 | 10 | 183 ± 3.5 | 7,144 ± 352 | 39.0 ± 1.6 |
| 3,750 | 10 | (d) 154 ± 2.6 | 6,412 ± 349 | 41.5 ± 1.9 |
| 7,500 | 10 | (d) 141 ± 4.4 | 6,225 ± 592 | 43.4 ± 3.0 |
| 15,000 | 10 | (d) 119 ± 2.8 | 5,943 ± 366 | (d) 49.5 ± 2.0 |

(a) Mean ± standard error; P values vs. the controls by Dunnett's test (Dunnett, 1955).
(b) One liver weight not recorded; ratio is based on nine animals.
(c) $P<0.05$
(d) $P<0.01$

at the two highest doses in male and female rats. This lesion consisted of tubular cell degeneration and regeneration with tubular dilatation. In some rats, minimal interstitial fibrosis and mononuclear cell inflammation were associated with the areas of regeneration. Nephrosis was present in 10/10 or 9/10 males that received 5,000 or 10,000 ppm and in 9/10 or 10/10 females that received 7,500 or 15,000 ppm. Mineralization at the renal corticomedullary junction was observed in all male rat groups receiving 625 ppm or more. The severity (minimal-to-mild) of the mineralization increased with increased dose. Mineralization was observed in all males that received 2,500, 5,000, or 10,000 ppm, in 8/10 that received 1,250 ppm, and in 1/10 that received 625 ppm.

*Dose Selection Rationale:* Because of kidney lesions observed in all dose groups above 625 ppm, dietary concentrations of furosemide selected for rats for the 2-year studies were 350 and 700 ppm.

## TWO-YEAR STUDIES

### Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of dosed and control rats were comparable throughout the studies (Table 10 and Figure 3). The average daily feed consumption per rat by low dose or high dose rats was 100% or 101% that by controls for males and 102% or 101% for females (Tables F1 and F2). The estimated average amount of furosemide consumed per day was approximately 14 or 29 mg/kg for low dose or high dose male rats and 16 or 31 mg/kg for low dose or high dose female rats. No compound-related clinical signs were observed, and there was no clear evidence of diuresis (such as wet bedding) in dosed groups.

## TABLE 10. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE

| Weeks on Study | Control | | 350 ppm | | | 700 ppm | | |
|---|---|---|---|---|---|---|---|---|
| | Av. Wt. (grams) | No. of Survivors | Av. Wt. (grams) | Wt. (percent of controls) | No. of Survivors | Av. Wt. (grams) | Wt. (percent of controls) | No. of Survivors |
| **MALE** | | | | | | | | |
| 0 | 139 | 50 | 139 | 100 | 50 | 138 | 99 | 50 |
| 1 | 179 | 50 | 178 | 99 | 50 | 174 | 97 | 50 |
| 2 | 217 | 50 | 216 | 100 | 50 | 211 | 97 | 50 |
| 3 | 243 | 50 | 245 | 101 | 50 | 240 | 99 | 50 |
| 4 | 266 | 50 | 269 | 101 | 50 | 264 | 99 | 50 |
| 5 | 285 | 50 | 287 | 101 | 50 | 282 | 99 | 50 |
| 6 | 301 | 50 | 302 | 100 | 50 | 298 | 99 | 50 |
| 7 | 315 | 50 | 318 | 101 | 50 | 312 | 99 | 50 |
| 8 | 330 | 50 | 330 | 100 | 50 | 326 | 99 | 50 |
| 9 | 340 | 50 | 342 | 101 | 50 | 338 | 99 | 50 |
| 10 | 349 | 50 | 351 | 101 | 50 | 345 | 99 | 50 |
| 11 | 360 | 50 | 364 | 101 | 50 | 359 | 100 | 50 |
| 12 | 368 | 50 | 373 | 101 | 50 | 367 | 100 | 50 |
| 13 | 376 | 50 | 381 | 101 | 50 | 374 | 99 | 50 |
| 16 | 393 | 50 | 402 | 102 | 50 | 394 | 100 | 50 |
| 20 | 413 | 50 | 421 | 102 | 50 | 411 | 100 | 50 |
| 24 | 433 | 50 | 444 | 103 | 50 | 433 | 100 | 50 |
| 28 | 446 | 50 | 455 | 102 | 50 | 445 | 100 | 50 |
| 32 | 463 | 50 | 472 | 102 | 50 | 461 | 100 | 50 |
| 37 | 472 | 50 | 485 | 103 | 50 | 473 | 100 | 50 |
| 42 | 490 | 50 | 501 | 102 | 50 | 490 | 100 | 50 |
| 46 | 490 | 50 | 502 | 102 | 50 | 490 | 100 | 50 |
| 50 | 495 | 49 | 509 | 103 | 48 | 499 | 101 | 49 |
| 54 | 502 | 49 | 516 | 103 | 48 | 504 | 100 | 49 |
| 58 | 503 | 48 | 516 | 103 | 48 | 504 | 100 | 48 |
| 62 | 506 | 48 | 516 | 102 | 48 | 500 | 99 | 48 |
| 67 | 499 | 48 | 510 | 102 | 44 | 496 | 99 | 47 |
| 71 | 499 | 46 | 512 | 103 | 43 | 491 | 98 | 46 |
| 75 | 500 | 44 | 513 | 103 | 43 | 492 | 98 | 44 |
| 79 | 496 | 42 | 505 | 102 | 42 | 488 | 98 | 43 |
| 83 | 493 | 40 | 496 | 101 | 39 | 480 | 97 | 42 |
| 90 | 485 | 35 | 487 | 100 | 28 | 471 | 97 | 34 |
| 93 | 475 | 31 | 486 | 102 | 24 | 463 | 97 | 32 |
| 98 | 449 | 26 | 467 | 104 | 19 | 454 | 101 | 27 |
| 103 | 443 | 17 | 462 | 104 | (a) 16 | 430 | 97 | 21 |
| **FEMALE** | | | | | | | | |
| 0 | 116 | 50 | 117 | 101 | 50 | 114 | 98 | 50 |
| 1 | 136 | 50 | 135 | 99 | 50 | 133 | 98 | 50 |
| 2 | 153 | 50 | 150 | 98 | 50 | 147 | 96 | 50 |
| 3 | 164 | 50 | 162 | 99 | 50 | 160 | 98 | 50 |
| 4 | 173 | 50 | 172 | 99 | 50 | 170 | 98 | 50 |
| 5 | 182 | 50 | 181 | 99 | 50 | 179 | 98 | 50 |
| 6 | 189 | 50 | 188 | 99 | 50 | 185 | 98 | 50 |
| 7 | 193 | 50 | 193 | 100 | 50 | 191 | 99 | 50 |
| 8 | 199 | 50 | 199 | 100 | 50 | 197 | 99 | 50 |
| 9 | 203 | 50 | 203 | 100 | 50 | 201 | 99 | 50 |
| 10 | 206 | 50 | 204 | 99 | 50 | 202 | 98 | 50 |
| 11 | 211 | 50 | 211 | 100 | 50 | 210 | 100 | 50 |
| 12 | 214 | 50 | 214 | 100 | 50 | 212 | 99 | 50 |
| 13 | 216 | 50 | 215 | 100 | 50 | 214 | 99 | 50 |
| 16 | 224 | 50 | 223 | 100 | 50 | 221 | 99 | 50 |
| 20 | 229 | 50 | 228 | 100 | 49 | 225 | 98 | 50 |
| 24 | 237 | 50 | 236 | 100 | 49 | 235 | 99 | 50 |
| 28 | 241 | 50 | 242 | 100 | 49 | 241 | 100 | 50 |
| 32 | 250 | 50 | 252 | 101 | 49 | 251 | 100 | 50 |
| 37 | 257 | 50 | 258 | 100 | 49 | 258 | 100 | 50 |
| 42 | 269 | 50 | 272 | 101 | 49 | 271 | 101 | 50 |
| 46 | 269 | 50 | 271 | 101 | 49 | 269 | 100 | 50 |
| 50 | 279 | 50 | 282 | 101 | 49 | 280 | 100 | 50 |
| 54 | 294 | 50 | 294 | 100 | 49 | 294 | 100 | 50 |
| 58 | 305 | 50 | 304 | 100 | 49 | 307 | 101 | 50 |
| 62 | 317 | 50 | 317 | 100 | 49 | 318 | 100 | 50 |
| 67 | 327 | 50 | 325 | 99 | 47 | 331 | 101 | 50 |
| 71 | 339 | 50 | 336 | 99 | 47 | 338 | 100 | 50 |
| 75 | 341 | 48 | 341 | 100 | 46 | 341 | 100 | 50 |
| 79 | 349 | 43 | 344 | 99 | 46 | 344 | 99 | 48 |
| 83 | 358 | 40 | 352 | 98 | 46 | 349 | 97 | 46 |
| 90 | 369 | 39 | 362 | 98 | 43 | 359 | 97 | 42 |
| 93 | 369 | 38 | 362 | 98 | 43 | 358 | 97 | 42 |
| 98 | 371 | 37 | 364 | 98 | 37 | 364 | 98 | 37 |
| 103 | 365 | 35 | 369 | 101 | 31 | 350 | 96 | 34 |

(a) One of 17 survivors at week 104 was not weighed.

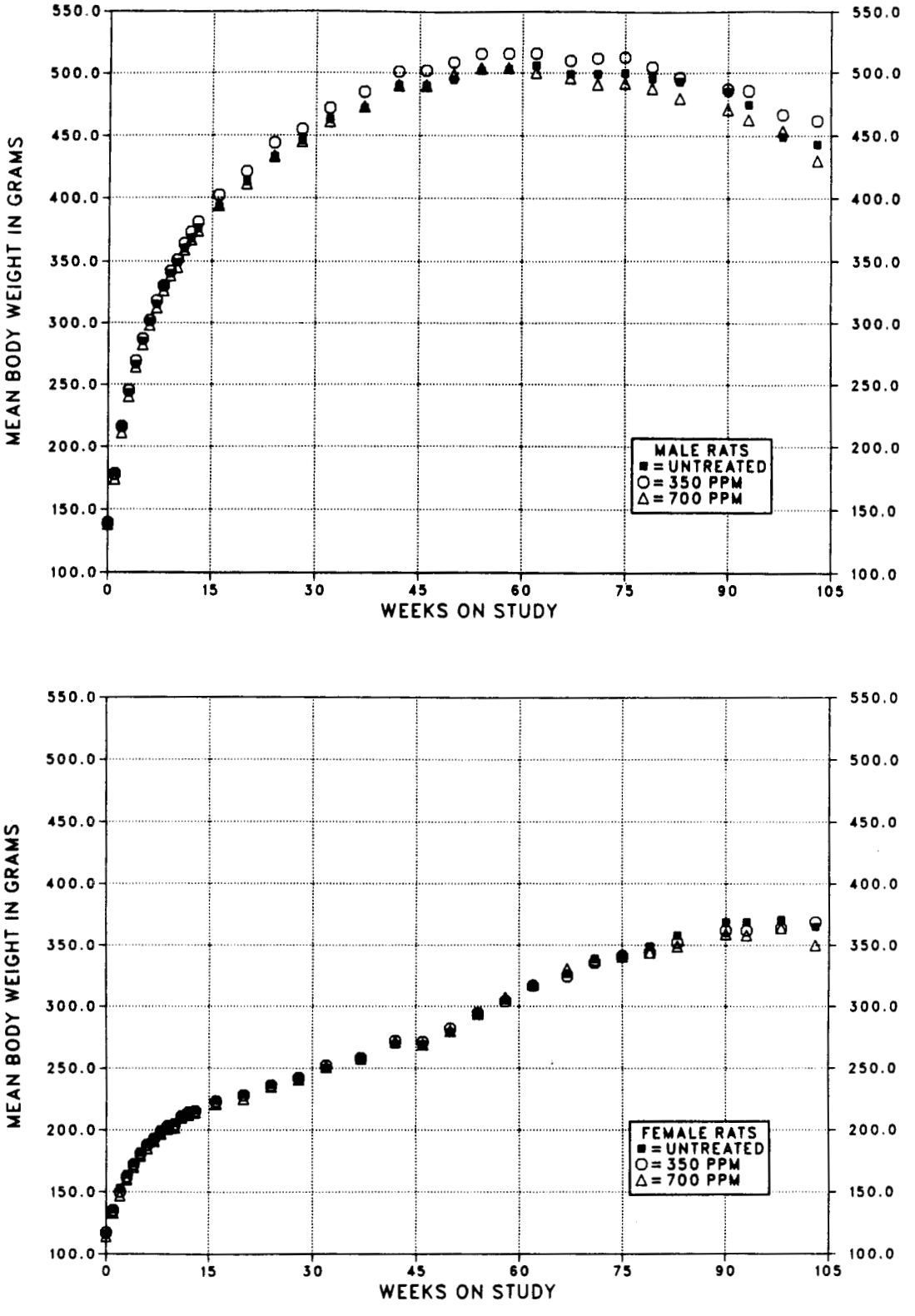



**FIGURE 3. GROWTH CURVES FOR RATS FED DIETS CONTAINING FUROSEMIDE FOR TWO YEARS**

## Survival

Estimates of the probabilities of survival for male and female rats fed diets containing furosemide at the concentrations used in these studies and for controls are shown in Table 11 and in the Kaplan and Meier curves in Figure 4. No significant differences in survival were observed between any groups of either sex. The number of male rats in all groups which were killed in a moribund condition compared with the number of those that died naturally was very high in this study.

## Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the brain, kidney, parathyroids, anterior pituitary gland, thyroid gland, and clitoral gland.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes A and B for male and female rats, respectively.

TABLE 11. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **MALE (a)** | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural deaths | 5 | 5 | 5 |
| Moribund kills | (b) 28 | (b) 28 | 25 |
| Animals surviving until study termination | 17 | 17 | 20 |
| Survival P values (b) | 0.720 | 0.623 | 0.769 |
| **FEMALE (a)** | | | |
| Animals initially in study | 50 | 50 | 504 |
| Natural deaths | 5 | 5 | 3 |
| Moribund kills | (b) 10 | 14 | (d) 13 |
| Animals surviving until study termination | 35 | 31 | 34 |
| Survival P values (b) | 1.000 | 0.634 | 0.999 |

(a) Terminal-kill period: weeks 104-106
(b) Three moribund animals were killed after the start of the study termination period; for statistical purposes, these animals have been pooled with those killed at termination.
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.
(d) One moribund animal was killed after the start of the study termination period; for statistical purposes, this animal has been pooled with those killed at termination.

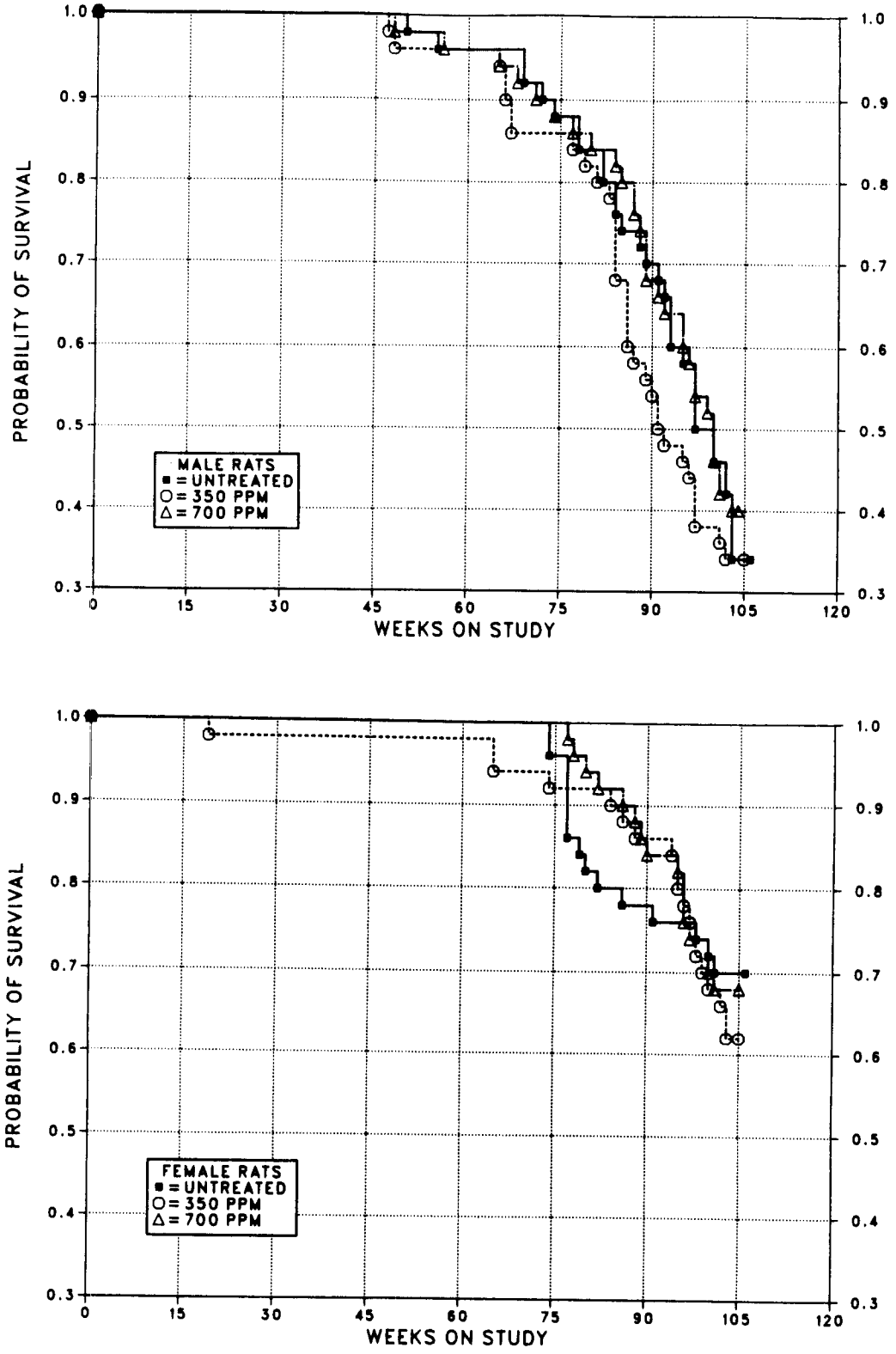



**FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING FUROSEMIDE FOR TWO YEARS**

*Brain:* Meningiomas were considered to be the cause of death in three low dose male rats that died during weeks 47, 48, and 97 (Table 12). The historical incidence of meningiomas in untreated male F344/N rats is 2/1,928 (0.1%) which is significantly different from the incidence observed in the low dose group ($P<0.001$ by the Fisher exact test), and no more than one meningioma has been seen in any comparable historical control group. Hyperplasia of the meninges was seen in three other low dose male rats, but these lesions were not morphologically similar (in cell type) to the meningiomas.

The meningiomas in two rats were similar. They were located in the dorsal caudal region of the cerebellum, were greater than 5 mm in diameter, and had extensively invaded and replaced much of the caudal part of the cerebellum. They consisted of solid sheets and intersecting bundles of cells with fusiform hyperchromatic nuclei and scant cytoplasm. The tumors were highly cellular, and necrotic cells and cells in mitosis were relatively frequent. The histologic appearance and location were suggestive of an extremely rare tumor, the medulloblastoma, but lacked the pseudorosettes characteristic of neuroblast differentiation. The meningioma in the third low dose male rat was a more typical meningioma. It was located in the region of the olfactory lobes and was about 4 mm in diameter. It consisted of solid sheets of epithelioid cells with a moderate amount of amphophilic cytoplasm and round vesicular nuclei containing one or two nucleoli. Small foci of mineralization were scattered throughout the tumor.

**TABLE 12. ANALYSIS OF MENINGIOMAS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (a,b)**

| | Control | 350 ppm (c) | 700 ppm (c) |
|---|---|---|---|
| Overall Rates | 0/50 (0%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted Rates | 0.0% | 8.4% | 0.0% |
| Terminal Rates | 0/17 (0%) | 0/17 (0%) | 0/20 (0%) |
| Week of First Observation | | 47 | |
| Life Table Tests | P=0.636 | P=0.107 | (d) |
| Incidental Tumor Tests | P=0.625 | P=0.095 | (d) |

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table A3 (footnotes).
(b) Historical incidence in NTP studies (mean ± SD): 2/1,928 (0.1% ± 0.5%)
(c) The estimated dose in milligrams per kilograms per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.
(d) No P value is reported because no tumors were observed in the 700-ppm and control groups.

*Kidney:* Cysts and epithelial hyperplasia of the renal pelvis were observed at increased incidences in high dose male rats (cysts: control, 6/50; low dose, 7/50; high dose, 12/50; epithelial hyperplasia: 17/50; 15/50; 27/50). Tubular cell hyperplasia was observed in 4/50 control, 2/50 low dose, and 4/50 high dose male rats. Tubular cell adenomas were seen in 1/50 control, 3/50 low dose, and 1/50 high dose male rats (Table 13). A tubular cell adenocarcinoma was seen in one additional low dose male rat and in another high dose male rat. The highest previously observed untreated control group incidence of tubular cell adenomas or adenocarcinomas (combined) is 3/50.

Nephropathy occurred at similar incidences in all groups of male rats, but the average severity of this lesion was greater in high dose rats than in controls (Table 15). The incidence and severity of nephropathy were not increased in dosed female rats. Nephropathy is a common spontaneous disease characterized by degeneration and regeneration of tubular epithelial cells, tubular dilatation and atrophy, granular and hyaline casts in tubular lumens, thickening of tubular basement membrane, interstitial fibrosis, and glomerulosclerosis.

*Note:* After review and approval of this Technical Report by the Peer Review Panel (April 19, 1988), furosemide was selected as one of several chemicals for which the kidney in male rats would receive additional evaluation by a more extensive sampling procedure to determine if the standard sampling procedures were giving an accurate assessment of the incidences of tubular cell tumors in the kidney.

The standard sampling method for microscopic examination involves single longitudinal sections taken from the center of the left and right kidney, plus additional sections of any grossly visible tumors. The additional pathology procedure involved embedding the remaining half of each kidney which had been retained as part of the wet tissues, step-sectioning the embedded tissue every 1 mm, and examining the resulting three or four sections. The numbers of additional kidney sections reviewed were 300 for the control group, 301 for the low dose group, and 299 for the the high dose group.

The results of the additional tissue review after duplicate diagnoses from the original review were eliminated are shown in Table 14. The composite results from Tables 13 and 14 are shown in Table 15.

It was the decision of the NTP staff that these new findings did not measurably change the level of evidence or affect the interpretation of the tubular cell tumors in the kidney as stated in this Report. The Peer Review Panel concurred with this decision after these new data were presented at a public meeting held on October 3, 1988.

*Parathyroids:* The incidence of hyperplasia of the parathyroids was increased in high dose male rats (control, 8/48; low dose, 8/46; high dose, 15/47). This effect is secondary to the nephropathy, and the incidences are in rough agreement with the average grade of severity in the dosed groups (Table 16).

*Anterior Pituitary Gland:* The incidence of adenomas in low dose male rats was significantly greater than that in controls (Table 17). The incidences in both dosed groups and in the controls were lower than the mean historical control incidence for these lesions, and anterior pituitary tumors were not considered related to furosemide administration.

TABLE 13. ANALYSIS OF RENAL TUBULAR CELL LESIONS (ORIGINAL SECTIONS) IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Hyperplasia** | | | |
| Overall Rates | 4/50 (8%) | 2/50 (4%) | 4/50 (8%) |
| **Adenoma** | | | |
| Overall Rates | 1/50 (2%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted Rates | 5.9% | 17.6% | 5.0% |
| Terminal Rates | 1/17 (6%) | 3/17 (18%) | 1/20 (5%) |
| Week of First Observation | 104 | 104 | 104 |
| Life Table Tests | P=0.550N | P=0.300 | P=0.727N |
| Incidental Tumor Tests | P=0.550N | P=0.300 | P=0.727N |
| **Adenocarcinoma** | | | |
| Overall Rates | 0/50 (0%) | 1/50 (2%) | 1/50 (2%) |
| **Adenoma or Adenocarcinoma (a)** | | | |
| Overall Rates | 1/50 (2%) | 4/50 (8%) | 2/50 (4%) |
| Adjusted Rates | 5.9% | 20.1% | 7.3% |
| Terminal Rates | 1/17 (6%) | 3/17 (18%) | 1/20 (5%) |
| Week of First Observation | 104 | 86 | 84 |
| Life Table Tests | P=0.467 | P=0.171 | P=0.543 |
| Incidental Tumor Tests | P=0.477 | P=0.217 | P=0.561 |

(a) Historical incidence in NTP studies (mean ± SD): 9/1,928 (0.5% ± 1%)

TABLE 14. RESULTS OF ANALYSIS OF RENAL TUBULAR CELL LESIONS (ADDITIONAL SECTIONS) IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Hyperplasia** | | | |
| Minimal | 1/50 | 2/50 | 0/50 |
| Mild | 1/50 | 1/50 | 4/50 |
| Moderate | 0/50 | 0/50 | 1/50 |
| Oncocytic | 0/50 | 0/50 | 4/50 |
| **Adenoma** | 2/50 | 1/50 | 4/50 |

TABLE 15. RESULTS OF ANALYSIS OF RENAL TUBULAR CELL LESIONS (COMPOSITE DATA) IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Hyperplasia** | 6/50 | 5/50 | 10/50 |
| **Adenoma** | 3/50 | 4/50 | 5/50 |
| **Adenocarcinoma** | 0/50 | 1/50 | 1/50 |
| **Adenoma or Adenocarcinoma** | | | |
| Overall Rates | 3/50 (6%) | 5/50 (10%) | 6/50 (12%) |
| Adjusted Rates | 17.6% | 25.8% | 22.6% |
| Terminal Rates | 3/17 (18%) | 4/17 (24%) | 3/20 (15%) |
| Week of First Observation | 104 | 86 | 84 |
| Life Table Tests | P=0.257 | P=0.344 | P=0.301 |
| Incidental Tumor Tests | P=0.250 | P=0.398 | P=0.303 |

TABLE 16. SEVERITY OF NEPHROPATHY IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (a)

| Grade | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| Minimal: 1 | 1 | 2 | 1 |
| Mild: 2 | 28 | 24 | 10 |
| Moderate: 3 | 14 | 20 | 29 |
| Marked: 4 | 5 | 3 | 9 |
| Average grade | 2.5 ± 0.1 | 2.5 ± 0.1 | (b,c) 2.9 ± 0.1 |

(a) The number of animals with the indicated grade of severity is given in the control and dosed group columns.
(b) One high dose rat with nephropathy was not graded.
(c) $P < 0.01$ vs. the controls

TABLE 17. ANALYSIS OF PROLIFERATIVE ANTERIOR PITUITARY GLAND LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Focal Hyperplasia** | | | |
| Overall Rates | 14/50 (28%) | 20/50 (40%) | 22/50 (44%) |
| **Adenoma (a)** | | | |
| Overall Rates | 4/50 (8%) | 11/50 (22%) | 8/50 (16%) |
| Adjusted Rates | 14.7% | 41.6% | 23.9% |
| Terminal Rates | 1/17 (6%) | 5/17 (29%) | 2/20 (10%) |
| Week of First Observation | 78 | 67 | 74 |
| Life Table Tests | P=0.229 | P=0.039 | P=0.221 |
| Incidental Tumor Tests | P=0.173 | P=0.044 | P=0.166 |

(a) Historical incidence of adenomas or carcinomas (combined) in NTP studies (mean ± SD): 459/1,830 (25% ± 10%)

*Thyroid Gland*: C-Cell adenomas in female rats occurred with a significant positive trend; the incidence in the high dose group was not significantly greater than that in the controls by the incidental tumor or logistic regression tests (Table 18). A C-cell carcinoma occurred in one other low dose female rat. C-Cell adenomas or carcinomas (combined) were seen in 7/50 control, 3/50 low dose, and 6/50 high dose male rats. Logistic regression analysis was used as a supplemental statistical test because of the differing results given for high dose female rats by the life table and incidental tumor tests.

*Clitoral Gland:* Hyperplasia was observed at increased incidences in dosed female rats (control, 1/48; low dose, 5/45; high dose, 6/49). The incidences of adenomas in dosed female rats were not significantly greater than that in controls (6/48; 10/45; 9/49).

TABLE 18. ANALYSIS OF THYROID GLAND C-CELL LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Hyperplasia** | | | |
| Overall Rates | 29/50 (58%) | 24/50 (48%) | 26/50 (52%) |
| **Adenoma** | | | |
| Overall Rates | 4/50 (8%) | 6/50 (12%) | 11/50 (22%) |
| Adjusted Rates | 11.0% | 19.4% | 29.2% |
| Terminal Rates | 3/35 (9%) | 6/31 (19%) | 8/34 (24%) |
| Week of First Observation | 98 | 104 | 88 |
| Life Table Tests | P=0.030 | P=0.302 | P=0.048 |
| Logistic Regression Tests | P=0.035 | P=0.368 | P=0.055 |
| Incidental Tumor Tests | P=0.042 | P=0.373 | P=0.090 |
| **Carcinoma** | | | |
| Overall Rates | 0/50 (0%) | 1/50 (2%) | 0/50 (0%) |
| **Adenoma or Carcinoma (a)** | | | |
| Overall Rates | 4/50 (8%) | 7/50 (14%) | 11/50 (22%) |
| Adjusted Rates | 11.0% | 22.6% | 29.2% |
| Terminal Rates | 3/35 (9%) | 7/31 (23%) | 8/34 (24%) |
| Week of First Observation | 98 | 104 | 88 |
| Life Table Tests | P=0.032 | P=0.200 | P=0.048 |
| Logistic Regression Tests | P=0.038 | P=0.255 | P=0.055 |
| Incidental Tumor Tests | P=0.044 | P=0.254 | P=0.090 |

(a) Historical incidence in NTP studies (mean ± SD): 218/1,938 (11% ± 7%)

## FOURTEEN-DAY STUDIES

All males and 1/5 females that received 46,000 ppm furosemide died before the end of the studies (Table 19). Male mice that received 15,300 ppm and female mice that received 46,000 ppm lost weight over the course of the studies. The final mean body weights of male mice that received 1,700 or 5,100 ppm were 16% or 14% lower than that of controls. The final mean body weight of females that received 15,300 was 13% lower than that of controls. Rough fur, a hunched appearance, depression, and weakness were observed for all mice that received 46,000 ppm. These effects appeared later in the study for females than for males. Minimal-to-mild nephrosis was observed in all mice that received 46,000 ppm, in 5/5 males and 1/5 females that received 15,300 ppm, and in 1/5 males that received 5,100 ppm. Microscopically, this lesion was a subcapsular or cortical tubular cell regeneration; tubular dilatation and casts and mineralization at the corticomedullary junction were also present.

TABLE 19. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY FEED STUDIES OF FUROSEMIDE

| Concentration (ppm) | Survival (a) | Mean Body Weights (grams) Initial (b) | Final | Change (c) | Final Weight Relative to Controls (percent) |
|---|---|---|---|---|---|
| **MALE** | | | | | |
| 0 | 5/5 | 24.8 ± 0.4 | 29.6 ± 0.7 | +4.8 ± 0.5 | |
| 570 | 5/5 | 24.4 ± 0.7 | 28.8 ± 0.4 | +4.4 ± 0.4 | 97.3 |
| 1,700 | 4/4 | 22.0 ± 0.4 | 24.8 ± 0.5 | +2.8 ± 0.3 | 83.8 |
| 5,100 | 5/5 | 24.4 ± 1.2 | 25.4 ± 0.8 | +1.0 ± 0.5 | 85.8 |
| 15,300 | 5/5 | 24.2 ± 0.6 | 22.8 ± 0.7 | −1.4 ± 0.4 | 77.0 |
| 46,000 | (d) 0/5 | 25.6 ± 0.8 | (e) | (e) | (e) |
| **FEMALE** | | | | | |
| 0 | 5/5 | 19.2 ± 0.4 | 22.4 ± 0.4 | +3.2 ± 0.2 | |
| 570 | 5/5 | 19.2 ± 0.2 | 21.2 ± 0.6 | +2.0 ± 0.5 | 94.6 |
| 1,700 | 5/5 | 18.6 ± 0.2 | 20.4 ± 0.2 | +1.8 ± 0.4 | 91.1 |
| 5,100 | 5/5 | 18.8 ± 0.6 | 21.0 ± 0.3 | +2.2 ± 0.4 | 93.8 |
| 15,300 | 5/5 | 18.4 ± 0.4 | 19.4 ± 0.5 | +1.0 ± 0.3 | 86.6 |
| 46,000 | (f) 4/5 | 19.4 ± 0.2 | 14.5 ± 0.5 | −4.7 ± 0.8 | 64.7 |

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Day of death: 7,7,8,8,8
(e) No data are reported due to 100% mortality in this group.
(f) Day of death: 14

## THIRTEEN-WEEK STUDIES

No compound-related deaths occurred (Table 20). The final mean body weights of males that received 3,750, 7,500, or 15,000 were 12%, 22%, or 17% lower than that of controls. Final mean body weights of dosed and control female mice were comparable. Feed consumption by dosed groups was lower than that by controls during the first week of the study and somewhat higher thereafter. The liver weight to body weight ratio was significantly increased for male mice that received 15,000 ppm and for females that received 5,000, 10,000, or 20,000 ppm (Table 21). Nephrosis was observed in 3/10 females that received 20,000 ppm, 2/10 males that received 15,000 ppm, and 1/10 males that received 7,500 ppm. Proteinaceous tubular casts were observed in the renal medulla of 10/10 females that received 20,000 ppm, 8/10 that received 10,000 ppm, 3/10 that received 5,000 ppm, and 1/10 that received 2,500 ppm. These tubular casts were observed in 9/10 males in the 3,750-, 7,500-, and 15,000-ppm groups.

*Dose Selection Rationale:* Because of lower body weight gain in males and kidney lesions in both males and females at higher doses, dietary concentrations of furosemide selected for mice for the 2-year studies were 700 and 1,400 ppm.

TABLE 20. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF FUROSEMIDE

| Concentration (ppm) | Survival (a) | Mean Body Weights (grams) | | | Final Weight Relative to Controls (percent) | Feed Consumption (d) | |
|---|---|---|---|---|---|---|---|
| | | Initial (b) | Final | Change (c) | | Week 7 | Week 13 |
| **MALE** | | | | | | | |
| 0 | (e) 9/10 | 23.0 ± 0.8 | 29.9 ± 1.0 | +6.8 ± 1.1 | | 3.5 | 3.8 |
| 938 | 10/10 | 23.1 ± 0.7 | 28.7 ± 0.7 | +5.6 ± 0.8 | 96.0 | 4.1 | 4.1 |
| 1,875 | 10/10 | 22.4 ± 0.9 | 28.1 ± 0.7 | +5.7 ± 0.9 | 94.0 | 4.1 | 4.1 |
| 3,750 | 10/10 | 22.5 ± 0.6 | 26.3 ± 0.4 | +3.8 ± 0.6 | 88.0 | 4.0 | 4.0 |
| 7,500 | 10/10 | 23.0 ± 0.7 | 23.3 ± 0.5 | +0.3 ± 1.0 | 77.9 | 3.7 | 4.0 |
| 15,000 | (f) 9/10 | 23.5 ± 0.8 | 24.8 ± 0.4 | +1.3 ± 1.0 | 82.9 | 3.8 | 5.0 |
| **FEMALE** | | | | | | | |
| 0 | 10/10 | 17.8 ± 0.4 | 22.7 ± 0.3 | +4.9 ± 0.3 | | 3.4 | 3.5 |
| 1,250 | (g) 8/10 | 17.9 ± 0.7 | 23.9 ± 0.8 | +5.9 ± 0.4 | 105.3 | 3.5 | 4.0 |
| 2,500 | 10/10 | 17.5 ± 0.5 | 22.0 ± 0.3 | +4.5 ± 0.3 | 96.9 | 3.7 | 3.5 |
| 5,000 | 10/10 | 17.7 ± 0.4 | 23.4 ± 0.4 | +5.7 ± 0.2 | 103.1 | 3.3 | 3.8 |
| 10,000 | 10/10 | 17.6 ± 0.4 | 22.0 ± 0.3 | +4.4 ± 0.2 | 96.9 | 3.8 | 4.0 |
| 20,000 | 10/10 | 17.6 ± 0.5 | 22.7 ± 0.4 | +5.1 ± 0.3 | 100.0 | 3.4 | 4.7 |

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.
(e) Week of death: 13
(f) One mouse escaped during week 1.
(g) Accidental deaths

TABLE 21. ANALYSIS OF LIVER WEIGHTS OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF FUROSEMIDE (a)

| Concentration (ppm) | No. of Animals Weighed | Necropsy Body Weight (grams) | Liver Weight (mg) | Liver Weight/ Body Weight (mg/g) |
|---|---|---|---|---|
| **MALE** | | | | |
| 0 | 9 | 26.7 ± 0.9 | 1,049 ± 25 | 39.6 ± 1.2 |
| 938 | 10 | 25.1 ± 0.7 | 1,074 ± 76 | 42.5 ± 2.3 |
| 1,875 | 10 | 24.0 ± 0.5 | 1,021 ± 70 | 42.3 ± 2.4 |
| 3,750 | 10 | (b) 22.9 ± 0.4 | 872 ± 55 | 37.9 ± 2.0 |
| 7,500 | 10 | (c) 22.3 ± 1.2 | 996 ± 97 | 44.0 ± 2.3 |
| 15,000 | 9 | (c) 22.4 ± 1.0 | 1,187 ± 112 | (c) 52.0 ± 2.7 |
| **FEMALE** | | | | |
| 0 | 10 | 20.5 ± 0.3 | 857 ± 22 | 41.8 ± 0.9 |
| 1,250 | 8 | 21.4 ± 0.7 | 950 ± 52 | 44.4 ± 1.9 |
| 2,500 | 10 | 19.1 ± 0.3 | 798 ± 36 | 41.8 ± 1.8 |
| 5,000 | 10 | 23.2 ± 1.3 | (b) 1,252 ± 113 | (c) 53.1 ± 2.0 |
| 10,000 | 10 | 23.0 ± 1.4 | (c) 1,278 ± 151 | (c) 53.9 ± 3.5 |
| 20,000 | 10 | 20.7 ± 0.9 | 1,178 ± 83 | (c) 56.4 ± 1.6 |

(a) Mean ± standard error; P values vs. the controls by Dunnett's test (Dunnett, 1955).
(b) $P<0.05$
(c) $P<0.01$

## TWO-YEAR STUDIES

### Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of high dose male mice were generally 5%-9% lower than those of controls before week 23 and 11%-17% lower thereafter (Table 22 and Figure 5). Mean body weights of low dose male mice were generally 5%-10% lower than those of controls after week 31. Mean body weights of high dose female mice were 11%-22% lower than those of controls after week 61, and those of low dose female mice were 5%-13% lower from week 82 to the end of the studies.

The average daily feed consumption by low dose or high dose male mice was 107% or 105% that by controls and by low dose or high dose female mice, 105% or 107% that by controls (Tables F3 and F4). The average amount of furosemide consumed per day was approximately 90 or 190 mg/kg for low dose or high dose male mice and 100 or 215 mg/kg for low dose or high dose female mice. Excessively wet bedding, presumably resulting from diuresis, was considered to be compound related. Fighting was apparent among males in all groups. Penile and preputial lesions found in many animals during the study may have contributed to the incidences of urogenital infection and inflammation.

TABLE 22. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE

| Weeks on Study | Control Av. Wt. (grams) | Control No. of Survivors | 700 ppm Av. Wt. (grams) | 700 ppm Wt. (percent of controls) | 700 ppm No. of Survivors | 1,400 ppm Av. Wt. (grams) | 1,400 ppm Wt. (percent of controls) | 1,400 ppm No. of Survivors |
|---|---|---|---|---|---|---|---|---|
| **MALE** | | | | | | | | |
| 0 | 23.6 | 50 | 23.3 | 99 | 50 | 23.2 | 98 | 50 |
| 1 | 22.8 | 50 | 23.2 | 102 | 50 | 23.2 | 102 | 50 |
| 2 | 26.0 | 50 | 25.0 | 96 | 50 | 24.5 | 94 | 50 |
| 3 | 27.3 | 50 | 26.0 | 95 | 50 | 25.9 | 95 | 50 |
| 4 | 27.7 | 50 | 25.9 | 94 | 50 | 25.3 | 91 | 50 |
| 5 | 28.5 | 50 | 26.8 | 94 | 50 | 26.8 | 94 | 50 |
| 6 | 30.0 | 50 | 29.5 | 98 | 50 | 27.8 | 93 | 50 |
| 7 | 30.2 | 50 | 29.4 | 97 | 50 | 28.5 | 94 | 50 |
| 8 | 30.9 | 50 | 29.8 | 96 | 50 | 29.4 | 95 | 50 |
| 9 | 30.0 | 50 | 28.9 | 96 | 50 | 27.4 | 91 | 50 |
| 10 | 31.5 | 50 | 30.4 | 97 | 50 | 30.0 | 95 | 50 |
| 11 | 32.9 | 49 | 31.0 | 94 | 50 | 30.6 | 93 | 50 |
| 12 | 32.4 | 49 | 31.4 | 97 | 50 | 30.6 | 94 | 50 |
| 13 | 32.7 | 49 | 31.1 | 95 | 50 | 30.6 | 94 | 50 |
| 15 | 33.4 | 49 | 32.5 | 97 | 50 | 32.5 | 97 | 50 |
| 19 | 34.7 | 48 | 33.8 | 97 | 50 | 32.5 | 94 | 50 |
| 23 | 35.8 | 48 | 33.9 | 95 | 50 | 32.7 | 91 | 50 |
| 27 | 37.3 | 48 | 36.4 | 98 | 49 | 33.3 | 89 | 50 |
| 31 | 38.8 | 48 | 36.5 | 94 | 49 | 34.0 | 88 | 50 |
| 36 | 38.7 | 48 | 36.9 | 95 | 49 | 34.4 | 89 | 49 |
| 41 | 40.6 | 48 | 38.5 | 95 | 48 | 35.9 | 88 | 48 |
| 45 | 39.7 | 48 | 37.1 | 93 | 48 | 34.4 | 87 | 48 |
| 49 | 40.6 | 48 | 38.7 | 95 | 48 | 35.4 | 87 | 48 |
| 53 | 40.9 | 48 | 38.7 | 95 | 48 | 34.6 | 85 | 48 |
| 57 | 40.8 | 48 | 37.8 | 93 | 48 | 34.2 | 84 | 48 |
| 61 | 40.3 | 48 | 36.9 | 92 | 45 | 34.4 | 85 | 46 |
| 66 | 41.3 | 48 | 38.6 | 93 | 45 | 34.7 | 84 | 46 |
| 70 | 40.6 | 47 | 38.4 | 95 | 43 | 34.6 | 85 | 45 |
| 74 | 40.6 | 47 | 38.5 | 95 | 43 | 34.9 | 86 | 41 |
| 78 | 39.8 | 46 | 38.8 | 97 | 41 | 34.8 | 87 | 40 |
| 82 | 41.3 | 45 | 38.3 | 93 | 40 | 34.8 | 84 | 38 |
| 89 | 39.8 | 39 | 37.5 | 94 | 37 | 33.2 | 83 | 34 |
| 92 | 40.7 | 37 | 38.5 | 95 | 34 | 34.5 | 85 | 32 |
| 97 | 39.9 | 34 | 36.1 | 90 | 26 | 33.1 | 83 | 30 |
| 101 | 38.4 | 33 | 36.3 | 95 | 26 | 32.2 | 84 | 29 |
| 104 | 36.7 | 31 | 34.6 | 94 | 24 | 31.0 | 84 | 26 |
| **FEMALE** | | | | | | | | |
| 0 | 18.9 | 50 | 18.6 | 98 | 50 | 18.4 | 97 | 50 |
| 1 | 19.6 | 50 | 19.1 | 97 | 50 | 18.6 | 95 | 50 |
| 2 | 20.4 | 50 | 19.9 | 98 | 50 | 19.9 | 98 | 50 |
| 3 | 21.8 | 50 | 21.2 | 97 | 50 | 21.1 | 97 | 50 |
| 4 | 21.8 | 50 | 21.6 | 99 | 50 | 21.5 | 99 | 50 |
| 5 | 22.5 | 50 | 22.4 | 100 | 50 | 22.1 | 98 | 50 |
| 6 | 23.5 | 50 | 23.2 | 99 | 50 | 22.8 | 97 | 50 |
| 7 | 23.6 | 50 | 23.8 | 101 | 50 | 23.6 | 100 | 50 |
| 8 | 24.1 | 50 | 24.0 | 100 | 50 | 24.1 | 100 | 50 |
| 9 | 23.6 | 50 | 22.7 | 96 | 50 | 23.2 | 98 | 50 |
| 10 | 24.4 | 50 | 24.4 | 100 | 50 | 24.5 | 100 | 50 |
| 11 | 25.0 | 50 | 24.8 | 99 | 50 | 24.9 | 100 | 50 |
| 12 | 25.3 | 50 | 25.2 | 100 | 50 | 25.4 | 100 | 50 |
| 13 | 26.2 | 50 | 25.4 | 97 | 50 | 25.9 | 99 | 50 |
| 15 | 26.4 | 50 | 26.5 | 100 | 50 | 26.6 | 101 | 50 |
| 19 | 27.6 | 50 | 27.4 | 99 | 50 | 27.1 | 98 | 50 |
| 23 | 28.6 | 50 | 27.9 | 98 | 50 | 27.8 | 97 | 50 |
| 27 | 29.6 | 50 | 29.1 | 98 | 50 | 28.1 | 95 | 50 |
| 31 | 31.0 | 50 | 30.3 | 98 | 50 | 29.5 | 95 | 50 |
| 36 | 32.5 | 50 | 30.9 | 95 | 50 | 30.7 | 94 | 50 |
| 41 | 33.7 | 50 | 33.0 | 98 | 49 | 32.4 | 96 | 50 |
| 45 | 34.6 | 50 | 33.3 | 96 | 49 | 32.1 | 93 | 50 |
| 49 | 36.1 | 50 | 35.4 | 98 | 49 | 33.7 | 93 | 50 |
| 53 | 36.3 | 50 | 36.2 | 100 | 49 | 34.3 | 94 | 50 |
| 57 | 36.6 | 50 | 36.3 | 99 | 49 | 34.6 | 95 | 50 |
| 61 | 36.8 | 50 | 36.0 | 98 | 49 | 34.2 | 93 | 50 |
| 66 | 39.1 | 48 | 37.5 | 96 | 48 | 34.8 | 89 | 50 |
| 70 | 39.6 | 47 | 37.9 | 96 | 47 | 34.7 | 88 | 50 |
| 74 | 39.6 | 47 | 38.6 | 97 | 46 | 35.2 | 89 | 48 |
| 78 | 40.0 | 45 | 38.2 | 96 | 45 | 35.1 | 88 | 46 |
| 82 | 40.5 | 44 | 38.4 | 95 | 43 | 35.3 | 87 | 44 |
| 89 | 40.7 | 43 | 38.4 | 94 | 39 | 33.4 | 82 | 41 |
| 92 | 42.6 | 40 | 38.7 | 91 | 37 | 33.6 | 79 | 38 |
| 97 | 41.5 | 39 | 36.9 | 89 | 34 | 33.0 | 80 | 29 |
| 101 | 41.7 | 37 | 36.9 | 88 | 32 | 32.5 | 78 | 23 |
| 104 | 39.9 | 36 | 34.8 | 87 | 29 | 31.4 | 79 | 19 |

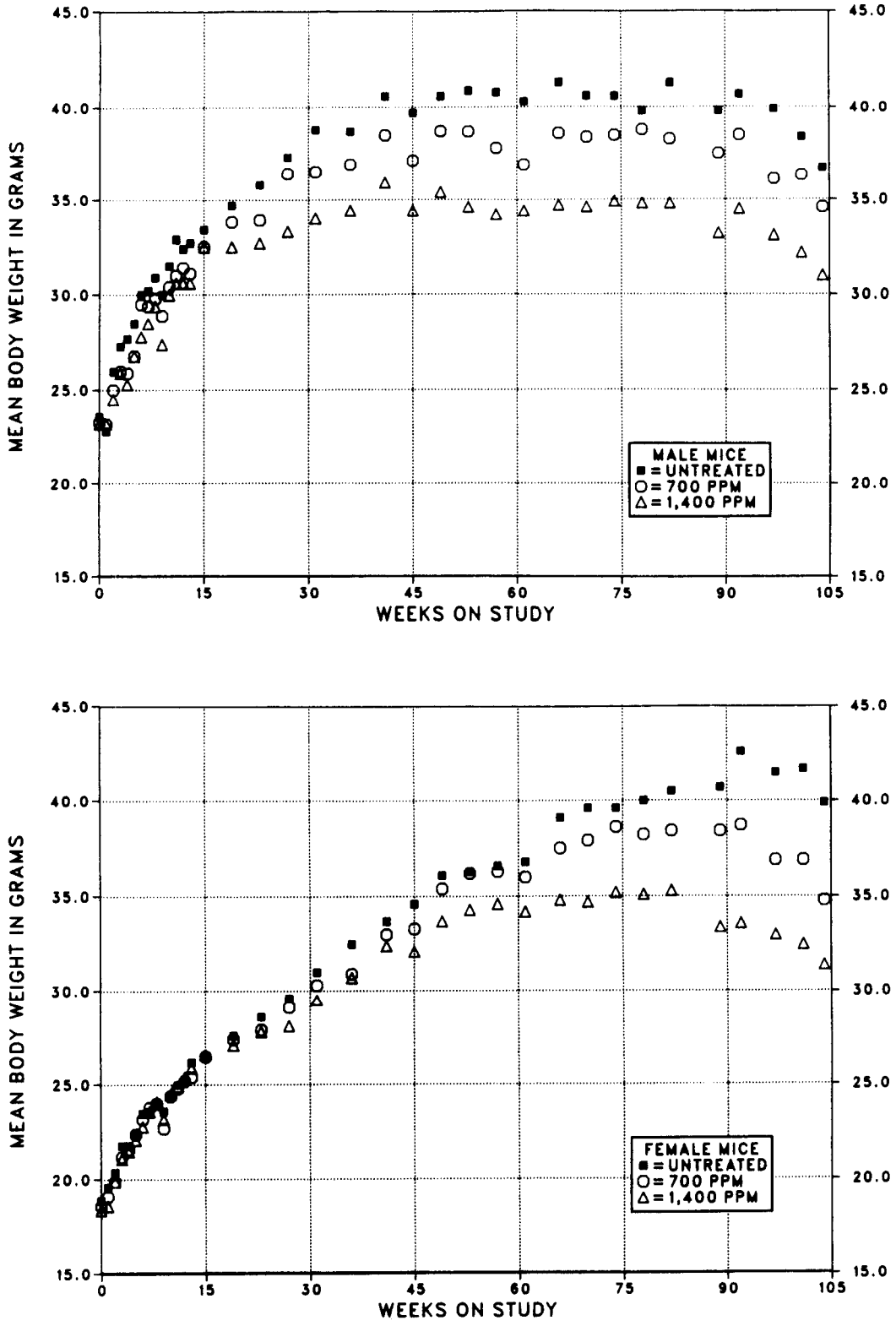



**FIGURE 5. GROWTH CURVES FOR MICE FED DIETS CONTAINING FUROSEMIDE FOR TWO YEARS**

### Survival

Estimates of the probabilities of survival for male and female mice fed diets containing furosemide at the concentrations used in these studies and for controls are shown in Table 23 and in the Kaplan and Meier curves in Figure 6. The survival of the high dose group of female mice was significantly lower than that of the controls after week 99.

### Pathology and Statistical Analyses of Results

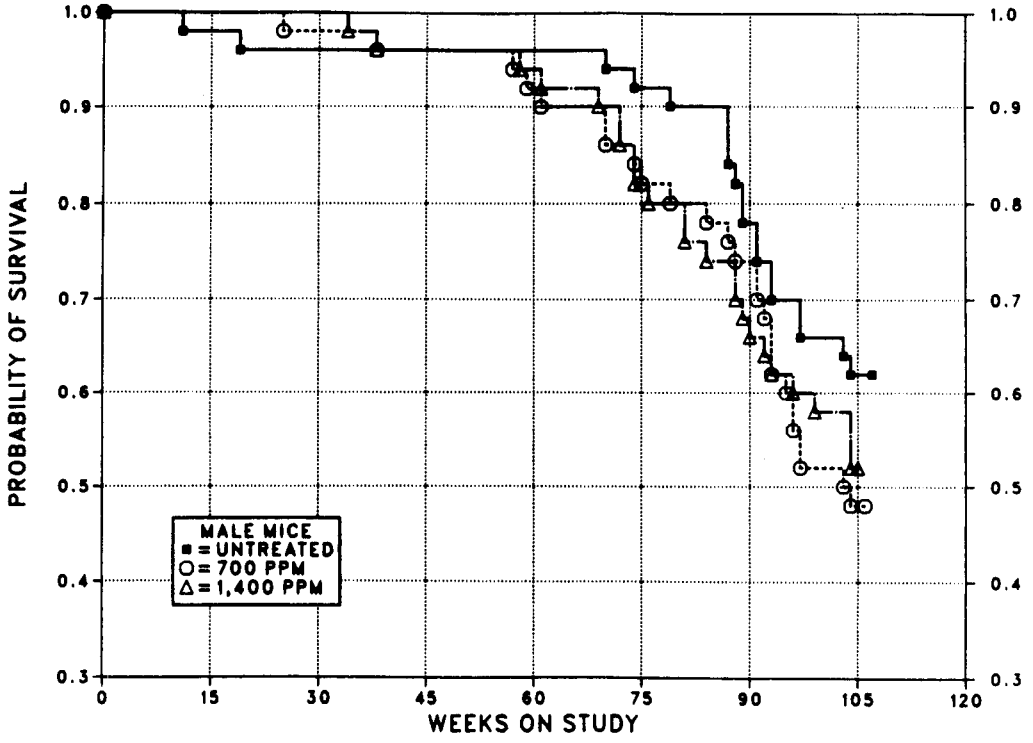
This section describes the significant or noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the mammary gland, hematopoietic system, urinary bladder, kidney, thyroid gland, liver, forestomach, lung, brain, ovary, uterus, and prostate.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes C and D for male and female mice, respectively.

TABLE 23. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **MALE** (a) | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural deaths | (b) 7 | 11 | 8 |
| Moribund kills | 12 | 15 | 16 |
| Animals surviving until study termination | 31 | 24 | 26 |
| Survival P values (c) | 0.302 | 0.206 | 0.333 |
| **FEMALE** (a) | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural deaths | (d) 4 | 9 | 19 |
| Moribund kills | (e) 10 | 12 | (e) 12 |
| Accidentally killed | 0 | 0 | 1 |
| Animals surviving until study termination | 36 | 29 | 18 |
| Survival P values (c) | 0.003 | 0.229 | 0.003 |

(a) Terminal-kill period: weeks 105-107
(b) One animal died after the start of the study termination period; for statistical purposes, this animal has been pooled with those killed at termination.
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.
(d) Three animals died after the start of the study termination period; for statistical purposes, these animals have been pooled with those killed at termination.
(e) One moribund animal was killed after the start of the study termination period; for statistical purposes, this animal has been pooled with those killed at termination.



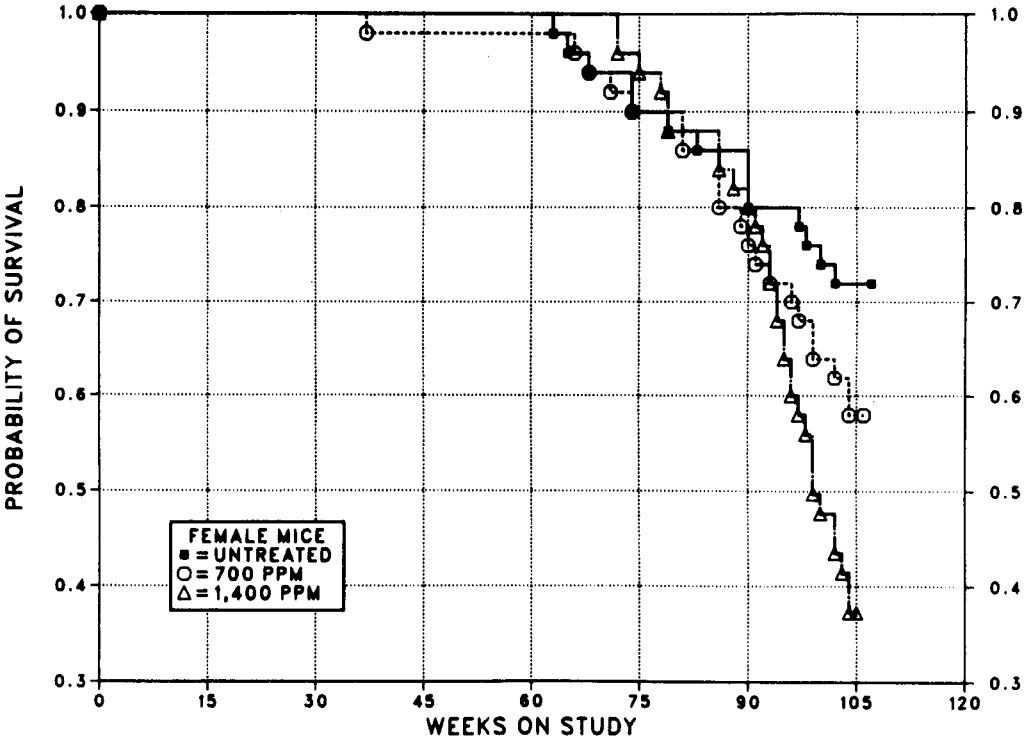




**FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING FUROSEMIDE FOR TWO YEARS**

*Mammary Gland:* Malignant mixed tumors (adenocarcinoma, type C) and one acinar cell tumor occurred in seven dosed female mice (Table 24). These tumors occurred with a positive trend, and the incidence in the high dose group was significantly greater than that in the controls by the logistic regression and life table tests. Logistic regression was used as a supplemental test in this instance because the incidental tumor test appeared to be overly affected by differences in survival in certain time intervals preselected for analysis.

Malignant mixed tumors (adenocarcinoma, type C) are the most common mammary gland tumor in $B6C3F_1$ mice. These tumors comprise small glandular structures consisting of an inner layer of cuboidal epithelial cells and surrounded by an outer layer of myoepithelial cells. A small-to-moderate amount of collagenous stroma is present. These tumors typically show little evidence of invasion at the margin, but some metastasize to the lungs. The tumors observed in this study did not differ from the spontaneous lesion; all neoplasms were well circumscribed, and none metastasized.

*Hematopoietic System:* Lymphomas in female mice occurred with a significant positive trend by life table analysis; the incidence in the high dose group was greater than that in the controls (Table 25). Hematopoiesis was observed at increased incidences in the spleen and liver of dosed male and high dose female mice and in the adrenal cortex of high dose female mice (Table 26). Hematopoiesis was characterized primarily by the accumulation of immature cells of the granulocytic series in the red pulp of the spleen and sinusoids of the liver and adrenal gland. This is a response to increased demand for leukocytes, as a result primarily of inflammatory processes in organs of the urogenital tract and elsewhere.

*Urinary Bladder:* Mucosal epithelial hyperplasia and submucosal chronic focal inflammation were observed at increased incidences in dosed male mice (Table 26).

**TABLE 24. ANALYSIS OF MALIGNANT MAMMARY GLAND TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (a, b)**

| | Control | 700 ppm (c) | 1,400 ppm (c) |
|---|---|---|---|
| Overall Rates | 0/50 (0%) | 2/50 (4%) | 5/48 (10%) |
| Adjusted Rates | 0.0% | 6.1% | 18.6% |
| Terminal Rates | 0/36 (0%) | 0/29 (0%) | 1/18 (6%) |
| Week of First Observation | | 99 | 72 |
| Life Table Tests | P=0.005 | P=0.212 | P=0.011 |
| Logistic Regression Tests | P=0.014 | P=0.226 | P=0.032 |
| Incidental Tumor Tests | P=0.102 | P=0.397 | P=0.090 |

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table D3 (footnotes).
(b) Historical incidence of malignant mixed tumors or adenocarcinomas (combined) in NTP studies (mean ± SD): 40/2,040 (2% ± 2%). In this study, all tumors, except an acinar cell tumor in one low dose mouse, were diagnosed as malignant mixed tumors.
(c) The estimated dose in milligrams per kilograms per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.

**TABLE 25. ANALYSIS OF MALIGNANT LYMPHOMAS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (a)**

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| Overall Rates | 16/50 (32%) | 13/50 (26%) | 20/48 (42%) |
| Adjusted Rates | 39.5% | 33.8% | 64.7% |
| Terminal Rates | 12/36 (33%) | 6/29 (21%) | 9/18 (50%) |
| Week of First Observation | 63 | 81 | 78 |
| Life Table Tests | P=0.019 | P=0.525N | P=0.013 |
| Logistic Regression Tests | P=0.171 | P=0.345N | P=0.188 |
| Incidental Tumor Tests | P=0.254 | P=0.275N | P=0.232 |

(a) Historical incidence of lymphomas or leukemia (combined) in NTP studies (mean ± SD): 636/2,040 (31% ± 13%)

**TABLE 26. INCIDENCES OF MICE WITH SELECTED NONNEOPLASTIC LESIONS IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE**

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Site/Lesion | Control | 700 ppm | 1,400 ppm | Control | 700 ppm | 1,400 ppm |
| Urinary bladder | | | | | | |
| Mucosal epithelial hyperplasia | 1/48 | 7/50 | 8/50 | 0/50 | 0/47 | 0/44 |
| Submucosal chronic focal inflammation | 16/48 | 21/50 | 31/50 | 23/50 | 25/47 | 19/44 |
| Spleen | | | | | | |
| Hematopoiesis | 15/49 | 22/50 | 27/50 | 8/50 | 14/49 | 23/47 |
| Liver | | | | | | |
| Hematopoiesis | 4/49 | 9/50 | 8/50 | 4/50 | 5/49 | 14/47 |
| Adrenal cortex | | | | | | |
| Hematopoiesis | 0/48 | 1/49 | 0/50 | 2/49 | 1/49 | 8/47 |
| Ovary or uterus | | | | | | |
| Suppurative inflammation | -- | -- | -- | 3/50 | 4/47 | 14/47 |
| Prostate | | | | | | |
| Suppurative inflammation | 2/48 | 4/50 | 9/50 | -- | -- | -- |

*Kidney:* The incidences of nephropathy were increased in dosed male and female mice (Table 27), and the nephropathy was subjectively judged to be more severe in dosed mice (Table 28). Nephropathy is a spontaneous disease of aging mice which is characterized by glomerulosclerosis, tubular degeneration and atrophy, regeneration of tubular epithelium, and interstitial fibrosis. The incidences of tubular cysts and suppurative inflammation were also increased in male mice. Variable degrees of dilatation of the kidney pelvis (hydronephrosis) occurred primarily in dosed male and female mice.

TABLE 27. INCIDENCES OF MICE WITH SELECTED NONNEOPLASTIC RENAL LESIONS IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE

| Site/Lesion | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Control | 700 ppm | 1,400 ppm | Control | 700 ppm | 1,400 ppm |
| Kidney | | | | | | |
| Cysts | 2/50 | 3/50 | 8/50 | 0/50 | 0/49 | 1/47 |
| Suppurative inflammation | 0/50 | 11/50 | 14/50 | 0/50 | 0/49 | 1/47 |
| Nephropathy | 24/50 | 44/50 | 49/50 | 11/50 | 46/49 | 47/47 |
| Kidney/pelvis | | | | | | |
| Dilatation | 1/50 | 13/50 | 42/50 | 1/50 | 3/49 | 17/47 |
| Epithelial hyperplasia | 0/50 | 5/50 | 3/50 | 0/50 | 0/49 | 1/47 |

TABLE 28. SEVERITY OF NEPHROPATHY IN MICE IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE (a)

| Grade | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Control | 700 ppm | 1,400 ppm | Control | 700 ppm | 1,400 ppm |
| Minimal: 1 | 18 | 16 | 0 | 6 | 3 | 0 |
| Mild: 2 | 3 | 21 | 6 | 2 | 38 | 8 |
| Moderate: 3 | 2 | 5 | 21 | 1 | 4 | 33 |
| Marked: 4 | 1 | 2 | 22 | 2 | 1 | 6 |
| Total number of animals with nephropathy | 24 | 44 | 49 | 11 | 46 | 47 |
| Average grade | 1.4 ± 0.2 | (b) 1.8 ± 0.1 | (c) 3.3 ± 0.1 | 1.9 ± 0.4 | 2.1 ± 0.1 | (c) 3.0 ± 0.1 |

(a) The number of animals with the indicated grade of severity is given in the control and dosed group columns.
(b) $P < 0.05$ vs. the controls
(c) $P < 0.01$ vs. the controls

*Thyroid Gland:* The incidence of follicular cell adenomas in low dose female mice was significantly greater than that in controls; the incidences of follicular cell adenomas or carcinomas (combined) in dosed female mice were not significantly greater than that in controls (Table 29).

*Liver:* Hepatocellular carcinomas in male mice occurred with a significant positive trend (control, 6/49; low dose, 8/50; high dose, 12/50; P=0.049). The incidences of hepatocellular carcinomas and adenomas or carcinomas (combined) in dosed male mice were not significantly greater than those in the controls (hepatocellular adenomas or carcinomas, combined: 15/49; 16/50; 20/50).

*Forestomach:* Epithelial hyperplasia was observed at an increased incidence in low dose female mice (male: control, 2/46; low dose, 4/22; high dose, 1/49; female: 1/47; 8/46; 1/46).

*Lung:* Congestion was seen at an increased incidence in dosed male mice (male: control, 6/50; low dose, 13/28; high dose, 11/50; female: 6/50; 3/47; 6/47). Interstitial inflammation was observed at an increased incidence in high dose female mice (male: 5/50; 4/28; 2/50; female: 1/50; 1/47; 6/47).

*Brain:* Mineralization was observed at increased incidences in dosed female mice (male: control, 27/47; low dose, 20/50; high dose, 19/50; female: 13/48; 22/46; 34/47).

*Ovary or Uterus:* Suppurative inflammation was observed at an increased incidence in high dose female mice (Table 26).

*Prostate:* Suppurative inflammation was observed at an increased incidence in high dose male mice (Table 26).

**TABLE 29. ANALYSIS OF THYROID GLAND FOLLICULAR CELL LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE**

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **Hyperplasia** | | | |
| Overall Rates | 2/50 (4%) | 7/47 (15%) | 5/47 (11%) |
| **Adenoma** | | | |
| Overall Rates | 0/50 (0%) | 4/47 (9%) | 3/47 (6%) |
| Adjusted Rates | 0.0% | 11.2% | 13.6% |
| Terminal Rates | 0/36 (0%) | 2/29 (7%) | 2/18 (11%) |
| Week of First Observation | | 74 | 94 |
| Life Table Tests | P=0.051 | P=0.050 | P=0.050 |
| Incidental Tumor Tests | P=0.074 | P=0.046 | P=0.093 |
| **Carcinoma** | | | |
| Overall Rates | 2/50 (4%) | 0/47 (0%) | 1/47 (2%) |
| **Adenoma or Carcinoma (a)** | | | |
| Overall Rates | 2/50 (4%) | 4/47 (9%) | 3/47 (6%) |
| Adjusted Rates | 5.6% | 11.2% | 13.6% |
| Terminal Rates | 2/36 (6%) | 2/29 (7%) | 2/18 (11%) |
| Week of First Observation | 105 | 74 | 94 |
| Life Table Tests | P=0.214 | P=0.269 | P=0.253 |
| Incidental Tumor Tests | P=0.273 | P=0.263 | P=0.366 |

(a) Historical incidence in NTP studies (mean ± SD): 49/1,937 (3% ± 3%)

Furosemide was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested at doses up to 10 mg/plate in both the presence and absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Table 30). Furosemide gave an equivocal response in the mouse lymphoma assay in the absence of S9 as indicated by the marginal increase in trifluorothymidine (Tft) resistance at the highest dose tested in trial one and an even smaller induction of Tft resistance in trial two at the second highest dose only (Table 31). In the presence of Aroclor 1254-induced male F344 rat liver S9, furosemide exposure resulted in an increase in Tft-resistant cells at the highest dose tested and with associated toxicity in each of two trials; overall, furosemide was judged to be positive in the mouse lymphoma assay. When tested for cytogenetic effects in Chinese hamster ovary cell cultures, furosemide induced sister chromatid exchanges and chromosomal aberrations in both the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 (Tables 32 and 33).

TABLE 30. MUTAGENICITY OF FUROSEMIDE IN *SALMONELLA TYPHIMURIUM* (a)

| Strain | Dose (μg/plate) | Revertants/Plate (b) | | | | | |
|---|---|---|---|---|---|---|---|
| | | −S9 | | +S9 (hamster) | | +S9 (rat) | |
| | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| TA100 | 0 | 114 ± 33.1 | 96 ± 1.5 | 171 ± 9.1 | 114 ± 3.2 | 166 ± 9.3 | 111 ± 9.4 |
| | 100 | 89 ± 9.8 | 83 ± 7.1 | 147 ± 13.6 | 111 ± 5.2 | 164 ± 3.8 | 99 ± 9.8 |
| | 333 | 103 ± 1.2 | 84 ± 0.9 | 125 ± 8.0 | 89 ± 4.5 | 175 ± 17.3 | 130 ± 5.5 |
| | 1,000 | 107 ± 8.7 | 93 ± 1.2 | 144 ± 12.1 | 106 ± 7.4 | 162 ± 18.4 | 115 ± 9.8 |
| | 3,333 | 90 ± 14.3 | 89 ± 5.6 | 138 ± 11.3 | 130 ± 6.9 | 161 ± 15.3 | 130 ± 4.8 |
| | 10,000 | 86 ± 4.4 | 70 ± 2.6 | 155 ± 17.7 | 137 ± 11.5 | 148 ± 22.4 | 120 ± 15.1 |
| | Trial summary | Negative | Negative | Negative | Negative | Negative | Negative |
| | Positive control (c) | 368 ± 14.2 | 808 ± 1.7 | 1,361 ± 61.0 | 1,874 ± 145.9 | 1,468 ± 37.6 | 1,275 ± 19.7 |
| TA1535 | 0 | 5 ± 1.7 | 10 ± 0.7 | 7 ± 2.9 | 12 ± 2.6 | 7 ± 2.1 | 20 ± 4.9 |
| | 100 | 7 ± 1.2 | 11 ± 1.3 | 9 ± 0.6 | 10 ± 3.2 | 4 ± 0.7 | 16 ± 2.6 |
| | 333 | 4 ± 1.8 | 12 ± 1.5 | 6 ± 0.6 | 13 ± 1.2 | 4 ± 0.3 | 16 ± 2.2 |
| | 1,000 | 3 ± 0.9 | 7 ± 1.2 | 4 ± 0.6 | 17 ± 1.2 | 11 ± 1.2 | 14 ± 1.9 |
| | 3,333 | 4 ± 1.5 | 15 ± 2.2 | 9 ± 1.2 | 14 ± 1.5 | 10 ± 1.2 | 23 ± 2.9 |
| | 10,000 | 2 ± 1.0 | 7 ± 0.9 | 6 ± 1.9 | 11 ± 2.4 | 3 ± 0.7 | 19 ± 3.1 |
| | Trial summary | Negative | Negative | Negative | Negative | Negative | Negative |
| | Positive control (c) | 83 ± 13.5 | 881 ± 45.1 | 146 ± 9.3 | 202 ± 26.3 | 129 ± 3.5 | 149 ± 11.3 |
| TA1537 | 0 | 5 ± 1.5 | 11 ± 3.5 | 6 ± 2.3 | 12 ± 2.0 | 9 ± 0.6 | 12 ± 1.0 |
| | 100 | 3 ± 0.6 | 7 ± 0.3 | 5 ± 1.2 | 12 ± 1.8 | 3 ± 0.7 | 18 ± 1.2 |
| | 333 | 2 ± 0.7 | 8 ± 0.7 | 8 ± 1.7 | 10 ± 1.7 | 2 ± 0.6 | 14 ± 1.7 |
| | 1,000 | 3 ± 2.2 | 12 ± 2.6 | 7 ± 1.3 | 16 ± 1.8 | 8 ± 2.5 | 13 ± 3.4 |
| | 3,333 | 4 ± 0.3 | 11 ± 2.5 | 4 ± 0.7 | 14 ± 0.9 | 4 ± 0.7 | 18 ± 1.5 |
| | 10,000 | 3 ± 1.2 | 11 ± 1.9 | 5 ± 1.0 | 13 ± 2.2 | 2 ± 0.3 | 15 ± 1.5 |
| | Trial summary | Negative | Negative | Negative | Negative | Negative | Negative |
| | Positive control (c) | 164 ± 16.7 | 157 ± 8.1 | 134 ± 3.9 | 176 ± 8.7 | 121 ± 19.5 | 100 ± 15.0 |
| TA98 | 0 | 9 ± 1.9 | 24 ± 2.8 | 16 ± 2.1 | 25 ± 1.2 | 11 ± 1.8 | 28 ± 0.9 |
| | 100 | 9 ± 1.3 | 23 ± 1.5 | 12 ± 1.5 | 22 ± 2.4 | 13 ± 2.3 | 30 ± 0.6 |
| | 333 | 10 ± 1.9 | 24 ± 1.7 | 14 ± 2.3 | 28 ± 3.3 | 9 ± 0.9 | 25 ± 1.5 |
| | 1,000 | 10 ± 1.2 | 19 ± 4.7 | 14 ± 2.9 | 27 ± 2.7 | 13 ± 1.3 | 29 ± 2.2 |
| | 3,333 | 9 ± 1.2 | 24 ± 3.0 | 11 ± 2.0 | 20 ± 2.7 | 13 ± 2.6 | 37 ± 3.1 |
| | 10,000 | Toxic | Toxic | 11 ± 3.0 | 22 ± 2.6 | 12 ± 1.5 | 31 ± 2.3 |
| | Trial summary | Negative | Negative | Negative | Negative | Negative | Negative |
| | Positive control (c) | 181 ± 3.7 | 368 ± 63.1 | 1,016 ± 47.4 | 1,881 ± 288.8 | 866 ± 15.3 | 862 ± 16.7 |

(a) Study performed at Case Western Reserve University. The detailed protocol is presented by Zeiger et al. (1987). Cells and study compound or solvent (dimethyl sulfoxide) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 μg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

TABLE 31. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE IN MOUSE L5178Y LYMPHOMA CELLS BY FUROSEMIDE (a,b)

| Compound | Concentration (μl/ml) | Cloning Efficiency (percent) | Relative Total Growth (percent) | Tft-Resistant Cells | Mutant Fraction (c) |
|---|---|---|---|---|---|
| **-S9** | | | | | |
| **Trial 1** | | | | | |
| Dimethyl sulfoxide (d) | | 103.8 ± 4.0 | 100.0 ± 3.9 | 119.5 ± 13.1 | 38.8 ± 4.0 |
| Furosemide | 125 | 85.0 ± 0.6 | 87.3 ± 1.8 | 78.0 ± 2.1 | 30.7 ± 0.9 |
| | (e,f) 250 | 93.5 ± 10.5 | 51.0 ± 3.0 | 97.5 ± 18.5 | 34.5 ± 2.5 |
| | (f) 500 | 89.0 ± 6.0 | 27.5 ± 7.5 | 128.0 ± 11.0 | 48.5 ± 7.5 |
| | (g) 750 | 98 | 23 | 120 | 41 |
| | 1,000 | 82.0 ± 2.3 | 11.7 ± 2.7 | 174.7 ± 27.0 | (h) 71.0 ± 10.1 |
| | 1,500 | Lethal | -- | -- | -- |
| Methyl methanesulfonate | (f) 5 | 89.0 ± 2.0 | 81.5 ± 7.5 | 335.0 ± 17.0 | (h) 126.0 ± 9.0 |
| **Trial 2** | | | | | |
| Dimethyl sulfoxide | | 113.7 ± 2.0 | 100.0 ± 5.3 | 92.0 ± 9.5 | 26.7 ± 2.6 |
| Furosemide | 62.5 | 113.0 ± 2.1 | 83.0 ± 3.2 | 99.3 ± 13.0 | 29.3 ± 3.3 |
| | 125 | 100.3 ± 2.2 | 69.3 ± 3.4 | 90.7 ± 9.9 | 30.3 ± 4.1 |
| | (e) 250 | 88.7 ± 5.8 | 63.0 ± 3.0 | 103.0 ± 5.6 | 39.3 ± 3.8 |
| | 500 | 105.3 ± 4.9 | 59.3 ± 3.3 | 138.3 ± 13.6 | (h) 44.0 ± 5.7 |
| | 750 | 102.7 ± 3.9 | 52.0 ± 5.5 | 97.0 ± 9.3 | 31.7 ± 3.4 |
| | (g) 1,000 | 94 | 47 | 96 | 34 |
| | 1,500 | Lethal | -- | -- | -- |
| Methyl methanesulfonate | 5 | 64.0 ± 4.7 | 38.3 ± 4.2 | 707.3 ± 44.4 | (h) 377.3 ± 53.3 |
| **+S9 (i)** | | | | | |
| **Trial 1** | | | | | |
| Dimethyl sulfoxide (f) | | 88.5 ± 3.5 | 100.0 ± 1.0 | 112.5 ± 16.5 | 42.5 ± 4.5 |
| Furosemide | (e) 250 | 94.0 ± 4.5 | 94.0 ± 0.6 | 106.7 ± 12.2 | 37.7 ± 2.7 |
| | (f) 500 | 76.5 ± 13.5 | 77.0 ± 3.0 | 98.0 ± 6.0 | 43.5 ± 5.5 |
| | (g) 750 | 88 | 92 | 83 | 32 |
| | (f) 1,000 | 89.5 ± 12.5 | 65.5 ± 10.5 | 128.0 ± 5.0 | 48.5 ± 4.5 |
| | (j) 1,500 | 60 | 10 | 330 | (h) 183 |
| | 1,800 | Lethal | -- | -- | -- |
| Methylcholanthrene | (f) 2.5 | 43.5 ± 9.5 | 13.5 ± 0.5 | 772.5 ± 93.5 | (h) 606.5 ± 65.5 |
| **Trial 2** | | | | | |
| Dimethyl sulfoxide (d) | | 93.0 ± 4.9 | 100.0 ± 5.0 | 81.8 ± 2.3 | 29.5 ± 0.9 |
| Furosemide | (e) 400 | 94.7 ± 6.4 | 82.0 ± 2.6 | 73.7 ± 5.0 | 26.3 ± 3.5 |
| | (f) 600 | 68.0 ± 18.0 | 59.5 ± 16.5 | 65.5 ± 8.5 | 35.5 ± 13.5 |
| | 800 | 92.3 ± 4.7 | 88.7 ± 16.2 | 71.0 ± 14.6 | 26.0 ± 6.7 |
| | 1,000 | 102.0 ± 3.0 | 72.7 ± 6.4 | 74.7 ± 10.1 | 24.7 ± 3.3 |
| | 1,200 | 96.0 ± 3.5 | 74.3 ± 5.5 | 93.7 ± 13.4 | 33.0 ± 6.0 |
| | (k) 1,500 | 63.5 ± 9.5 | 21.0 ± 15.0 | 441.5 ± 11.5 | (h) 236.0 ± 28.0 |
| Methylcholanthrene | 2.5 | 68.3 ± 8.9 | 34.7 ± 2.7 | 809.7 ± 77.9 | (h) 398.3 ± 11.8 |

TABLE 31. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE IN MOUSE L5178Y LYMPHOMA CELLS BY FUROSEMIDEMUTAGENICITY OF FUROSEMIDE IN MOUSE L5178Y LYMPHOMA CELLS (*Continued*)

(a) Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. All doses are tested in triplicate unless otherwise specified; the average for the three tests is presented in the table. Cells ($6 \times 10^5$/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, $3 \times 10^6$ cells were plated in medium and soft agar supplemented with trifluorothymidine (Tft) for selection of Tft-resistant cells, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.
(b) Mean ± standard error of replicate trials for approximately $1 \times 10^6$ cells each. All data are evaluated statistically for both trend and peak response ($P<0.05$ for at least one of the three highest dose sets). Both responses must be significantly ($P<0.05$) positive for a chemical to be considered capable of inducing Tft resistance. If only one of these responses is significant, the call is "equivocal"; the absence of both trend and peak response results in a "negative" call.
(c) Mutant fraction (frequency) is a ratio of the Tft-resistant cells to the cloning efficiency, divided by 3 (to arrive at MF per $1 \times 10^6$ cells treated); MF = mutant fraction.
(d) Data presented are the average of four tests.
(e) Acidic pH shift at this and all higher doses
(f) Data presented are the average of two tests.
(g) Data presented are for one test.
(h) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.
(i) Tests conducted with metabolic activation were performed as described in (a) except that S9, prepared from the liver of Aroclor 1254-induced F344 rats, was added at the same time as the study chemical and/or solvent.
(j) Data presented are for one test. The dose in two tests was lethal.
(k) Data presented are the average of two tests. The dose in one test was lethal.

**TABLE 32. INDUCTION OF SISTER-CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY FUROSEMIDE (a)**

| | Dose (µg/ml) | Total Cells | No. of Chromo-somes | No. of SCEs | SCEs/ Chromo-some | SCEs/ Cell | Hours in BrdU | Relative SCEs/Cell (percent) (b) |
|---|---|---|---|---|---|---|---|---|
| −S9 (c) | | | | | | | | |
| Trial 1--Summary: Weakly positive | | | | | | | | |
| Dimethyl sulfoxide | | 50 | 1,049 | 398 | 0.38 | 8.0 | 25.7 | |
| Furosemide | 50 | 50 | 1,047 | 445 | 0.43 | 8.9 | 25.7 | 111.3 |
| | 166.7 | 50 | 1,050 | 446 | 0.42 | 8.9 | 25.7 | 111.3 |
| | 500 | 50 | 1,050 | 565 | 0.54 | 11.3 | 25.7 | 141.3 |
| | 1,700 | 0 | 0 | | | | | |
| Mitomycin C | 0.001 | 50 | 1,049 | 590 | 0.56 | 11.8 | 25.7 | 147.5 |
| | 0.01 | 5 | 105 | 186 | 1.77 | 37.2 | 25.7 | 465.0 |
| Trial 2--Summary: Positive | | | | | | | | |
| Dimethyl sulfoxide | | 25 | 524 | 175 | 0.33 | 7.0 | 26.1 | |
| Furosemide | 502.5 | 25 | 524 | 219 | 0.42 | 8.8 | 26.1 | 125.7 |
| | 750 | 25 | 523 | 263 | 0.50 | 10.5 | 26.1 | 150.0 |
| | 1,000 | 25 | 524 | 365 | 0.70 | 14.6 | 26.1 | 208.6 |
| Mitomycin C | 0.001 | 25 | 523 | 287 | 0.55 | 11.5 | 26.1 | 164.3 |
| | 0.01 | 5 | 105 | 200 | 1.90 | 40.0 | 26.1 | 571.4 |
| +S9 (d)--Summary: Positive | | | | | | | | |
| Dimethyl sulfoxide | | 50 | 1,050 | 369 | 0.35 | 7.4 | 25.7 | |
| Furosemide | 500 | 50 | 1,050 | 413 | 0.39 | 8.3 | 25.7 | 112.2 |
| | 1,700 | 50 | 1,049 | 454 | 0.43 | 9.1 | 25.7 | 123.0 |
| | 5,000 | 50 | 1,049 | 562 | 0.54 | 11.2 | 25.7 | 151.4 |
| Cyclophosphamide | 0.4 | 50 | 1,050 | 566 | 0.54 | 11.3 | 25.7 | 152.7 |
| | 2 | 5 | 105 | 206 | 1.96 | 41.2 | 25.7 | 556.8 |

(a) Study performed at Litton Bionetics, Inc.; SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as described in (c) and (d) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake off, fixed, air dried, and stained.
(b) SCEs/cell of culture exposed to study chemical relative to those of culture exposed to solvent
(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.
(d) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE 33. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY FUROSEMIDE (a)

| Trial 1 | | | | | Trial 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose (µg/ml) | Total Cells | No. of Abs | Abs/ Cell | Percent Cells with Abs | Dose (µg/ml) | Total Cells | No. of Abs | Abs/ Cell | Percent Cells with Abs |
| −S9 (b) Harvest time: 10.0 hours | | | | | −S9 (b) Harvest time: 12.1 hours | | | | |
| Dimethyl sulfoxide | | | | | Dimethyl sulfoxide | | | | |
| | 200 | 3 | 0.02 | 1.5 | | 200 | 3 | 0.02 | 1.5 |
| Furosemide | | | | | Furosemide | | | | |
| 1,257 | 200 | 4 | 0.02 | 2.0 | 1,263 | 200 | 8 | 0.04 | 4.0 |
| (c) 3,750 | 200 | 11 | 0.06 | 5.5 | 1,885 | 200 | 10 | 0.05 | 5.0 |
| (c) 5,000 | 150 | 17 | 0.11 | 8.0 | 2,513 | 200 | 21 | 0.11 | 10.5 |
| | | | | | 3,141 | 0 | | | |
| Summary: Positive | | | | | Summary: Positive | | | | |
| Mitomycin C | | | | | Mitomycin C | | | | |
| 0.25 | 200 | 20 | 0.10 | 9.5 | 0.25 | 200 | 59 | 0.30 | 23.5 |
| 0.75 | 25 | 13 | 0.52 | 48.0 | 0.75 | 25 | 26 | 1.04 | 64.0 |
| +S9 (d) Harvest time: 12.0 hours | | | | | | | | | |
| Dimethyl sulfoxide | | | | | | | | | |
| | 200 | 5 | 0.03 | 2.0 | | | | | |
| Furosemide | | | | | | | | | |
| 2,500 | 200 | 10 | 0.05 | 5.0 | | | | | |
| 3,750 | 200 | 33 | 0.17 | 15.5 | | | | | |
| 5,000 | 200 | 35 | 0.18 | 15.5 | | | | | |
| Summary: Positive | | | | | | | | | |
| Cyclophosphamide | | | | | | | | | |
| 7.5 | 200 | 22 | 0.11 | 10.5 | | | | | |
| 37.5 | 25 | 32 | 1.28 | 56.0 | | | | | |

(a) Study performed at Litton Bionetics, Inc.; Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as indicated in (b) and (d). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake off, fixed, and stained in 6% Giemsa.
(b) In the absence of S9, cells were incubated with study compound or solvent for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.
(c) Precipitate at this and all higher doses
(d) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

# IV. DISCUSSION AND CONCLUSIONS

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Furosemide administered in feed was evaluated in 14-day, 13-week, and 2-year studies in rats and mice. The chemical exhibited rather low toxicity in short-term studies, with deaths observed only at the highest dose of 46,000 ppm in the 14-day studies. There was concern during the design of these studies that electrolyte imbalances resulting from the diuretic action of furosemide would be so severe as to preclude the use of doses sufficiently high to reveal organ-specific pathologic effects. This was not the case because chemically related kidney effects were seen in rats and mice of each sex at doses that did not substantially influence body weight gain. With the exception of parathyroid hyperplasia secondary to nephrotoxicity, no effects of furosemide on other organs were observed in the short-term studies.

Urine production was not monitored in these studies, but wet bedding was observed in rats after week 4 of the 13-week studies and appeared to be related to dose. No other clinical signs could be related specifically to administration of the chemical, as observations of hunched backs, depression, and nasal exudate were recorded only for animals in groups in which death occurred or for which weight gain was severely depressed.

In the 14-day studies, rats consuming feed containing 15,000 ppm or higher furosemide lost weight, and furosemide at concentrations as low as 1,700 ppm in feed resulted in substantial reductions in weight gain. Kidney lesions in rats included mineralization at the corticomedullary junction and slight nephrosis characterized by tubular cell regeneration. Generally similar dose effects on weight gain were seen for mice and rats. Nephrosis was also the primary toxic effect in mice. The lesion was characterized as tubular cell regeneration accompanied by tubular dilatation and proteinaceous casts.

In the 13-week studies, a dose-related nephrosis occurred in rats which consisted of tubular cell degeneration and regeneration; interstitial fibrosis and mononuclear cell inflammation were present at the higher doses. Mineralization at the corticomedullary junction was also dose related in male rats. In mice, nephrosis characterized by tubular cell regeneration occurred only in a few mice at the higher doses, but evidence of less severe injury, proteinaceous casts in the tubules of the renal medulla, was present at lower concentrations and appeared to be dose related.

The top concentrations selected for the 2-year studies in rats and mice were lower than those concentrations that gave evidence of kidney lesions in the 13-week studies. The selected top concentrations, 700 ppm for rats and 1,400 ppm for mice, were also lower than those that resulted in clear effects on weight gain in the short-term studies. Liver weight to body weight ratios were increased for rats and mice in the higher dose groups in the 13-week studies. However, there were no microscopic changes in the liver of these animals, and body weights of higher dose rats and mice were substantially different from those of controls, so these findings did not influence dose selection.

In the 2-year studies in rats, administration of furosemide did not affect body weight or feed consumption. No chemical-related clinical signs or convincing evidence of diuresis were observed. However, survival of all groups of male rats was low (control, 17/50; low dose, 17/50; high dose, 20/50). In a summary of 2-year National Toxicology Program (NTP) studies evaluated up to 1985, average survival of F344/N male rats was 66% in untreated control groups (Haseman et al., 1985). More recent compilations have indicated a decline in survival to approximately 52% in seven more recent studies (NTP, unpublished). However, the 34% survival seen in control and low dose groups and the 40% survival in the high dose group in the current study are still significantly lower than that observed in contemporary studies. Serologic analysis and clinical signs did not suggest the presence of infection. An aggressive moribund kill program was in effect at the study laboratory, and this may, at least in part, account for the lower than usual survival of male rats. Survival of female rats was not affected by administration of furosemide and was similar to that usually observed in 2-year studies.

The kidney of rats was affected by furosemide in the 2-year studies, but this was evident only as an increase in the severity of lesions and was

indistinguishable from the nephropathy commonly found in aging F344/N rats. In males, cortical cyst formation and epithelial hyperplasia of the renal pelvis were more severe in high dose animals, and tubular cell tumors appeared to be marginally increased in dosed rats. Tubular cell adenomas were seen in three control, four low dose, and five high dose male rats (composite data, see Table 15). Tubular cell adenocarcinomas occurred in one low and one high dose male rat. The original incidence of renal tubular cell adenomas or adenocarcinomas (combined) in low dose rats (4/50) (appropriate for comparison with historial control incidences) is greater than has been observed previously in any single untreated control group (3/50) and is significantly greater than the mean historical control rate of 0.5% ($P<0.001$). However, the marginal overall increase in the incidences of tubular cell hyperplasia (control, 6/50; low dose, 5/50; high dose, 10/50) and of tubular cell neoplasms do not support an unequivocal association of these lesions with furosemide administration.

Malignant meningiomas were found in the brain of three low dose male rats. These tumors are extremely rare with only two observed previously in approximately 2,000 control male rats. Malignant meningiomas occur in humans at a frequency that increases linearly with age (the average annual age-adjusted incidence per 100,000 persons is 0.13) (Velema and Percy, 1987). All three tumors in the current study were found in animals that died early; two of the tumors occurred quite early, at weeks 47 and 48, and in cage mates. Although this cluster of rare tumors is highly unusual and is suggestive of an exogenous cause, the absence of tumors in the high dose group makes it difficult to relate these tumors to furosemide administration.

In female rats, C-cell adenomas of the thyroid gland occurred with a positive trend, but C-cell hyperplasia was not increased. C-Cell neoplasms are relatively common in F344/N rats and occur at a mean incidence of 11%; the highest observed incidence in untreated control females is 38% (Table B4). In the current study, the incidences of 4/50 in control, 6/50 in low dose, and 11/50 in high dose female rats represent a marginal increase in a variable and common tumor. This increase was not attributed to furosemide administration.

The dietary concentrations for mice in the 2-year studies were twice those for rats, but the doses in milligrams per kilogram body weight were approximately six times higher. The average daily doses consumed by rats were approximately 15 and 30 mg/kg compared with approximately 100 or 200 mg/kg consumed by mice. The usual therapeutic dose for humans is 3 mg/kg per day, and the maximal dose is about 10 mg/kg per day.

Administration of furosemide at the higher doses in mice resulted in observable diuresis, necessitating more frequent changing of bedding than usual. Body weights were also notably lower in low and high dose mice of each sex than in controls. Lower body weights may be related to diuresis, since estimated feed consumption by dosed mice differed from that by controls by 10% or less. The mice in these studies were group housed; fighting, which is common in male $B6C3F_1$ mice, resulted in frequent penile and preputial lesions and probably contributed to the observed incidences of urinary bladder and prostate inflammation in males. Survival of high dose female mice was low (18/50), but survival of other groups was typical for 2-year studies and did not appear to be affected by furosemide administration.

As in rats, the kidney was the major target organ in mice. Both the incidence and severity of nephropathy were increased in dosed mice. There was also a chemically related increase in the incidence of hydronephrosis in both sexes, a condition that may have resulted from an impediment in the urine flow lower in the urinary tract. The increased severity of nephropathy may have been the cause of the lower survival in high dose females late in the study.

Garthoff et al. (1982) studied kidney lesions in beagle dogs given furosemide or muzolimine (another "loop" diuretic) with and without electrolyte replacement. In 13-week studies, characteristic kidney lesions were observed after the animals received pharmacologically active doses of both drugs (0.6-9.6 mg/kg per day of furosemide). These lesions included dilated renal tubules with partially flattened epithelia.

Desquamated epithelia were observed in some tubules in the cortex, as was an interstitial infiltration with round cells. The cortex contained fibrotic areas with atrophic nephrons, and subcapsular cysts were observed. In similar studies of 13 weeks' duration with electrolyte replacement in feed and in 52-week studies with electrolyte replacement in drinking water, these lesions were reduced in intensity. These data suggest that certain of the kidney lesions observed in the current studies could be associated with electrolyte depletion rather than with a direct toxic action of furosemide. Electrolyte replacement was considered during the design of the current studies and was not judged necessary, based on the findings from the 14-day studies.

In the current 2-year studies, extramedullary hematopoiesis was diagnosed in the spleen, liver, and adrenal cortex of mice and consisted primarily of a granulocyte response. The hematopoiesis appeared to be related to increases in inflammation of the prostate, ovary, urinary bladder, and kidney in dosed animals.

Female mice receiving diets containing furosemide showed an increase in malignant mixed tumors (adenocarcinoma, type C) of the mammary gland. One acinar cell carcinoma was also observed in a low dose female mouse. Adenocarcinoma, type C, is the most frequently observed spontaneous mammary gland tumor in untreated $B6C3F_1$ mice (historical incidence of malignant mixed tumors or adenocarcinomas, combined, 2%; range, up to 8%; Table D4a), but none was seen in controls in this study. These tumors are not generally considered to be fatal, but their presence can lead to a moribund animal being killed before the end of the study.

A substantial difference occurred in the P values for the life table test and the incidental tumor test for the mammary gland tumors in female mice (see Table 24). The high P value for the incidental tumor test was due in part to the fact that the mammary gland tumors in three of the five high dose mice were given little weight by this procedure, since they were observed during a time interval (weeks 93-104) when few control animals died. The tumor incidences in this time interval were 0/4 and 3/19 for control and high dose groups, respectively. Under similar circumstances in the past, the NTP has used the logistic regression trend test as an additional assessment. This statistical test is appropriate for nonfatal tumors and is less influenced by differences in survival than is the incidental tumor test. By logistic regression analysis, the positive trend for mixed malignant tumors was significant, and the incidence in high dose female mice was significantly greater than that in the controls. The incidences increased with dose, and the incidence for high dose females was about fivefold the mean historical incidence. This increase was seen despite the fact that six of the seven tumors occurred in animals killed or dying after week 97, a period when survival was significantly lower in dosed mice than in controls.

Additional information that supports the statistical significance of this finding involves comparisons of the observed incidences with the historical incidences of malignant mammary gland neoplasms in groups of female control mice at the laboratory that performed these studies. Two other feed studies (hydrochlorothiazide, NTP, 1989a, and diphenhydramine hydrochloride, NTP, 1989b) have been completed at this laboratory. The results from these studies are not incorporated into the NTP historical data base compilation of April 1987, which is presented in Table D4a. In these studies, the incidences of malignant mammary neoplasms in control female mice were 0/49 (hydrochlorothiazide) and 2/50 (diphenylhydramine hydrochloride). If the combined incidence from these two studies is used as an estimate of the specific laboratory historical control incidence for malignant mammary neoplasms (2/99) and if the incidences observed in the dosed female mice in the furosemide study are compared with this incidence, then both the dose response trend (control, 2/99; low dose, 2/50; high dose, 5/50) and the increased incidence in the high dose group are statistically significant ($P<0.05$). Based on these considerations, it is concluded that the increased incidence of malignant mammary gland neoplasms in female mice is associated with furosemide administration.

Other chemicals that have been found to cause increased incidences of mammary neoplasms in female mice in NTP and National Cancer

Institute studies include benzene (NTP, 1986), reserpine (NCI, 1982), 1,2-dibromoethane (NTP, 1982), 1,2-dichloroethane (NCI, 1978a), and sulfallate (NCI, 1978b). It should be noted that the B6C3F$_1$ mice used in these studies do not carry known murine mammary tumor viruses.

Several neoplasms occurred at statistically significant incidences but were not considered biologically significant or related to furosemide administration. These included malignant lymphomas and follicular cell adenomas of the thyroid gland in female mice and hepatocellular carcinomas in male mice. The combined incidences of adenomas and carcinomas of the liver or thyroid gland were not increased in dosed mice, and the marginal increase in malignant lymphomas was not dose related.

Studies with Salmonella indicate that furosemide does not induce reverse gene mutations in bacteria; however, furosemide is clearly clastogenic in in vitro studies, and limited evidence suggests similar actions in in vivo studies. There are three reports in the literature of cytogenetic effects induced by furosemide in cultured mammalian cells and one report of aberrations induced in the germ cells of mice. Two of the in vitro studies were performed by the same laboratory with Chinese hamster fibroblasts (Ishidate et al., 1978; Matsuoka et al., 1979). A summary of the data from these studies was presented by Ishidate (1984) which lists, in addition to several equivocal responses obtained both in the absence and presence of S9, one positive response resulting primarily from induction of chromosomal breaks in 11% and 9% of cells from cultures treated with 2.0 mg/ml furosemide for 24 and 48 hours, respectively. The third study, with human lymphocytes, reported induction of abnormal metaphases in up to 49% of cells, including exchanges in approximately 3% of the cells. These results are supported by those from NTP-sponsored tests in which increases in both SCEs and chromosomal aberrations were induced by furosemide in the presence and absence of S9. The in vivo study reported induction of translocations by furosemide detected as multivalents observed in meiotic cells at the first metaphase division. This one demonstration of clastogenicity in vivo, however, is less convincing than the in vitro data. In this study, predominantly univalent chromosomes were observed during weeks 1-5 after treatment, and a major increase in translocations was observed only in the first week after treatment. Further, the observation of approximately 2% translocations in controls is unusually high.

The experimental and tabulated data for the NTP Technical Report on furosemide were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix H, the audit revealed no major problems with the conduct of the studies or with collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

Under the conditions of these 2-year studies, there was *equivocal evidence of carcinogenic activity** of furosemide for male F344/N rats, as shown by marginal increases in uncommon tubular cell neoplasms of the kidney and meningiomas of the brain. There was *no evidence of carcinogenic activity* of furosemide for female F344/N rats fed diets containing 350 or 700 ppm furosemide for 2 years. There was *no evidence of carcinogenic activity* for male B6C3F$_1$ mice fed diets containing 700 or 1,400 ppm furosemide for 2 years. There was *some evidence of carcinogenic activity* of furosemide for female mice, as shown by an increase in malignant tumors of the mammary gland.

Nephropathy was more severe in the kidney of male rats and of male and female mice fed diets containing furosemide than in controls.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 7.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 10-11.

# V. REFERENCES

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# APPENDIX A

# SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE


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**TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (50) | (50) | (50) |
| Squamous cell papilloma | | 2 (4%) | 2 (4%) |
| Squamous cell carcinoma | | | 1 (2%) |
| Basal cell tumor | 2 (4%) | 1 (2%) | 2 (4%) |
| Keratoacanthoma | | | 2 (4%) |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Sarcoma, NOS | 2 (4%) | 2 (4%) | 2 (4%) |
| Fibroma | 3 (6%) | 5 (10%) | 1 (2%) |
| Lipoma | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #Lung | (50) | (41) | (50) |
| Carcinoma, NOS, metastatic | | 1 (2%) | |
| Alveolar/bronchiolar adenoma | 2 (4%) | 1 (2%) | 1 (2%) |
| Alveolar/bronchiolar carcinoma | 1 (2%) | 1 (2%) | 1 (2%) |
| Pheochromocytoma, metastatic | | | 1 (2%) |
| Sarcoma, NOS, metastatic | | | 1 (2%) |
| Osteosarcoma, metastatic | | 1 (2%) | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Malignant lymphoma, NOS | | | 1 (2%) |
| Leukemia, mononuclear cell | 23 (46%) | 21 (42%) | 20 (40%) |
| CIRCULATORY SYSTEM | | | |
| None | | | |
| DIGESTIVE SYSTEM | | | |
| *Palate | (50) | (50) | (50) |
| Squamous cell papilloma | | 1 (2%) | |
| Squamous cell carcinoma | 1 (2%) | | |
| *Tongue | (50) | (50) | (50) |
| Squamous cell papilloma | | 1 (2%) | 1 (2%) |
| #Duodenum | (50) | (34) | (50) |
| Adenocarcinoma, NOS | 1 (2%) | | |
| URINARY SYSTEM | | | |
| #Kidney | (50) | (50) | (50) |
| Tubular cell adenoma | 1 (2%) | 3 (6%) | 1 (2%) |
| Tubular cell adenocarcinoma | | 1 (2%) | 1 (2%) |
| #Urinary bladder/mucosa | (50) | (34) | (46) |
| Transitional cell papilloma | 1 (2%) | | |
| ENDOCRINE SYSTEM | | | |
| #Anterior pituitary | (50) | (50) | (50) |
| Adenoma, NOS | 4 (8%) | 11 (22%) | 8 (16%) |
| #Adrenal medulla | (50) | (36) | (49) |
| Pheochromocytoma | 17 (34%) | 6 (17%) | 16 (33%) |
| Pheochromocytoma, malignant | 1 (2%) | | 1 (2%) |

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ENDOCRINE SYSTEM (Continued) | | | |
| #Thyroid | (50) | (50) | (50) |
| Follicular cell adenoma | 1 (2%) | | 1 (2%) |
| Follicular cell carcinoma | | | 1 (2%) |
| C-cell adenoma | 6 (12%) | 3 (6%) | 6 (12%) |
| C-cell carcinoma | 1 (2%) | | |
| #Pancreatic islets | (49) | (34) | (50) |
| Islet cell adenoma | 1 (2%) | 1 (3%) | |
| Islet cell carcinoma | 1 (2%) | 1 (3%) | |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (50) |
| Carcinoma, NOS | | 1 (2%) | |
| Adenoma, NOS | | | 1 (2%) |
| Fibroadenoma | 1 (2%) | 2 (4%) | 1 (2%) |
| #Preputial gland | (48) | (12) | (47) |
| Carcinoma, NOS | 1 (2%) | | 1 (2%) |
| Adenoma, NOS | 2 (4%) | 3 (25%) | 2 (4%) |
| #Testis | (50) | (50) | (49) |
| Interstitial cell tumor | 47 (94%) | 43 (86%) | 42 (86%) |
| NERVOUS SYSTEM | | | |
| #Brain/meninges | (50) | (50) | (50) |
| Meningioma | | 3 (6%) | |
| #Brain | (50) | (50) | (50) |
| Granular cell tumor, benign | | | 1 (2%) |
| Astrocytoma | 1 (2%) | 1 (2%) | |
| *Cranial nerve | (50) | (50) | (50) |
| Neurilemoma | | | 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| *Zymbal gland | (50) | (50) | (50) |
| Carcinoma, NOS | | 1 (2%) | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *Skull | (50) | (50) | (50) |
| Osteoma | | 1 (2%) | |
| *Vertebra | (50) | (50) | (50) |
| Chordoma | | 1 (2%) | |
| BODY CAVITIES | | | |
| *Thoracic cavity | (50) | (50) | (50) |
| Osteosarcoma | | | 1 (2%) |
| *Abdominal cavity | (50) | (50) | (50) |
| Mesothelioma, NOS | 1 (2%) | 2 (4%) | 2 (4%) |
| *Tunica vaginalis | (50) | (50) | (50) |
| Mesothelioma, NOS | 5 (10%) | 4 (8%) | 2 (4%) |
| ALL OTHER SYSTEMS | | | |
| None | | | |

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMAL DISPOSITION SUMMARY | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural death | 5 | 5 | 5 |
| Moribund sacrifice | 31 | 31 | 25 |
| Terminal sacrifice | 14 | 14 | 20 |
| TUMOR SUMMARY | | | |
| Total animals with primary tumors** | 49 | 49 | 49 |
| Total primary tumors | 127 | 124 | 124 |
| Total animals with benign tumors | 48 | 46 | 47 |
| Total benign tumors | 88 | 85 | 89 |
| Total animals with malignant tumors | 29 | 31 | 29 |
| Total malignant tumors | 33 | 33 | 31 |
| Total animals with secondary tumors## | | 2 | 3 |
| Total secondary tumors | | 2 | 3 |
| Total animals with tumors uncertain-- benign or malignant | 5 | 4 | 3 |
| Total uncertain tumors | 6 | 6 | 4 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
## Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

## TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: UNTREATED CONTROL

| ANIMAL NUMBER | 02 27 | 01 14 | 01 19 | 03 38 | 03 34 | 04 45 | 01 17 | 02 28 | 02 21 | 02 23 | 03 30 | 04 43 | 01 16 | 04 46 | 00 02 | 02 24 | 00 09 | 01 15 | 02 26 | 04 47 | 03 31 | 00 01 | 00 06 | 02 22 | 03 38 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 05 50 | 05 55 | 06 69 | 06 69 | 07 72 | 07 74 | 07 78 | 07 78 | 08 22 | 08 22 | 08 84 | 08 44 | 08 85 | 08 88 | 08 89 | 09 91 | 09 92 | 09 93 | 09 33 | 09 33 | 09 35 | 09 97 | 09 97 | 09 97 | 09 97 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Basal cell tumor | | | | | | | | | | | | | | | | | | | | | | | X | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS | | | | | | | X | | | | | | | | | | | | | | | | | | |
| Fibroma | | | | | | | | | | | | | | X | | | | | | | | | | X | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | X | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Squamous cell carcinoma | | | | | | | | | | | X | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS | | X | | | | | | | | | | | | | | | | | | | | | | | |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tubular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Transitional cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | X | | | | | | | | | | | | X | | | | | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | X | | | X | X | | X |
| Pheochromocytoma, malignant | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | X | |
| C-cell adenoma | | | | | | | | | | | X | | | X | | | | | | | | | | | |
| C-cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + |
| Islet cell adenoma | | | | | | | | | | | | | | | | X | | | | | | | | | |
| Islet cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Interstitial cell tumor | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | X | X | X | X | X |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Preputial/clitoral gland | − | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Astrocytoma | | | | | | | | | | | | | | | X | | | | | | | | | | |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Mesothelioma, NOS | | | | | | | | | | | | | | X | | | | | | | | | | | |
| Tunica vaginalis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Mesothelioma, NOS | | | X | X | | | | | | | | | | X | X | | X | | | | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Leukemia, mononuclear cell | | | | | | X | | X | X | X | | X | X | | X | | | X | X | X | X | | X | | |

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

## TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

| ANIMAL NUMBER | 0007 | 0107 | 0120 | 0202 | 0302 | 0352 | 0422 | 0449 | 0111 | 0118 | 0225 | 0033 | 0044 | 0055 | 0058 | 0138 | 0259 | 0336 | 0337 | 0339 | 0440 | 0441 | 0444 | 0448 | 0550 | TOTAL: TISSUES TUMORS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 100 | 100 | 102 | 102 | 103 | 103 | 103 | 103 | 104 | 104 | 104 | 105 | 105 | 105 | 105 | 105 | 105 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Basal cell tumor | | | | | | | | | | | | | | | | | | | | | | | | | X | 2 |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Sarcoma NOS | | | | | | | | | | | | | | | | | X | | | | | | | | | 2 |
| Fibroma | | | | | | | | | | | | | | | | | | | | | X | | | | | 3 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar adenoma | | | | | | | | | X | | | | | | | X | | | | | | | | | | 2 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Tubular cell adenoma | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Transitional cell papilloma | | X | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | | | X | | | | | | | | | | | | | | | | | | | | | X | | 4 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pheochromocytoma | | | | X | X | X | | X | X | X | | X | X | X | | | X | | X | X | | | X | | | 17 |
| Pheochromocytoma, malignant | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| C cell adenoma | | X | | | | X | | | | | | | X | | | | | | | X | | | | | | 6 |
| C cell carcinoma | | | X | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Parathyroid | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Islet cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Islet cell carcinoma | | | | | | | | | X | | | | | | | | | | | | | | | | | 1 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Fibroadenoma | | | | | | | | | | | | | | | | | X | | | | | | | | | 1 |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Interstitial cell tumor | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 47 |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Preputial/clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Carcinoma, NOS | | | | | | | | | | | | X | | | | | | | | | | | | | | 1 |
| Adenoma, NOS | | | | | | X | | | | | | | | X | | | | | | | | | | | | 2 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Tunica vaginalis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 5 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Leukemia, mononuclear cell | X | X | | X | | | X | | | | | | | X | X | X | X | X | | X | | X | | | | 23 |

* Animals necropsied

## TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: LOW DOSE

| ANIMAL NUMBER | 019 | 017 | 004 | 002 | 045 | 009 | 039 | 034 | 012 | 037 | 038 | 001 | 003 | 018 | 043 | 047 | 013 | 014 | 027 | 035 | 023 | 041 | 021 | 005 | 011 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 047 | 048 | 065 | 066 | 066 | 067 | 067 | 077 | 079 | 081 | 083 | 084 | 084 | 084 | 084 | 084 | 086 | 086 | 086 | 086 | 087 | 089 | 090 | 091 | 091 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | X | | | | | |
| Basal cell tumor | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS | | | | | X | | | | | | | | | | | | | | | | | | | | |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lipoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS, metastatic | | | X | | | | | | | | | | | | | | | | | | | | | | |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | X | | | | | | | | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Osteosarcoma, metastatic | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | + | + | + | − | + | + | − | + | + | − | + | + | + | + | + | + | − | + | + | + | + | − |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | X | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tubular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tubular cell adenocarcinoma | | | | | | | | | | | | | | | | | | | | X | | | | | |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | X | | | X | | | | | X | | | | | X | | X | | | | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | | | | | | | | | | | X | | | | | | | | | X | | X | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | + | + | + | + |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Islet cell adenoma | | | | | | | | | | | | | | | | | X | | | | | | | | |
| Islet cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | N | + | + | + | + | + | + | + | + | + | N | + | N | + | + | + | N | + | + | N | + | + |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Interstitial cell tumor | | | | X | X | | | X | | X | X | X | X | X | X | X | | X | X | X | X | X | X | X | X |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Preputial/clitoral gland | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Astrocytoma | | | | | X | | | | | | | | | | | | | | | | | | | | |
| Meningioma | X | X | | | | | | | | | | | | | | | | | | | | | | | |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Zymbal gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Carcinoma, NOS | | | X | | | | | | | | | | | | | | | | | | | | | | |
| **MUSCULOSKELETAL SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Osteoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Chordoma | | | | | | | | | | | | | | | | | | | | | | X | | | |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Mesothelioma, NOS | | | | X | | | | | | | | | | | | | | | | X | | | | | |
| Tunica vaginalis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Mesothelioma, NOS | | | | X | | X | | | | | | | | | | | | | | X | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Leukemia, mononuclear cell | | | | | | | | X | | X | X | X | | X | X | X | X | | X | | X | | X | X | X |

## TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

| ANIMAL NUMBER | 026 | 025 | 029 | 040 | 044 | 048 | 046 | 030 | 007 | 008 | 049 | 006 | 010 | 015 | 016 | 020 | 022 | 024 | 028 | 031 | 032 | 033 | 036 | 042 | 050 | TOTAL: |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 092 | 095 | 096 | 097 | 097 | 097 | 101 | 102 | 104 | 104 | 104 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | TISSUES TUMORS |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | N | N | N | + | N | N | N | + | N | N | N | + | N | N | + | N | N | *50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | X | | | | | | | | | | 2 |
| Basal cell tumor | | | | | | | | | | | | | | | | | | | | X | | | | | | 1 |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | N | N | N | + | N | N | N | + | N | N | N | + | N | N | + | N | N | *50 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | X | | | | | | 2 |
| Fibroma | | | X | | | | | | | | | | X | X | | | X | | | X | | | | | | 5 |
| Lipoma | | | | | | | | | | | X | | | | | | | | | | | | | | | 1 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | – | + | – | – | – | – | + | + | + | – | + | + | + | – | – | – | 41 |
| Carcinoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | X | | | | | | | | | | 1 |
| Osteosarcoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | – | + | + | – | – | – | – | + | – | – | + | + | + | + | + | + | – | 42 |
| Nasal cavity | – | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 8 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 33 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | – | + | – | + | + | + | – | + | + | – | – | + | + | – | 44 |
| Lymph nodes | + | + | + | + | + | + | + | + | – | + | + | + | – | + | + | – | + | – | + | + | – | + | – | + | + | 44 |
| Thymus | – | + | + | + | + | + | – | + | – | + | – | + | – | – | + | – | – | – | + | + | – | + | – | + | + | 34 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | + | – | + | – | – | + | – | 38 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Squamous cell papilloma | | | | | | | | X | | | | | | | | | | | | | | | | | | 2 |
| Salivary gland | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | + | – | + | – | – | – | – | + | – | 36 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | – | – | + | – | – | + | + | + | + | + | + | + | + | 46 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | – | – | + | – | – | + | + | + | + | + | + | + | + | 46 |
| Pancreas | + | + | + | + | + | + | + | + | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 34 |
| Esophagus | + | + | + | + | + | + | + | + | + | – | + | – | – | – | + | + | + | – | + | + | + | + | + | + | + | 45 |
| Stomach | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 33 |
| Small intestine | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | – | – | – | – | + | – | – | – | – | 34 |
| Large intestine | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | – | – | – | – | + | – | – | – | – | 34 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Tubular cell adenoma | | | | | | | | | X | | | X | | | | | | | | | | | | X | | 3 |
| Tubular cell adenocarcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Urinary bladder | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | – | | 34 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | | | | | | | X | | | | X | | | | | | | | | | X | X | X | X | X | 11 |
| Adrenal | + | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | – | – | – | – | + | – | – | + | – | 36 |
| Pheochromocytoma | X | | X | | | | | | X | | | | | | | | | | | | | | | | | 6 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| C-cell adenoma | | | | | X | | | | X | X | | | | | | | | | | | | | | | | 3 |
| Parathyroid | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | 46 |
| Pancreatic islets | + | + | + | + | + | + | + | + | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 34 |
| Islet cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Islet cell carcinoma | | | | | | | | | | X | | | | | | | | | | | | | | | | 1 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | N | N | N | N | N | + | N | N | N | N | N | N | N | + | + | N | | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | X | | | 1 |
| Fibroadenoma | | | | | | | | | | | | | | X | | | | | | | | X | | | | 2 |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Interstitial cell tumor | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 43 |
| Prostate | + | + | + | + | + | + | + | + | – | – | – | – | – | – | – | + | – | – | – | – | – | – | – | + | – | 35 |
| Preputial/clitoral gland | – | + | + | + | + | + | + | + | + | + | – | – | – | + | + | – | – | – | – | – | – | + | – | – | | 12 |
| Adenoma, NOS | | X | | | | | | | | | | | | X | X | | | | | | | | | | | 3 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Meningioma | | | | X | | | | | | | | | | | | | | | | | | | | | | 3 |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Zymbal gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **MUSCULOSKELETAL SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Osteoma | | | | | | | | | | | | | | | | | | X | | | | | | | | 1 |
| Chordoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Tunica vaginalis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | X | | | | | | 4 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Leukemia, mononuclear cell | X | | | | X | | | | X | | X | | X | | X | | | | | | | | X | X | | 21 |

* Animals necropsied

## TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: HIGH DOSE

| ANIMAL NUMBER | 0003 | 0444 | 0112 | 0004 | 0338 | 0227 | 0333 | 0116 | 0447 | 0118 | 0223 | 0448 | 0221 | 0005 | 0009 | 0220 | 0228 | 0330 | 0007 | 0115 | 0006 | 0332 | 0335 | 0449 | 0113 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 0488 | 0596 | 0665 | 0668 | 0711 | 0744 | 0777 | 0800 | 0844 | 0855 | 0887 | 0877 | 0888 | 0899 | 0899 | 0899 | 0911 | 0922 | 0955 | 0955 | 0966 | 0977 | 0997 | 0999 | 1000 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | X | | |
| Squamous cell carcinoma | | | | | | | | | | | | X | | | | | | | | | | | | | |
| Basal cell tumor | | | | | | | | | | | | | | | | | | | | | | | | | |
| Keratoacanthoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | X | | X | | | | | | |
| Fibroma | | | | | | | | | | | | | X | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | X | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pheochromocytoma, metastatic | | | | | | | | | | | | | | | | | | | | | X | | | | |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | X | | | | | | |
| Osteosarcoma, metastatic | | X | | | | | | | | | | | | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | + | + | – | + | + | + | + | + | – | + | + | + | + | + | – | + | + | + | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | X | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tubular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tubular cell adenocarcinoma | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | – | |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | X | | | | X | X | X | | | | | | | | X | | | | X | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + |
| Pheochromocytoma | | | | | | | | X | | | | | | | | X | | | | X | | | | X | |
| Pheochromocytoma, malignant | | | | | | | | | | | | | | | | | | | | | X | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Follicular cell carcinoma | | | | | | | | | | | | | | | | | | | | | | X | | | |
| C-cell adenoma | | | | | | | X | | | | X | | | | | | | X | | | | | | | |
| Parathyroid | + | + | + | + | + | – | + | + | + | + | + | + | + | + | – | – | + | + | + | + | + | + | + | + | + |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + |
| Interstitial cell tumor | | | | | X | | X | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | | X |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Preputial/clitoral gland | – | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | – | + |
| Carcinoma, NOS | | | | | | | X | | | | | | | | | | | | | | | | | | |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | X | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Nerves | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Neurilemoma | X | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Granular cell tumor, benign | | | | | | | | | | | | | | | | | | | | | | | | | |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Zymbal gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Carcinoma, NOS | | | X | | | | | | | | | | | | | | | | | | | | | | |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pleura | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Osteosarcoma | | X | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | X | | | |
| Tunica vaginalis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | X | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Malignant lymphoma, NOS | | | | | | | | | | | | | | | | X | | | | | | | | | |
| Leukemia, mononuclear cell | | | | | X | | | X | | | | X | | X | X | | | X | | X | | X | | | |

## TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

| ANIMAL NUMBER | 0292 | 0466 | 0261 | 0433 | 0038 | 0001 | 0002 | 0110 | 0111 | 0114 | 0117 | 0119 | 0222 | 0244 | 0255 | 0331 | 0334 | 0361 | 0367 | 0393 | 0401 | 0411 | 0422 | 0445 | 0450 | TOTAL: TISSUES TUMORS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 100 | 100 | 101 | 101 | 103 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | X | | | | | | | | 2 |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Basal cell tumor | | | | | | | | | | | | | | | | | | X | | | X | | | | | 2 |
| Keratoacanthoma | | | | | | | | X | | | | X | | | | | | | | | | | | | | 2 |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | X | | | 1 |
| Pheochromocytoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Osteosarcoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Salivary gland | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Large intestine | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Tubular cell adenoma | | | | | | | | | | | | | | | | X | | | | | | | | | | 1 |
| Tubular cell adenocarcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Urinary bladder | + | + | + | – | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | | | | | | X | | | | | | | | | | | | X | | | | | | | | 8 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Pheochromocytoma | | | X | | X | X | X | | X | | X | | | | X | | | | X | | X | | X | X | X | 16 |
| Pheochromocytoma, malignant | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Follicular cell adenoma | | | | | | | | X | | | | | | | | | | | | | | | | | | 1 |
| Follicular cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| C-cell adenoma | | | | | | | | | | X | | X | X | | | | | | | | | | | | | 6 |
| Parathyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | N | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Adenoma, NOS | | X | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Fibroadenoma | | | | | | | | | | | | | | X | | | | | | | | | | | | 1 |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Interstitial cell tumor | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 42 |
| Prostate | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Preputial/clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Adenoma, NOS | | | | | | X | | | | | | | | | | | | | | | | | | | | 2 |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Nerves | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Neurilemoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Granular cell tumor, benign | | | X | | | | | | | | | | | | | | | | | | | | | | | 1 |
| SPECIAL SENSE ORGANS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Zymbal gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| BODY CAVITIES | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pleura | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Osteosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | X | | | | | | | 2 |
| Tunica vaginalis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Mesothelioma, NOS | | | | | | | | | X | | | | | | | | | | | | | | | | | 2 |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Malignant lymphoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Leukemia, mononuclear cell | X | X | X | | | X | | X | X | | | | | | X | | X | | | X | X | | X | X | | 20 |

* Animals necropsied

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Skin: Squamous Cell Papilloma or Carcinoma** | | | |
| Overall Rates (a) | 0/50 (0%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted Rates (b) | 0.0% | 8.7% | 10.6% |
| Terminal Rates (c) | 0/17 (0%) | 1/17 (6%) | 1/20 (5%) |
| Week of First Observation | | 86 | 87 |
| Life Table Tests (d) | P=0.105 | P=0.230 | P=0.139 |
| Incidental Tumor Tests (d) | P=0.088 | P=0.314 | P=0.127 |
| Cochran-Armitage Trend Test (d) | P=0.082 | | |
| Fisher Exact Test (d) | | P=0.247 | P=0.121 |
| **Subcutaneous Tissue: Fibroma** | | | |
| Overall Rates (a) | 3/50 (6%) | 5/50 (10%) | 1/50 (2%) |
| Adjusted Rates (b) | 11.6% | 26.9% | 2.6% |
| Terminal Rates (c) | 1/17 (6%) | 4/17 (24%) | 0/20 (0%) |
| Week of First Observation | 88 | 96 | 88 |
| Life Table Tests (d) | P=0.231N | P=0.306 | P=0.289N |
| Incidental Tumor Tests (d) | P=0.244N | P=0.291 | P=0.281N |
| Cochran-Armitage Trend Test (d) | P=0.264N | | |
| Fisher Exact Test (d) | | P=0.357 | P=0.309N |
| **Subcutaneous Tissue: Fibroma or Sarcoma** | | | |
| Overall Rates (a) | 5/50 (10%) | 6/50 (12%) | 3/50 (6%) |
| Adjusted Rates (b) | 19.0% | 28.4% | 8.4% |
| Terminal Rates (c) | 2/17 (12%) | 4/17 (24%) | 0/20 (0%) |
| Week of First Observation | 78 | 66 | 88 |
| Life Table Tests (d) | P=0.271N | P=0.449 | P=0.335N |
| Incidental Tumor Tests (d) | P=0.300N | P=0.422 | P=0.344N |
| Cochran-Armitage Trend Test (d) | P=0.303N | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.357N |
| **Lung: Alveolar/Bronchiolar Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 3/50 (6%) | 2/41 (5%) | 2/50 (4%) |
| Adjusted Rates (b) | 14.4% | 14.7% | 8.3% |
| Terminal Rates (c) | 2/17 (12%) | 1/8 (13%) | 1/20 (5%) |
| Week of First Observation | 93 | 84 | 97 |
| Life Table Tests (d) | P=0.367N | P=0.640 | P=0.459N |
| Incidental Tumor Tests (d) | P=0.397N | P=0.664 | P=0.508N |
| Cochran-Armitage Trend Test (d) | P=0.409N | | |
| Fisher Exact Test (d) | | P=0.594N | P=0.500N |
| **Hematopoietic System: Mononuclear Cell Leukemia** | | | |
| Overall Rates (a) | 23/50 (46%) | 21/50 (42%) | 20/50 (40%) |
| Adjusted Rates (b) | 64.9% | 59.8% | 61.2% |
| Terminal Rates (c) | 7/17 (41%) | 6/17 (35%) | 9/20 (45%) |
| Week of First Observation | 74 | 77 | 71 |
| Life Table Tests (d) | P=0.260N | P=0.538 | P=0.280N |
| Incidental Tumor Tests (d) | P=0.266N | P=0.268N | P=0.337N |
| Cochran-Armitage Trend Test (d) | P=0.307N | | |
| Fisher Exact Test (d) | | P=0.420N | P=0.343N |
| **Kidney: Tubular Cell Adenoma** | | | |
| Overall Rates (a) | 1/50 (2%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted Rates (b) | 5.9% | 17.6% | 5.0% |
| Terminal Rates (c) | 1/17 (6%) | 3/17 (18%) | 1/20 (5%) |
| Week of First Observation | 104 | 104 | 104 |
| Life Table Tests (d) | P=0.550N | P=0.300 | P=0.727N |
| Incidental Tumor Tests (d) | P=0.550N | P=0.300 | P=0.727N |
| Cochran-Armitage Trend Test (d) | P=0.610 | | |
| Fisher Exact Test (d) | | P=0.309 | P=0.753 |

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Kidney: Tubular Cell Adenoma or Adenocarcinoma** | | | |
| Overall Rates (a) | 1/50 (2%) | 4/50 (8%) | 2/50 (4%) |
| Adjusted Rates (b) | 5.9% | 20.1% | 7.3% |
| Terminal Rates (c) | 1/17 (6%) | 3/17 (18%) | 1/20 (5%) |
| Week of First Observation | 104 | 86 | 84 |
| Life Table Tests (d) | P=0.467 | P=0.171 | P=0.543 |
| Incidental Tumor Tests (d) | P=0.477 | P=0.217 | P=0.561 |
| Cochran-Armitage Trend Test (d) | P=0.406 | | |
| Fisher Exact Test (d) | | P=0.181 | P=0.500 |
| **Anterior Pituitary Gland: Adenoma** | | | |
| Overall Rates (a) | 4/50 (8%) | 11/50 (22%) | 8/50 (16%) |
| Adjusted Rates (b) | 14.7% | 41.6% | 23.9% |
| Terminal Rates (c) | 1/17 (6%) | 5/17 (29%) | 2/20 (10%) |
| Week of First Observation | 78 | 67 | 74 |
| Life Table Tests (d) | P=0.229 | P=0.039 | P=0.221 |
| Incidental Tumor Tests (d) | P=0.173 | P=0.044 | P=0.166 |
| Cochran-Armitage Trend Test (d) | P=0.166 | | |
| Fisher Exact Test (d) | | P=0.045 | P=0.178 |
| **Adrenal Gland Medulla: Pheochromocytoma** | | | |
| Overall Rates (a) | 17/50 (34%) | (e) 6/36 (17%) | 16/49 (33%) |
| Adjusted Rates (b) | 66.5% | | 59.6% |
| Terminal Rates (c) | 9/17 (53%) | | 10/20 (50%) |
| Week of First Observation | 93 | | 80 |
| Life Table Test (d) | | | P=0.375N |
| Incidental Tumor Test (d) | | | P=0.545N |
| Fisher Exact Test (d) | | | P=0.528N |
| **Adrenal Gland Medulla: Pheochromocytoma or Malignant Pheochromocytoma** | | | |
| Overall Rates (a) | 18/50 (36%) | (e) 6/36 (17%) | 17/49 (35%) |
| Adjusted Rates (b) | 70.6% | | 61.0% |
| Terminal Rates (c) | 10/17 (59%) | | 10/20 (50%) |
| Week of First Observation | 93 | | 80 |
| Life Table Test (d) | | | P=0.370N |
| Incidental Tumor Test (d) | | | P=0.555N |
| Fisher Exact Test (d) | | | P=0.530N |
| **Thyroid Gland: C-Cell Adenoma** | | | |
| Overall Rates (a) | 6/50 (12%) | 3/50 (6%) | 6/50 (12%) |
| Adjusted Rates (b) | 23.5% | 15.8% | 21.5% |
| Terminal Rates (c) | 2/17 (12%) | 2/17 (12%) | 3/20 (15%) |
| Week of First Observation | 84 | 97 | 77 |
| Life Table Tests (d) | P=0.507N | P=0.306N | P=0.560N |
| Incidental Tumor Tests (d) | P=0.539N | P=0.295N | P=0.568N |
| Cochran-Armitage Trend Test (d) | P=0.566 | | |
| Fisher Exact Test (d) | | P=0.243N | P=0.620 |
| **Thyroid Gland: C-Cell Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 7/50 (14%) | 3/50 (6%) | 6/50 (12%) |
| Adjusted Rates (b) | 26.8% | 15.8% | 21.5% |
| Terminal Rates (c) | 2/17 (12%) | 2/17 (12%) | 3/20 (15%) |
| Week of First Observation | 84 | 97 | 77 |
| Life Table Tests (d) | P=0.390N | P=0.222N | P=0.450N |
| Incidental Tumor Tests (d) | P=0.425N | P=0.232N | P=0.465N |
| Cochran-Armitage Trend Test (d) | P=0.436N | | |
| Fisher Exact Test (d) | | P=0.159N | P=0.500N |

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Preputial Gland: Adenoma** | | | |
| Overall Rates (a) | 2/48 (4%) | (e) 3/12 (25%) | 2/47 (4%) |
| Adjusted Rates (b) | 10.4% | | 8.2% |
| Terminal Rates (c) | 1/17 (6%) | | 1/20 (5%) |
| Week of First Observation | 103 | | 96 |
| Life Table Test (d) | | | P=0.661N |
| Incidental Tumor Test (d) | | | P=0.658 |
| Fisher Exact Test (d) | | | P=0.683 |
| **Preputial Gland: Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 3/48 (6%) | (e) 3/12 (25%) | 3/47 (6%) |
| Adjusted Rates (b) | 16.0% | | 10.3% |
| Terminal Rates (c) | 2/17 (12%) | | 1/20 (5%) |
| Week of First Observation | 103 | | 77 |
| Life Table Test (d) | | | P=0.621N |
| Incidental Tumor Test (d) | | | P=0.643 |
| Fisher Exact Test (d) | | | P=0.651 |
| **Testis: Interstitial Cell Tumor** | | | |
| Overall Rates (a) | 47/50 (94%) | 43/50 (86%) | 42/49 (86%) |
| Adjusted Rates (b) | 100.0% | 100.0% | 100.0% |
| Terminal Rates (c) | 17/17 (100%) | 17/17 (100%) | 20/20 (100%) |
| Week of First Observation | 69 | 66 | 71 |
| Life Table Tests (d) | P=0.141N | P=0.522 | P=0.153N |
| Incidental Tumor Tests (d) | P=0.053N | P=0.194N | P=0.070N |
| Cochran-Armitage Trend Test (d) | P=0.127N | | |
| Fisher Exact Test (d) | | P=0.159N | P=0.151N |
| **Brain: Meningioma** | | | |
| Overall Rates (a) | 0/50 (0%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted Rates (b) | 0.0% | 8.4% | 0.0% |
| Terminal Rates (c) | 0/17 (0%) | 0/17 (0%) | 0/20 (0%) |
| Week of First Observation | | 47 | |
| Life Table Tests (d) | P=0.636 | P=0.107 | (f) |
| Incidental Tumor Tests (d) | P=0.625 | P=0.095 | (f) |
| Cochran-Armitage Trend Test (d) | P=0.640 | | |
| Fisher Exact Test (d) | | P=0.121 | (f) |
| **All Sites: Mesothelioma** | | | |
| Overall Rates (a) | 5/50 (10%) | 4/50 (8%) | 3/50 (6%) |
| Adjusted Rates (b) | 12.0% | 12.6% | 13.1% |
| Terminal Rates (c) | 0/17 (0%) | 1/17 (6%) | 2/20 (10%) |
| Week of First Observation | 69 | 66 | 97 |
| Life Table Tests (d) | P=0.282N | P=0.577N | P=0.344N |
| Incidental Tumor Tests (d) | P=0.278N | P=0.350N | P=0.334N |
| Cochran-Armitage Trend Test (d) | P=0.290N | | |
| Fisher Exact Test (d) | | P=0.500N | P=0.358N |
| **All Sites: Benign Tumors** | | | |
| Overall Rates (a) | 48/50 (96%) | 46/50 (92%) | 47/50 (94%) |
| Adjusted Rates (b) | 100.0% | 100.0% | 100.0% |
| Terminal Rates (c) | 17/17 (100%) | 17/17 (100%) | 20/20 (100%) |
| Week of First Observation | 69 | 66 | 48 |
| Life Table Tests (d) | P=0.306N | P=0.405 | P=0.329N |
| Incidental Tumor Tests (d) | P=0.330N | P=0.442N | P=0.439N |
| Cochran-Armitage Trend Test (d) | P=0.417N | | |
| Fisher Exact Test (d) | | P=0.339N | P=0.500N |

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **All Sites: Malignant Tumors** | | | |
| Overall Rates (a) | 29/50 (58%) | 31/50 (62%) | 29/50 (58%) |
| Adjusted Rates (b) | 75.0% | 76.1% | 69.8% |
| Terminal Rates (c) | 9/17 (53%) | 9/17 (53%) | 9/20 (45%) |
| Week of First Observation | 55 | 47 | 56 |
| Life Table Tests (d) | P=0.428N | P=0.290 | P=0.461N |
| Incidental Tumor Tests (d) | P=0.528N | P=0.548N | P=0.555 |
| Cochran-Armitage Trend Test (d) | P=0.541 | | |
| Fisher Exact Test (d) | | P=0.419 | P=0.580 |
| **All Sites: All Tumors** | | | |
| Overall Rates (a) | 49/50 (98%) | 49/50 (98%) | 49/50 (98%) |
| Adjusted Rates (b) | 100.0% | 100.0% | 100.0% |
| Terminal Rates (c) | 17/17 (100%) | 17/17 (100%) | 20/20 (100%) |
| Week of First Observation | 55 | 47 | 48 |
| Life Table Tests (d) | P=0.357N | P=0.311 | P=0.382N |
| Incidental Tumor Tests (d) | P=0.616N | P=0.706 | P=0.743N |
| Cochran-Armitage Trend Test (d) | P=0.640 | | |
| Fisher Exact Test (d) | | P=0.752 | P=0.752 |

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) Incomplete sampling of tissues
(f) No P value is reported because no tumors were observed in the 700-ppm and control groups.

**TABLE A4a. HISTORICAL INCIDENCE OF BRAIN MENINGIOMAS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)**

| | Incidence in Controls |
|---|---|
| No 2-year studies by SRI International are included in the historical data base. | |
| **Overall Historical Incidence** | |
| TOTAL | 2/1,928 (0.1%) |
| SD (b) | 0.45% |
| Range (c) | |
| High | 1/49 |
| Low | 0/50 |

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

**TABLE A4b. HISTORICAL INCIDENCE OF RENAL TUBULAR CELL TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)**

| | Incidence in Controls |
|---|---|
| No 2-year studies by SRI International are included in the historical data base. | |
| **Overall Historical Incidence** | |
| TOTAL | (b) 9/1,928 (0.5%) |
| SD (c) | 1.17% |
| Range (d) | |
| High | (e) 3/50 |
| Low | 0/50 |

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes one adenoma, NOS, six tubular cell adenomas, one tubular adenocarcinoma, and one tubular cell adenocarcinoma
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Second highest: 1/50

TABLE A4c. HISTORICAL INCIDENCE OF ANTERIOR PITUITARY GLAND TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

| | Incidence in Controls | | |
|---|---|---|---|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| No 2-year studies by SRI International are included in the historical data base. | | | |
| **Overall Historical Incidence** | | | |
| TOTAL | (b) 417/1,830 (22.8%) | (c) 42/1,830 (2.3%) | (b,c) 459/1,830 (25.1%) |
| SD (d) | 10.75% | 2.85% | 10.32% |
| Range (e) | | | |
| High | 24/46 | 5/45 | 25/46 |
| Low | 2/39 | 0/50 | 2/39 |

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes 32 chromophobe adenomas and 1 acidophil adenoma
(c) Includes seven chromophobe carcinomas and one adenocarcinoma, NOS
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (50) | (50) | (50) |
| Inflammation, acute focal | | 1 (2%) | |
| Inflammation, chronic focal | 1 (2%) | 1 (2%) | |
| Hyperplasia, epithelial | 1 (2%) | 3 (6%) | 1 (2%) |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Epidermal inclusion cyst | 1 (2%) | ‡3 (6%) | 3 (6%) |
| Edema, NOS | | 1 (2%) | |
| Abscess, NOS | 1 (2%) | | |
| Inflammation, chronic focal | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #Nasal cavity | (50) | (8) | (49) |
| Foreign body, NOS | 1 (2%) | 1 (13%) | |
| Congestion, NOS | 1 (2%) | | |
| Edema, NOS | 1 (2%) | | |
| Hemorrhage | 2 (4%) | | 4 (8%) |
| Inflammation, acute focal | 10 (20%) | 1 (13%) | 6 (12%) |
| Inflammation, chronic focal | 1 (2%) | | 1 (2%) |
| Infection, fungal | 2 (4%) | 1 (13%) | 2 (4%) |
| *Tracheal lumen | (50) | (50) | (50) |
| Foreign body, NOS | 1 (2%) | | |
| Inflammation, acute | 1 (2%) | | |
| #Tracheal submucosa | (50) | (42) | (50) |
| Cyst, NOS | 1 (2%) | | |
| Inflammation, acute focal | | 2 (5%) | 1 (2%) |
| Inflammation, chronic focal | 5 (10%) | 1 (2%) | |
| #Lung/bronchiole | (50) | (41) | (50) |
| Hyperplasia, epithelial | 1 (2%) | | |
| #Lung | (50) | (41) | (50) |
| Foreign body, NOS | 1 (2%) | | |
| Mineralization | 1 (2%) | | |
| Congestion, NOS | 4 (8%) | 9 (22%) | 3 (6%) |
| Edema, NOS | 1 (2%) | | |
| Hemorrhage | 7 (14%) | 5 (12%) | 6 (12%) |
| Inflammation, interstitial | | 1 (2%) | 2 (4%) |
| Inflammation, acute focal | 2 (4%) | | |
| Inflammation, chronic focal | 5 (10%) | | 1 (2%) |
| Crystals, NOS | 3 (6%) | 2 (5%) | |
| Pigmentation, NOS | 1 (2%) | 2 (5%) | |
| Alveolar macrophages | 12 (24%) | 2 (5%) | 6 (12%) |
| Hyperplasia, alveolar epithelium | 3 (6%) | 1 (2%) | 2 (4%) |
| Metaplasia, osseous | 1 (2%) | | 2 (4%) |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Hematopoiesis | | | 1 (2%) |
| #Bone marrow | (50) | (33) | (50) |
| Cyst, NOS | | | 2 (4%) |
| Atrophy, focal | | 1 (3%) | |
| Myelofibrosis | | 1 (3%) | 2 (4%) |
| Hyperplasia, hematopoietic | 2 (4%) | | 2 (4%) |
| Hyperplasia, granulocytic | 3 (6%) | 3 (9%) | 5 (10%) |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #Spleen | (50) | (44) | (50) |
| Congestion, NOS | 2 (4%) | 7 (16%) | 3 (6%) |
| Fibrosis, focal | | 1 (2%) | |
| Adhesion, NOS | | | 1 (2%) |
| Necrosis, focal | 1 (2%) | | 1 (2%) |
| Atrophy, focal | 8 (16%) | 12 (27%) | 4 (8%) |
| Atrophy, diffuse | 1 (2%) | | |
| Hematopoiesis | 10 (20%) | 6 (14%) | 16 (32%) |
| #Lymph node | (50) | (44) | (50) |
| Cyst, NOS | 1 (2%) | | |
| #Mandibular lymph node | (50) | (44) | (50) |
| Cyst, NOS | 7 (14%) | 4 (9%) | 3 (6%) |
| Congestion, NOS | 2 (4%) | 1 (2%) | 1 (2%) |
| Hemorrhage | 4 (8%) | 2 (5%) | |
| Pigmentation, NOS | 1 (2%) | | |
| Hyperplasia, plasma cell | 3 (6%) | 3 (7%) | 4 (8%) |
| #Thoracic lymph node | (50) | (44) | (50) |
| Cyst, NOS | 1 (2%) | | |
| Pigmentation, NOS | 1 (2%) | 3 (7%) | 2 (4%) |
| Hyperplasia, NOS | | | 1 (2%) |
| Angiectasis | | 1 (2%) | |
| Hyperplasia, plasma cell | | | 1 (2%) |
| Hyperplasia, lymphoid | | | 1 (2%) |
| #Mediastinal lymph node | (50) | (44) | (50) |
| Pigmentation, NOS | 1 (2%) | | |
| #Abdominal lymph node | (50) | (44) | (50) |
| Cyst, NOS | | 1 (2%) | |
| #Hepatic lymph node | (50) | (44) | (50) |
| Hemorrhage | | | 1 (2%) |
| #Pancreatic lymph node | (50) | (44) | (50) |
| Edema, NOS | | 1 (2%) | |
| Hemorrhage | 1 (2%) | | |
| #Lumbar lymph node | (50) | (44) | (50) |
| Hyperplasia, plasma cell | | 1 (2%) | |
| #Mesenteric lymph node | (50) | (44) | (50) |
| Edema, NOS | 1 (2%) | 1 (2%) | 2 (4%) |
| Hemorrhage | 5 (10%) | 4 (9%) | 1 (2%) |
| Inflammation, acute | 1 (2%) | | |
| Hyperplasia, lymphoid | 2 (4%) | 1 (2%) | |
| #Renal lymph node | (50) | (44) | (50) |
| Cyst, NOS | | 1 (2%) | |
| Congestion, NOS | | 1 (2%) | |
| Edema, NOS | | 1 (2%) | 1 (2%) |
| Hemorrhage | 3 (6%) | | |
| Pigmentation, NOS | 1 (2%) | 1 (2%) | 3 (6%) |
| #Sacral lymph node | (50) | (44) | (50) |
| Cyst, NOS | 2 (4%) | | |
| #Liver | (50) | (46) | (50) |
| Hematopoiesis | 3 (6%) | | 4 (8%) |
| #Peyer's patch | (50) | (34) | (50) |
| Hematopoiesis | | 1 (3%) | |
| #Adrenal cortex | (50) | (36) | (49) |
| Hematopoiesis | | 1 (3%) | |
| #Adrenal medulla | (50) | (36) | (49) |
| Hematopoiesis | | 1 (3%) | |
| #Thymus | (49) | (34) | (47) |
| Hemorrhage | 1 (2%) | 4 (12%) | 1 (2%) |
| Involution, NOS | 28 (57%) | 28 (82%) | 35 (74%) |
| Hyperplasia, epithelial | 2 (4%) | | 2 (4%) |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| CIRCULATORY SYSTEM | | | |
| *Abdominal cavity | (50) | (50) | (50) |
| Periarteritis | | 1 (2%) | 2 (4%) |
| *Adipose tissue | (50) | (50) | (50) |
| Periarteritis | 1 (2%) | | |
| #Nasal cavity | (50) | (8) | (49) |
| Thrombosis, NOS | | | 1 (2%) |
| #Heart | (50) | (38) | (50) |
| Mineralization | | 1 (3%) | |
| Hemorrhage | 2 (4%) | | 1 (2%) |
| Inflammation, chronic focal | 48 (96%) | 34 (89%) | 47 (94%) |
| Fibrosis, focal | 1 (2%) | | |
| Degeneration, NOS | 1 (2%) | | |
| Necrosis, focal | | 1 (3%) | |
| Metaplasia, osseous | 1 (2%) | | |
| #Heart/atrium | (50) | (38) | (50) |
| Mineralization | 3 (6%) | 1 (3%) | |
| Dilatation, NOS | 1 (2%) | | |
| Thrombosis, NOS | 7 (14%) | 5 (13%) | 3 (6%) |
| Hemorrhage | 1 (2%) | | |
| Inflammation, chronic focal | | | 1 (2%) |
| Hyperplasia, epithelial | | | 1 (2%) |
| #Heart/ventricle | (50) | (38) | (50) |
| Thrombosis, NOS | 1 (2%) | | |
| *Artery | (50) | (50) | (50) |
| Periarteritis | | | 1 (2%) |
| *Aorta | (50) | (50) | (50) |
| Mineralization | | | 1 (2%) |
| Perivasculitis | | 1 (2%) | |
| *Aortic arch | (50) | (50) | (50) |
| Hemorrhage | | | 1 (2%) |
| *Pulmonary artery | (50) | (50) | (50) |
| Mineralization | 37 (74%) | 34 (68%) | 30 (60%) |
| Thrombosis, NOS | 1 (2%) | | |
| Hypertrophy, NOS | | 1 (2%) | 1 (2%) |
| #Pancreas | (49) | (34) | (50) |
| Periarteritis | 1 (2%) | | 2 (4%) |
| DIGESTIVE SYSTEM | | | |
| *Palate | (50) | (50) | (50) |
| Hyperplasia, epithelial | 4 (8%) | | 4 (8%) |
| #Salivary gland | (50) | (36) | (49) |
| Inflammation, chronic focal | 2 (4%) | 2 (6%) | 3 (6%) |
| Basophilic cyto change | 1 (2%) | 3 (8%) | 2 (4%) |
| Atrophy, focal | 1 (2%) | 1 (3%) | |
| Hyperplasia, intraductal | 4 (8%) | | 5 (10%) |
| #Liver | (50) | (46) | (50) |
| Abnormal curvature | | 1 (2%) | 1 (2%) |
| Cyst, NOS | 1 (2%) | | |
| Congestion, NOS | 1 (2%) | 2 (4%) | 1 (2%) |
| Hemorrhage | 3 (6%) | 3 (7%) | 1 (2%) |
| Inflammation, chronic focal | 7 (14%) | 7 (15%) | 6 (12%) |
| Peliosis hepatis | 17 (34%) | 12 (26%) | 14 (28%) |
| Necrosis, focal | 5 (10%) | 8 (17%) | 3 (6%) |
| Pigmentation, NOS | 2 (4%) | | |
| Eosinophilic cyto change | 1 (2%) | | |
| Hyperplasia, focal | 1 (2%) | | |
| Angiectasis | | | 1 (2%) |
| #Liver/hepatocytes | (50) | (46) | (50) |
| Degeneration, NOS | 2 (4%) | 2 (4%) | 1 (2%) |
| Peliosis hepatis | | 1 (2%) | |
| Nuclear enlargement | | | 1 (2%) |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| DIGESTIVE SYSTEM | | | |
| #Liver/hepatocytes (Continued) | (50) | (46) | (50) |
| Cytoplasmic vacuolization | 15 (30%) | 11 (24%) | 7 (14%) |
| Basophilic cyto change | 19 (38%) | 11 (24%) | 18 (36%) |
| Eosinophilic cyto change | 1 (2%) | 1 (2%) | |
| Clear cell change | 13 (26%) | | 10 (20%) |
| #Bile duct | (50) | (46) | (50) |
| Inflammation, chronic focal | 1 (2%) | | |
| Hyperplasia, focal | 49 (98%) | 41 (89%) | 46 (92%) |
| #Pancreas | (49) | (34) | (50) |
| Edema, NOS | | | 1 (2%) |
| Hemorrhage | 1 (2%) | | |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 8 (16%) | 6 (18%) | 11 (22%) |
| Pigmentation, NOS | | 2 (6%) | 3 (6%) |
| #Pancreatic acinus | (49) | (34) | (50) |
| Necrosis, focal | 1 (2%) | | |
| Basophilic cyto change | 1 (2%) | | 1 (2%) |
| Atrophy, focal | 23 (47%) | 20 (59%) | 30 (60%) |
| Hyperplasia, focal | | | 1 (2%) |
| *Esophageal lumen | (50) | (50) | (50) |
| Inflammation, acute | | 1 (2%) | |
| #Esophagus | (50) | (45) | (50) |
| Inflammation, chronic focal | 1 (2%) | | |
| #Glandular stomach | (50) | (33) | (50) |
| Mineralization | | 1 (3%) | 2 (4%) |
| Edema, NOS | | 1 (3%) | |
| Hemorrhage | 1 (2%) | | |
| Ulcer, NOS | 2 (4%) | 2 (6%) | 1 (2%) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 4 (8%) | 2 (6%) | 1 (2%) |
| Fibrosis | | 4 (12%) | |
| Degeneration, cystic | | 1 (3%) | |
| Necrosis, focal | 1 (2%) | | |
| Atrophy, focal | 19 (38%) | 6 (18%) | 22 (44%) |
| #Forestomach | (50) | (33) | (50) |
| Edema, NOS | 2 (4%) | 2 (6%) | 1 (2%) |
| Ulcer, NOS | 2 (4%) | 5 (15%) | 1 (2%) |
| Inflammation, acute focal | 1 (2%) | 1 (3%) | 1 (2%) |
| Inflammation, chronic focal | | 2 (6%) | |
| Necrosis, focal | | 1 (3%) | |
| Hyperplasia, epithelial | 19 (38%) | 22 (67%) | 21 (42%) |
| #Peyer's patch | (50) | (34) | (50) |
| Congestion, NOS | 1 (2%) | | |
| #Duodenum | (50) | (34) | (50) |
| Inflammation, suppurative | 1 (2%) | | |
| #Duodenal mucosa | (50) | (34) | (50) |
| Hyperplasia, NOS | | 1 (3%) | |
| #Duodenal submucosa | (50) | (34) | (50) |
| Inflammation, chronic focal | 1 (2%) | | |
| #Jejunum | (50) | (34) | (50) |
| Inflammation, suppurative | 1 (2%) | | |
| #Ileum | (50) | (34) | (50) |
| Inflammation, suppurative | 1 (2%) | | |
| #Colon | (50) | (34) | (48) |
| Dilatation, NOS | | 1 (3%) | |
| Inflammation, chronic focal | | 1 (3%) | |
| Erosion | | 1 (3%) | |
| Parasitism | 4 (8%) | 3 (9%) | 8 (17%) |
| #Cecum | (50) | (34) | (48) |
| Dilatation, NOS | | 1 (3%) | |
| Impaction, fecal | | 1 (3%) | |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| DIGESTIVE SYSTEM | | | |
| #Cecum (Continued) | (50) | (34) | (48) |
| Congestion, NOS | | 1 (3%) | |
| Parasitism | 1 (2%) | | |
| *Rectum | (50) | (50) | (50) |
| Parasitism | 3 (6%) | | 2 (4%) |
| Polypoid hyperplasia | | 1 (2%) | |
| URINARY SYSTEM | | | |
| #Kidney | (50) | (50) | (50) |
| Mineralization | 2 (4%) | 1 (2%) | 1 (2%) |
| Cyst, NOS | 6 (12%) | 7 (14%) | 12 (24%) |
| Congestion, NOS | 1 (2%) | 3 (6%) | 2 (4%) |
| Inflammation, acute focal | 1 (2%) | | |
| Nephropathy | 48 (96%) | 49 (98%) | 50 (100%) |
| Necrosis, focal | | 2 (4%) | |
| Pigmentation, NOS | 25 (50%) | 28 (56%) | 23 (46%) |
| Hyperplasia, tubular cell | 4 (8%) | 2 (4%) | 4 (8%) |
| #Kidney/pelvis | (50) | (50) | (50) |
| Dilatation, NOS | 2 (4%) | 3 (6%) | 1 (2%) |
| Inflammation, suppurative | | 1 (2%) | 1 (2%) |
| Hyperplasia, epithelial | 17 (34%) | 15 (30%) | 27 (54%) |
| #Urinary bladder/mucosa | (50) | (34) | (46) |
| Inflammation, acute focal | | | 1 (2%) |
| Hyperplasia, epithelial | | 1 (3%) | 1 (2%) |
| #Urinary bladder/submucosa | (50) | (34) | (46) |
| Inflammation, acute focal | | | 1 (2%) |
| Inflammation, chronic | | 1 (3%) | |
| Inflammation, chronic focal | | 2 (6%) | 2 (4%) |
| #Urinary bladder/muscularis | (50) | (34) | (46) |
| Hemorrhage | | 2 (6%) | |
| ENDOCRINE SYSTEM | | | |
| #Pituitary intermedia | (50) | (50) | (50) |
| Cyst, NOS | 9 (18%) | 4 (8%) | 6 (12%) |
| Hyperplasia, focal | | | 2 (4%) |
| #Anterior pituitary | (50) | (50) | (50) |
| Cyst, NOS | 6 (12%) | 4 (8%) | 4 (8%) |
| Hemorrhage | 1 (2%) | | |
| Necrosis, focal | 1 (2%) | | |
| Pigmentation, NOS | 2 (4%) | 2 (4%) | |
| Atrophy, diffuse | | 1 (2%) | |
| Hyperplasia, focal | 14 (28%) | 20 (40%) | 22 (44%) |
| Angiectasis | 1 (2%) | | |
| #Pituitary posterior | (50) | (50) | (50) |
| Gliosis | | 1 (2%) | 3 (6%) |
| #Adrenal cortex | (50) | (36) | (49) |
| Cyst, NOS | 2 (4%) | | 1 (2%) |
| Congestion, NOS | 5 (10%) | 1 (3%) | 1 (2%) |
| Hemorrhage | | 1 (3%) | |
| Degeneration, NOS | 6 (12%) | 8 (22%) | 11 (22%) |
| Necrosis, focal | 1 (2%) | | |
| Pigmentation, NOS | 1 (2%) | | |
| Focal cellular change | 1 (2%) | | |
| Atrophy, diffuse | 1 (2%) | | |
| Hyperplasia, focal | 16 (32%) | 6 (17%) | 14 (29%) |
| Angiectasis | | | 2 (4%) |
| #Adrenal medulla | (50) | (36) | (49) |
| Cyst, NOS | | 1 (3%) | 1 (2%) |
| Congestion, NOS | | 1 (3%) | |

**TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ENDOCRINE SYSTEM | | | |
| #Adrenal medulla (Continued) | (50) | (36) | (49) |
| Degeneration, NOS | 1 (2%) | | 1 (2%) |
| Hyperplasia, NOS | 13 (26%) | 5 (14%) | 9 (18%) |
| Hyperplasia, focal | | 1 (3%) | |
| #Thyroid | (50) | (50) | (50) |
| Cyst, NOS | 1 (2%) | 1 (2%) | 4 (8%) |
| Follicular cyst, NOS | 1 (2%) | 1 (2%) | 1 (2%) |
| Hyperplasia, C-cell | 18 (36%) | 10 (20%) | 12 (24%) |
| #Parathyroid | (48) | (46) | (47) |
| Hyperplasia, NOS | 8 (17%) | 8 (17%) | 15 (32%) |
| #Pancreatic islets | (49) | (34) | (50) |
| Hyperplasia, focal | 2 (4%) | 2 (6%) | |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (50) |
| Pigmentation, NOS | 2 (4%) | | |
| Hyperplasia, cystic | 15 (30%) | 13 (26%) | 14 (28%) |
| *Penis | (50) | (50) | (50) |
| Ulcer, NOS | | 1 (2%) | |
| #Preputial gland | (48) | (12) | (47) |
| Cyst, NOS | 4 (8%) | | 2 (4%) |
| Inflammation, suppurative | 5 (10%) | | 2 (4%) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | | | 4 (9%) |
| Atrophy, NOS | 44 (92%) | 7 (58%) | 40 (85%) |
| Hyperplasia, NOS | 5 (10%) | 2 (17%) | 5 (11%) |
| #Prostate | (50) | (35) | (48) |
| Inflammation, suppurative | 3 (6%) | 8 (23%) | 4 (8%) |
| Inflammation, chronic focal | 2 (4%) | 3 (9%) | 3 (6%) |
| Corpora amylacea | 1 (2%) | | |
| Hyperplasia, epithelial | 6 (12%) | 4 (11%) | 9 (19%) |
| *Seminal vesicle | (50) | (50) | (50) |
| Dilatation, NOS | | 1 (2%) | 1 (2%) |
| Spermatocele | | 1 (2%) | |
| Edema, NOS | 1 (2%) | | |
| Inflammation, suppurative | | 1 (2%) | |
| Inflammation, chronic focal | 1 (2%) | 1 (2%) | |
| Atrophy, NOS | 14 (28%) | 10 (20%) | 14 (28%) |
| #Testis | (50) | (50) | (49) |
| Mineralization | 6 (12%) | 5 (10%) | 2 (4%) |
| Spermatocele | 1 (2%) | | |
| Hemorrhage | | 1 (2%) | |
| Necrosis, focal | 1 (2%) | | |
| Necrosis, fat | 1 (2%) | | |
| Atrophy, focal | 35 (70%) | 38 (76%) | 36 (73%) |
| Atrophy, diffuse | 14 (28%) | 9 (18%) | 11 (22%) |
| Hyperplasia, interstitial cell | 9 (18%) | 17 (34%) | 16 (33%) |
| #Testis/tubule | (50) | (50) | (49) |
| Atrophy, diffuse | | 1 (2%) | |
| *Epididymis | (50) | (50) | (50) |
| Inflammation, suppurative | | 1 (2%) | |
| Degeneration, NOS | 14 (28%) | 6 (12%) | 13 (26%) |
| Hyperplasia, epithelial | 1 (2%) | | 1 (2%) |
| NERVOUS SYSTEM | | | |
| #Brain/meninges | (50) | (50) | (50) |
| Congestion, NOS | 1 (2%) | | |
| Hemorrhage | 1 (2%) | 1 (2%) | |
| Hyperplasia, NOS | | 3 (6%) | |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| NERVOUS SYSTEM (Continued) | | | |
| #Brain | (50) | (50) | (50) |
| Mineralization | 1 (2%) | | 1 (2%) |
| Hydrocephalus, NOS | 1 (2%) | 5 (10%) | 3 (6%) |
| Congestion, NOS | 1 (2%) | | |
| Hemorrhage | 2 (4%) | 5 (10%) | 1 (2%) |
| Necrosis, focal | 2 (4%) | | |
| Pigmentation, NOS | | | 1 (2%) |
| *Spinal cord | (50) | (50) | (50) |
| Hemorrhage | | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| *Eye | (50) | (50) | (50) |
| Phthisis bulbi | | 1 (2%) | |
| *Eye/anterior chamber | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| Inflammation, acute | 1 (2%) | | |
| *Eye/sclera | (50) | (50) | (50) |
| Mineralization | 3 (6%) | 5 (10%) | 4 (8%) |
| *Eye/cornea | (50) | (50) | (50) |
| Inflammation, acute | 1 (2%) | | |
| Inflammation, acute focal | | | 2 (4%) |
| Vascularization | | | 1 (2%) |
| *Eye/retina | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| Inflammation, chronic focal | 1 (2%) | | |
| Atrophy, focal | 3 (6%) | 3 (6%) | 1 (2%) |
| Atrophy, diffuse | 6 (12%) | 2 (4%) | |
| *Eye/crystalline lens | (50) | (50) | (50) |
| Degeneration, NOS | 8 (16%) | 3 (6%) | 1 (2%) |
| *Eye/lacrimal gland | (50) | (50) | (50) |
| Pigmentation, NOS | 1 (2%) | | |
| *Nasolacrimal duct | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| Inflammation, acute focal | 1 (2%) | | 1 (2%) |
| *Harderian gland | (50) | (50) | (50) |
| Inflammation, chronic focal | | 2 (4%) | |
| Pigmentation, NOS | | | 1 (2%) |
| *Zymbal gland | (50) | (50) | (50) |
| Cyst, NOS | | 1 (2%) | |
| MUSCULOSKELETAL SYSTEM | | | |
| *Bone | (50) | (50) | (50) |
| Cyst, NOS | | | 1 (2%) |
| Inflammation, acute focal | 1 (2%) | | |
| Necrosis, focal | 1 (2%) | | 2 (4%) |
| Fibrous osteodystrophy | 4 (8%) | 1 (2%) | 5 (10%) |
| *Muscle of perineum | (50) | (50) | (50) |
| Hemorrhage | | | 1 (2%) |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | 2 (4%) |
| *Abdominal cavity | (50) | (50) | (50) |
| Hematoma, NOS | 1 (2%) | | |
| Inflammation, chronic focal | | | 1 (2%) |
| Necrosis, fat | 8 (16%) | 4 (8%) | 6 (12%) |
| *Pleura | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| Pigmentation, NOS | | 1 (2%) | |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| BODY CAVITIES (Continued) | | | |
| *Mesentery | (50) | (50) | (50) |
| Inflammation, chronic | | 1 (2%) | |
| Pigmentation, NOS | 1 (2%) | | |
| ALL OTHER SYSTEMS | | | |
| *Multiple organs | (50) | (50) | (50) |
| Mineralization | 5 (10%) | 2 (4%) | 5 (10%) |
| Tail | | | |
| Necrosis, focal | 1 | | |
| Necrosis, diffuse | | 1 | |
| Adipose tissue | | | |
| Mineralization | 1 | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| None | | | |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site
‡ Multiple occurrence of morphology; tissue is counted only once.

# APPENDIX B

# SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE


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**TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Fibrous histiocytoma | 1 (2%) | | |
| *Skin | (50) | (50) | (50) |
| Squamous cell carcinoma | | 2 (4%) | |
| Keratoacanthoma | | 1 (2%) | |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Sarcoma, NOS | | 1 (2%) | |
| Fibroma | 2 (4%) | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #Lung | (50) | (27) | (50) |
| Alveolar/bronchiolar adenoma | 2 (4%) | 1 (4%) | 4 (8%) |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Leukemia, mononuclear cell | 8 (16%) | 14 (28%) | 10 (20%) |
| #Spleen | (50) | (50) | (49) |
| Carcinoma, NOS, metastatic | | 1 (2%) | |
| #Mandibular lymph node | (50) | (32) | (50) |
| Sarcoma, NOS, metastatic | 1 (2%) | | |
| CIRCULATORY SYSTEM | | | |
| #Heart | (50) | (20) | (50) |
| Neurilemoma | 1 (2%) | | 1 (2%) |
| DIGESTIVE SYSTEM | | | |
| *Tongue | (50) | (50) | (50) |
| Squamous cell carcinoma | 1 (2%) | | |
| #Forestomach | (50) | (18) | (50) |
| Squamous cell papilloma | | | 1 (2%) |
| URINARY SYSTEM | | | |
| None | | | |
| ENDOCRINE SYSTEM | | | |
| #Anterior pituitary | (49) | (50) | (50) |
| Carcinoma, NOS | | | 1 (2%) |
| Adenoma, NOS | 17 (35%) | 9 (18%) | 19 (38%) |
| #Adrenal | (50) | (22) | (50) |
| Cortical carcinoma | 1 (2%) | | |
| #Adrenal cortex | (50) | (22) | (50) |
| Adenoma, NOS | 1 (2%) | | |
| #Adrenal medulla | (50) | (22) | (50) |
| Pheochromocytoma | 4 (8%) | | 3 (6%) |
| #Thyroid | (50) | (50) | (50) |
| Follicular cell adenoma | 1 (2%) | | 1 (2%) |
| C-cell adenoma | 4 (8%) | 6 (12%) | 11 (22%) |
| C-cell carcinoma | | 1 (2%) | |
| #Pancreatic islets | (50) | (17) | (50) |
| Islet cell adenoma | | | 1 (2%) |

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (50) |
| Carcinoma, NOS | 2 (4%) | 3 (6%) | 2 (4%) |
| Adenoma, NOS | 1 (2%) | | 1 (2%) |
| Fibroadenoma | 21 (42%) | 25 (50%) | 21 (42%) |
| #Clitoral gland | (48) | (45) | (49) |
| Adenoma, NOS | 6 (13%) | 10 (22%) | 9 (18%) |
| *Vagina | (50) | (50) | (50) |
| Sarcoma, NOS | | 1 (2%) | |
| #Uterus | (49) | (28) | (49) |
| Endometrial stromal polyp | 13 (27%) | 13 (46%) | 16 (33%) |
| #Uterus/endometrium | (49) | (28) | (49) |
| Adenocarcinoma, NOS | | | 1 (2%) |
| #Ovary | (48) | (22) | (49) |
| Cystadenoma, NOS | 1 (2%) | | |
| Granulosa cell tumor | 1 (2%) | | |
| Granulosa cell carcinoma | | | 2 (4%) |
| Sertoli cell tumor | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| #Brain | (50) | (20) | (49) |
| Astrocytoma | 1 (2%) | 1 (5%) | |
| SPECIAL SENSE ORGANS | | | |
| None | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| None | | | |
| BODY CAVITIES | | | |
| *Abdominal cavity | (50) | (50) | (50) |
| Sarcoma, NOS | | | 1 (2%) |
| ALL OTHER SYSTEMS | | | |
| None | | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural death | 5 | 5 | 3 |
| Moribund sacrifice | 13 | 14 | 14 |
| Terminal sacrifice | 32 | 31 | 33 |

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| Total animals with primary tumors** | 47 | 45 | 46 |
| Total primary tumors | 90 | 89 | 105 |
| Total animals with benign tumors | 45 | 40 | 43 |
| Total benign tumors | 76 | 66 | 88 |
| Total animals with malignant tumors | 12 | 22 | 12 |
| Total malignant tumors | 13 | 23 | 17 |
| Total animals with secondary tumors## | 1 | 1 | |
| Total secondary tumors | 1 | 1 | |
| Total animals with tumors uncertain-- benign or malignant | 1 | | |
| Total uncertain tumors | 1 | | |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
## Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

## TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: UNTREATED CONTROL

| ANIMAL NUMBER | 047 | 050 | 003 | 012 | 015 | 019 | 024 | 031 | 045 | 034 | 027 | 043 | 018 | 032 | 035 | 029 | 036 | 041 | 001 | 002 | 004 | 005 | 006 | 007 | 008 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 074 | 074 | 077 | 077 | 077 | 077 | 077 | 079 | 080 | 082 | 086 | 091 | 098 | 100 | 101 | 104 | 104 | 104 | 105 | 105 | 105 | 105 | 105 | 105 | 105 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibroma | | | | | | | | | | | | | | X | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | + | + | + | – | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | X | | | | | | | | | | |
| Thymus | + | + | + | + | + | + | + | + | + | – | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Neurilemoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Squamous cell carcinoma | | | | | | | | | | | X | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | – | + | – | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | – | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | X | | | | | | X | | X | | | | | | X | X | | | | | | | X | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | X | | |
| Cortical carcinoma | | | | | | X | | | | | | | | | | | | | | | | | | | |
| Pheochromocytoma | X | | | | | | | | | | | | | | | | | X | | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Follicular cell adenoma | | | | | | | X | | | | | | | | | | | | | | | | | | |
| C-cell adenoma | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Parathyroid | + | + | + | + | + | – | + | + | + | – | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | | | | | | X | | | | | | | | | |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroadenoma | | | X | | X | | | | | | | X | | | | X | | | X | | | X | | X | |
| Preputial/clitoral gland | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | X | | | | | | | | | | | | | | | | | | | X | | X | | | |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + |
| Endometrial stromal polyp | | | X | | | | | | | | X | | | | | X | | X | | | | | | | X |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + |
| Cystadenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Granulosa cell tumor | | | | | | | | | | | | | | | | | | | | X | | | | | |
| Sertoli cell tumor | | | | | | | | | | | | | | | | X | | | | | | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | X | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Multiple organs, NOS* | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Fibrous histiocytoma | X | | | | | | | | | | | | | | | | | | | | | | | | |
| Leukemia, mononuclear cell | | | | | X | | | | X | X | X | | X | | | | | X | | | | | | | |

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
.: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

## TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

| ANIMAL NUMBER | 0009 | 0010 | 0011 | 0013 | 0014 | 0016 | 0017 | 0020 | 0021 | 0022 | 0023 | 0025 | 0026 | 0028 | 0030 | 0033 | 0037 | 0038 | 0039 | 0040 | 0042 | 0044 | 0046 | 0048 | 0049 | TOTAL: TISSUES TUMORS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Fibroma | | | | | | | | | | | | | | | | | X | | | | | | | | | 2 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar adenoma | X | | | | | | | | | | | | | X | | | | | | | | | | | | 2 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Neurilemoma | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | 47 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Adenoma, NOS | X | X | X | | X | | | X | X | | X | | X | X | | | | | | | X | X | | | | 17 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Cortical carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | X | | | X | | | | 4 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| C-cell adenoma | | | | | | | | | | | | | | | | X | | | | X | | | X | | | 4 |
| Parathyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Carcinoma, NOS | | | | | | | | | | X | | | | | | | | | | | | | | | | 2 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| Fibroadenoma | | X | | | X | | | X | | X | X | | X | X | X | X | | X | | | X | X | X | X | | 21 |
| Preputial/clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | X | X | | | | X | | | | 6 |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Endometrial stromal polyp | | | | X | | X | X | | X | | | X | X | | | | | | | X | | | | | X | 13 |
| Ovary | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Cystadenoma, NOS | | | | | | | | | | | | | | | | | | | | | X | | | | | 1 |
| Granulosa cell tumor | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Sertoli cell tumor | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Fibrous histiocytoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Leukemia, mononuclear cell | | X | | | | | X | | | | | | | | | | | | | | | | | | | 8 |

* Animals necropsied

## TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: LOW DOSE

| ANIMAL NUMBER | 00 06 | 00 01 | 01 14 | 04 44 | 03 33 | 01 15 | 03 31 | 03 35 | 01 18 | 03 32 | 02 27 | 03 39 | 00 03 | 05 00 | 00 07 | 03 36 | 01 10 | 00 08 | 04 49 | 00 02 | 00 04 | 00 05 | 00 09 | 01 11 | 01 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 01 99 | 06 65 | 06 65 | 07 44 | 08 44 | 08 66 | 08 88 | 09 44 | 09 55 | 09 55 | 09 66 | 09 97 | 09 98 | 09 98 | 09 99 | 10 00 | 10 02 | 10 33 | 10 33 | 10 55 | 10 55 | 10 55 | 10 55 | 10 55 | 10 55 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | + | + | N | N |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | X | | | |
| Keratoacanthoma | | | | | | | | | | | | | | | | | | X | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | + | + | N | N |
| Sarcoma, NOS | | | | | X | | | | | | | | | | | | | | | | | | | | |
| Fibroma | | | | | | | X | | | | | | | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | + | − | − |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | − | − | − | − | − | − | − | − | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − |
| Thymus | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | − | − | − | − | − | − |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | − | − | − | − | − | − |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | X | | | | | | | X | | | | | | X | | | | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell adenoma | | | | | | | | | | | | | | | | | | | | | | X | | X | X |
| C-cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | − | − | − | + | − | − |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | X | X |
| Fibroadenoma | | X | | | | | | | | | X | | | | X | | | X | X | X | | | X | X | |
| Preputial/clitoral gland | − | + | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | − | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | X | | | | | | | | | | | | | X | X | X | X | |
| Vagina | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | − | + | + | − | + |
| Endometrial stromal polyp | | | | X | | X | X | | X | | | X | X | | | | | | | X | | X | | | X |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Leukemia, mononuclear cell | | | X | X | | | | X | X | X | | X | X | X | X | X | | X | X | | | | | | |

## TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

| ANIMAL NUMBER | 0 1 3 | 0 1 6 | 0 1 7 | 0 1 9 | 0 2 0 | 0 2 1 | 0 2 2 | 0 2 3 | 0 2 4 | 0 2 5 | 0 2 6 | 0 2 8 | 0 2 9 | 0 3 0 | 0 3 4 | 0 3 7 | 0 3 8 | 0 4 0 | 0 4 1 | 0 4 2 | 0 4 3 | 0 4 5 | 0 4 6 | 0 4 7 | 0 4 8 | TOTAL: |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | TISSUES TUMORS |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | N | N | N | N | + | N | N | N | N | N | N | + | N | N | N | N | N | + | N | N | N | + | N | + | + | *50 |
| Squamous cell carcinoma | | | | | X | | | | | | | | | | | | | | | | | | | | | 2 |
| Keratoacanthoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Subcutaneous tissue | N | N | N | N | + | N | N | N | N | N | N | + | N | N | N | N | N | + | N | N | N | + | N | + | + | *50 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | − | − | − | − | − | − | − | − | − | − | − | − | + | + | − | − | + | − | + | − | − | + | − | + | 27 |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | X | | | | | | 1 |
| Trachea | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Nasal cavity | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 9 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 17 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Carcinoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | X | | | | | 1 |
| Lymph nodes | + | − | + | + | − | − | + | + | − | − | + | − | − | + | + | + | − | + | + | + | − | − | + | − | − | 32 |
| Thymus | + | − | + | − | − | − | − | + | − | − | + | − | − | + | + | + | − | − | + | − | − | − | + | − | − | 25 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | + | − | − | − | − | − | 20 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 18 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pancreas | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 17 |
| Esophagus | + | − | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Stomach | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 18 |
| Small intestine | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 17 |
| Large intestine | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 19 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | − | + | + | − | − | + | − | + | + | + | − | − | + | + | − | + | + | − | + | 38 |
| Urinary bladder | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 17 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | | X | | | | X | | | | | | | | | | X | | X | | | | | | X | X | 9 |
| Adrenal | + | − | − | − | − | − | − | − | − | − | − | − | − | − | + | + | − | − | − | − | − | − | + | − | − | 22 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| C-cell adenoma | X | | | | | | | | | | | | | | | | | | | | | X | | | X | 6 |
| C-cell carcinoma | | | | | | | | | | X | | | | | | | | | | | | | | | | 1 |
| Parathyroid | − | + | + | − | + | − | − | + | − | − | − | − | − | − | − | − | − | − | + | − | − | + | + | + | + | 28 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | N | + | + | + | N | + | N | + | + | + | + | + | + | + | N | + | + | + | + | + | + | N | + | + | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | X | | | | | 3 |
| Fibroadenoma | X | | X | X | X | | X | | X | X | X | | X | X | X | | X | | X | X | X | X | | X | | 25 |
| Preputial/clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | 45 |
| Adenoma, NOS | | | | X | | | X | | | X | | | | | | X | | X | | | | | | | | 10 |
| Vagina | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | X | | 1 |
| Uterus | − | − | − | − | − | − | − | − | − | − | + | + | − | − | − | − | − | + | + | + | + | + | − | − | − | 28 |
| Endometrial stromal polyp | | | | | | | | | | | X | | | | | | | | X | | X | X | | | | 13 |
| Ovary | − | − | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | 22 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | − | − | − | − | − | − | − | − | + | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 20 |
| Astrocytoma | | | | | | | | | | | X | | | | | | | | | | | | | | | 1 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Leukemia, mononuclear cell | | | | | | | | | X | | | | | | | | | | | | | | | X | | 14 |

* Animals necropsied

## TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: HIGH DOSE

| ANIMAL NUMBER | 0013 | 0020 | 0019 | 0037 | 0028 | 0025 | 0017 | 0032 | 0044 | 0018 | 0038 | 0045 | 0003 | 0021 | 0034 | 0012 | 0001 | 0002 | 0004 | 0005 | 0006 | 0007 | 0008 | 0009 | 0010 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 0777 | 0778 | 0880 | 0882 | 0886 | 0888 | 0889 | 0900 | 0955 | 0966 | 0966 | 0966 | 0977 | 1000 | 1000 | 1001 | 1014 | 1024 | 1044 | 1044 | 1044 | 1044 | 1044 | 1044 | 1044 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | X | | | | | | | | | | | | | | | | | | X | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Neurilemoma | | | | | | | | | | | | | | | | | | | | | | | | X | |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | − | + | + | − |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Squamous cell papilloma | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | X | | | | | | |
| Adenoma, NOS | X | X | X | X | | X | | X | X | | | X | X | X | X | X | | | | | | X | | | X |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | | | | | | | | X | | | | | | | | | X | | | | | | X | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | X | | | | | | | | |
| C-cell adenoma | | | | | | X | | | | | | | X | | X | | | | | X | | | X | X | |
| Parathyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Islet cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS | | | | | | X | | | | | | | | | | X | | | | | | | | | |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroadenoma | | | | | | X | | | | | | X | | X | | X | X | X | X | X | X | X | X | | |
| Preputial/clitoral gland | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | X | | | | | | | | | | | | | | X | | |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Endometrial stromal polyp | | | X | | | | | | | X | | | | | X | | | | X | | | | | X | X |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Granulosa cell carcinoma | | | | | | | | | | | | | | | | X | | | | | | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Sarcoma, NOS | | | | | | | | | | | | | | | | X | | | | | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Leukemia, mononuclear cell | | | | | X | | | | X | | X | | | | | X | | | X | | | | | | |

## TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

| ANIMAL NUMBER | 0111 | 0114 | 0115 | 0116 | 0222 | 0223 | 0224 | 0226 | 0227 | 0229 | 0230 | 0231 | 0233 | 0235 | 0236 | 0239 | 0240 | 0241 | 0242 | 0243 | 0246 | 0247 | 0248 | 0249 | 0250 | TOTAL TISSUES TUMORS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 104 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar adenoma | | | | | | | X | | | X | | | | | | | | | | | | | | | | 4 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Neurilemoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Adenoma, NOS | X | X | | | X | | | | | | | | | X | | | | | | | | | | X | | 19 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | | | | | | | | 3 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| C cell adenoma | | | | | | | | X | | | | | | | | | | | | X | X | X | | | X | 11 |
| Parathyroid | – | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | – | + | + | 46 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Islet cell adenoma | | | | | | | | | | | | X | | | | | | | | | | | | | | 1 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | X | | | | | | 1 |
| Fibroadenoma | X | X | X | | | X | X | X | | | | | | X | | | | | | X | X | | | X | | 21 |
| Preputial/clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Adenoma, NOS | X | | | | | X | | X | | | | | X | X | | | | X | | | | | | | | 9 |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | X | | | | | 1 |
| Endometrial stromal polyp | | X | X | | | X | | X | | | | | | X | X | | | X | X | | | X | X | | | 16 |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Granulosa cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | X | | | 2 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Leukemia, mononuclear cell | | | | X | | | | | | X | | | | | | | X | | | X | | | X | | 10 |

* Animals necropsied

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Lung: Alveolar/Bronchiolar Adenoma** | | | |
| Overall Rates (a) | 2/50 (4%) | (b) 1/27 (4%) | 4/50 (8%) |
| Adjusted Rates (c) | 5.7% | | 10.8% |
| Terminal Rates (d) | 2/35 (6%) | | 3/34 (9%) |
| Week of First Observation | 105 | | 86 |
| Life Table Test (e) | | | P=0.337 |
| Incidental Tumor Test (e) | | | P=0.340 |
| Fisher Exact Test (e) | | | P=0.339 |
| **Hematopoietic System: Mononuclear Cell Leukemia** | | | |
| Overall Rates (a) | 8/50 (16%) | 14/50 (28%) | 10/50 (20%) |
| Adjusted Rates (c) | 19.1% | 31.7% | 25.5% |
| Terminal Rates (d) | 3/35 (9%) | 2/31 (6%) | 6/34 (18%) |
| Week of First Observation | 77 | 65 | 86 |
| Life Table Tests (e) | P=0.386 | P=0.134 | P=0.425 |
| Incidental Tumor Tests (e) | P=0.552 | P=0.421 | P=0.553 |
| Fisher Exact Test (e) | P=0.357 | P=0.113 | P=0.397 |
| **Anterior Pituitary Gland: Adenoma** | | | |
| Overall Rates (a) | 17/49 (35%) | 9/50 (18%) | 19/50 (38%) |
| Adjusted Rates (c) | 44.0% | 26.5% | 40.5% |
| Terminal Rates (d) | 14/35 (40%) | 7/31 (23%) | 7/34 (21%) |
| Week of First Observation | 74 | 94 | 77 |
| Life Table Tests (e) | P=0.391 | P=0.093N | P=0.444 |
| Incidental Tumor Tests (e) | P=0.468 | P=0.071N | P=0.582 |
| Cochran-Armitage Trend Test (e) | P=0.397 | | |
| Fisher Exact Test (e) | | P=0.048N | P=0.447 |
| **Anterior Pituitary Gland: Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 17/49 (35%) | 9/50 (18%) | 20/50 (40%) |
| Adjusted Rates (c) | 44.0% | 26.5% | 42.7% |
| Terminal Rates (d) | 14/35 (40%) | 7/31 (23%) | 8/34 (24%) |
| Week of First Observation | 74 | 94 | 77 |
| Life Table Tests (e) | P=0.318 | P=0.093N | P=0.373 |
| Incidental Tumor Tests (e) | P=0.381 | P=0.071N | P=0.495 |
| Cochran-Armitage Trend Test (e) | P=0.317 | | |
| Fisher Exact Test (e) | | P=0.048N | P=0.368 |
| **Adrenal Gland Medulla: Pheochromocytoma** | | | |
| Overall Rates (a) | 4/50 (8%) | (b) 0/22 (0%) | 3/50 (6%) |
| Adjusted Rates (c) | 10.4% | | 8.1% |
| Terminal Rates (d) | 3/35 (9%) | | 2/34 (6%) |
| Week of First Observation | 74 | | 95 |
| Life Table Test (e) | | | P=0.503N |
| Incidental Tumor Test (e) | | | P=0.528N |
| Fisher Exact Test (e) | | | P=0.500N |
| **Thyroid Gland: C-Cell Adenoma** | | | |
| Overall Rates (a) | 4/50 (8%) | 6/50 (12%) | 11/50 (22%) |
| Adjusted Rates (c) | 11.0% | 19.4% | 29.2% |
| Terminal Rates (d) | 3/35 (9%) | 6/31 (19%) | 8/34 (24%) |
| Week of First Observation | 98 | 104 | 88 |
| Life Table Tests (e) | P=0.030 | P=0.302 | P=0.048 |
| Incidental Tumor Tests (e) | P=0.042 | P=0.373 | P=0.090 |
| Cochran-Armitage Trend Test (e) | P=0.031 | | |
| Fisher Exact Test (e) | | P=0.370 | P=0.045 |

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **Thyroid Gland: C-Cell Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 4/50 (8%) | 7/50 (14%) | 11/50 (22%) |
| Adjusted Rates (c) | 11.0% | 22.6% | 29.2% |
| Terminal Rates (d) | 3/35 (9%) | 7/31 (23%) | 8/34 (24%) |
| Week of First Observation | 98 | 104 | 88 |
| Life Table Tests (e) | P=0.032 | P=0.200 | P=0.048 |
| Incidental Tumor Tests (e) | P=0.044 | P=0.254 | P=0.090 |
| Cochran-Armitage Trend Test (e) | P=0.033 | | |
| Fisher Exact Test (e) | | P=0.262 | P=0.045 |
| **Mammary Gland: Fibroadenoma** | | | |
| Overall Rates (a) | 21/50 (42%) | 25/50 (50%) | 21/50 (42%) |
| Adjusted Rates (c) | 54.6% | 69.0% | 54.9% |
| Terminal Rates (d) | 18/35 (51%) | 20/31 (65%) | 17/34 (50%) |
| Week of First Observation | 77 | 65 | 88 |
| Life Table Tests (e) | P=0.506 | P=0.138 | P=0.554 |
| Incidental Tumor Tests (e) | P=0.531 | P=0.172 | P=0.578 |
| Cochran-Armitage Trend Test (e) | P=0.540 | | |
| Fisher Exact Test (e) | | P=0.274 | P=0.580 |
| **Mammary Gland: Carcinoma** | | | |
| Overall Rates (a) | 2/50 (4%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted Rates (c) | 5.7% | 9.7% | 5.0% |
| Terminal Rates (d) | 2/35 (6%) | 3/31 (10%) | 0/34 (0%) |
| Week of First Observation | 104 | 104 | 88 |
| Life Table Tests (e) | P=0.589 | P=0.444 | P=0.687N |
| Incidental Tumor Tests (e) | P=0.549N | P=0.444 | P=0.599N |
| Cochran-Armitage Trend Test (e) | P=0.594 | | |
| Fisher Exact Test (e) | | P=0.500 | P=0.691 |
| **Mammary Gland: Adenoma or Carcinoma (f)** | | | |
| Overall Rates (a) | 3/50 (6%) | 3/50 (6%) | 3/50 (6%) |
| Adjusted Rates (c) | 8.6% | 9.7% | 7.8% |
| Terminal Rates (d) | 3/35 (9%) | 3/31 (10%) | 1/34 (3%) |
| Week of First Observation | 104 | 104 | 88 |
| Life Table Tests (e) | P=0.577 | P=0.607 | P=0.661 |
| Incidental Tumor Tests (e) | P=0.547N | P=0.607 | P=0.586N |
| Cochran-Armitage Trend Test (e) | P=0.583 | | |
| Fisher Exact Test (e) | | P=0.661 | P=0.661 |
| **Clitoral Gland: Adenoma** | | | |
| Overall Rates (a) | 6/48 (13%) | 10/45 (22%) | 9/49 (18%) |
| Adjusted Rates (c) | 16.0% | 32.6% | 25.4% |
| Terminal Rates (d) | 5/35 (14%) | 9/31 (31%) | 8/34 (24%) |
| Week of First Observation | 74 | 94 | 95 |
| Life Table Tests (e) | P=0.240 | P=0.117 | P=0.274 |
| Incidental Tumor Tests (e) | P=0.224 | P=0.111 | P=0.251 |
| Cochran-Armitage Trend Test (e) | P=0.268 | | |
| Fisher Exact Test (e) | | P=0.167 | P=0.303 |
| **Uterus: Endometrial Stromal Polyp** | | | |
| Overall Rates (a) | 13/49 (27%) | (b) 13/28 (46%) | 16/49 (33%) |
| Adjusted Rates (c) | 35.4% | | 42.6% |
| Terminal Rates (d) | 11/34 (32%) | | 13/34 (38%) |
| Week of First Observation | 77 | | 80 |
| Life Table Test (e) | | | P=0.345 |
| Incidental Tumor Test (e) | | | P=0.362 |
| Fisher Exact Test (e) | | | P=0.329 |

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 350 ppm | 700 ppm |
|---|---|---|---|
| **All Sites: Benign Tumors** | | | |
| Overall Rates (a) | 45/50 (90%) | 40/50 (80%) | 43/50 (86%) |
| Adjusted Rates (c) | 97.8% | 92.9% | 89.5% |
| Terminal Rates (d) | 34/35 (97%) | 28/31 (90%) | 29/34 (85%) |
| Week of First Observation | 74 | 65 | 77 |
| Life Table Tests (e) | P=0.408N | P=0.466N | P=0.436N |
| Incidental Tumor Tests (e) | P=0.346N | P=0.266N | P=0.363N |
| Cochran-Armitage Trend Test (e) | P=0.336N | | |
| Fisher Exact Test (e) | | P=0.132N | P=0.380N |
| **All Sites: Malignant Tumors** | | | |
| Overall Rates (a) | 12/50 (24%) | 22/50 (44%) | 12/50 (24%) |
| Adjusted Rates (c) | 28.2% | 49.3% | 29.7% |
| Terminal Rates (d) | 6/35 (17%) | 9/31 (29%) | 7/34 (21%) |
| Week of First Observation | 77 | 65 | 86 |
| Life Table Tests (e) | P=0.524N | P=0.040 | P=0.563N |
| Incidental Tumor Tests (e) | P=0.402N | P=0.083 | P=0.503N |
| Cochran-Armitage Trend Test (e) | P=0.543 | | |
| Fisher Exact Test (e) | | P=0.028 | P=0.592 |
| **All Sites: All Tumors** | | | |
| Overall Rates (a) | 47/50 (94%) | 45/50 (90%) | 46/50 (92%) |
| Adjusted Rates (c) | 97.9% | 93.7% | 93.9% |
| Terminal Rates (d) | 34/35 (97%) | 28/31 (90%) | 31/34 (91%) |
| Week of First Observation | 74 | 65 | 77 |
| Life Table Tests (e) | P=0.472N | P=0.469 | P=0.501N |
| Incidental Tumor Tests (e) | P=0.426N | P=0.591N | P=0.489N |
| Cochran-Armitage Trend Test (e) | P=0.427N | | |
| Fisher Exact Test (e) | | P=0.358N | P=0.500N |

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Incomplete sampling of tissues
(c) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(d) Observed tumor incidence at terminal kill
(e) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(f) Except for one low dose animal with a carcinoma, a fibroadenoma was also present in each animal.

TABLE B4. HISTORICAL INCIDENCE OF THYROID GLAND C-CELL TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

| | Incidence in Controls | | |
|---|---|---|---|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| No 2-year studies by SRI International are included in the historical data base. | | | |
| **Overall Historical Incidence** | | | |
| TOTAL | 155/1,938 (8.0%) | 66/1,938 (3.4%) | 218/1,938 (11.2%) |
| SD (b) | 7.21% | 2.75% | 7.20% |
| Range (c) | | | |
| High | 17/50 | 5/50 | 19/50 |
| Low | 0/50 | 0/50 | 0/50 |

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Epidermal inclusion cyst | | 1 (2%) | |
| Hemorrhage | 1 (2%) | 1 (2%) | |
| Inflammation, chronic focal | | 1 (2%) | |
| Crystals, NOS | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #Nasal cavity | (48) | (9) | (50) |
| Foreign body, NOS | 1 (2%) | | |
| Hemorrhage | 3 (6%) | 1 (11%) | 3 (6%) |
| Inflammation, acute focal | 2 (4%) | | 5 (10%) |
| *Larynx | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| #Tracheal submucosa | (50) | (49) | (50) |
| Inflammation, chronic focal | | | 2 (4%) |
| #Lung/bronchus | (50) | (27) | (50) |
| Foreign body, NOS | 1 (2%) | | |
| #Lung/bronchiole | (50) | (27) | (50) |
| Hyperplasia, epithelial | 1 (2%) | | |
| #Lung | (50) | (27) | (50) |
| Mineralization | 1 (2%) | | |
| Congestion, NOS | 5 (10%) | 5 (19%) | 5 (10%) |
| Edema, NOS | | 1 (4%) | |
| Hemorrhage | 3 (6%) | 4 (15%) | 4 (8%) |
| Inflammation, chronic focal | 4 (8%) | 1 (4%) | 1 (2%) |
| Pigmentation, NOS | | 4 (15%) | |
| Alveolar macrophages | 30 (60%) | 3 (11%) | 28 (56%) |
| Hyperplasia, alveolar epithelium | 4 (8%) | | 5 (10%) |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Hematopoiesis | | 2 (4%) | |
| #Bone marrow | (50) | (17) | (50) |
| Cyst, NOS | 1 (2%) | | |
| Myelofibrosis | 2 (4%) | | |
| Hyperplasia, granulocytic | 1 (2%) | | 2 (4%) |
| Hyperplasia, reticulum cell | 2 (4%) | | 1 (2%) |
| #Spleen | (50) | (50) | (49) |
| Congestion, NOS | | 1 (2%) | 1 (2%) |
| Fibrosis | 1 (2%) | | |
| Necrosis, focal | 1 (2%) | 1 (2%) | |
| Infarct, NOS | | 1 (2%) | |
| Atrophy, focal | 2 (4%) | 2 (4%) | 1 (2%) |
| Hyperplasia, lymphoid | 3 (6%) | | 1 (2%) |
| Hematopoiesis | 8 (16%) | 4 (8%) | 9 (18%) |
| #Mandibular lymph node | (50) | (32) | (50) |
| Cyst, NOS | 3 (6%) | | 2 (4%) |
| Hemorrhage | 1 (2%) | | 3 (6%) |
| Pigmentation, NOS | | | 1 (2%) |
| Hyperplasia, plasma cell | 1 (2%) | | 2 (4%) |
| Hyperplasia, lymphoid | 1 (2%) | | |
| #Retropharyngeal lymph node | (50) | (32) | (50) |
| Hyperplasia, lymphoid | | | 1 (2%) |

**TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #Thoracic lymph node | (50) | (32) | (50) |
| Edema, NOS | 1 (2%) | | |
| Pigmentation, NOS | | 1 (3%) | 1 (2%) |
| Hyperplasia, plasma cell | | | 1 (2%) |
| Hyperplasia, reticulum cell | | | 1 (2%) |
| Hyperplasia, lymphoid | 1 (2%) | | |
| #Bronchial lymph node | (50) | (32) | (50) |
| Hyperplasia, plasma cell | | | 1 (2%) |
| #Mesenteric lymph node | (50) | (32) | (50) |
| Congestion, NOS | | 1 (3%) | 2 (4%) |
| Edema, NOS | 3 (6%) | 3 (9%) | 4 (8%) |
| Hemorrhage | 3 (6%) | 1 (3%) | |
| Pigmentation, NOS | 1 (2%) | | |
| #Renal lymph node | (50) | (32) | (50) |
| Congestion, NOS | | 1 (3%) | |
| Edema, NOS | | | 2 (4%) |
| Pigmentation, NOS | | | 1 (2%) |
| #Inguinal lymph node | (50) | (32) | (50) |
| Cyst, NOS | | 1 (3%) | |
| Hyperplasia, plasma cell | | 1 (3%) | |
| #Liver | (50) | (50) | (50) |
| Hematopoiesis | 1 (2%) | 3 (6%) | 3 (6%) |
| #Adrenal cortex | (50) | (22) | (50) |
| Hematopoiesis | | 1 (5%) | |
| #Adrenal medulla | (50) | (22) | (50) |
| Hyperplasia, lymphoid | | | 1 (2%) |
| #Thymus | (48) | (25) | (49) |
| Cyst, NOS | | | 1 (2%) |
| Congestion, NOS | 1 (2%) | | |
| Hemorrhage | 1 (2%) | | 1 (2%) |
| Involution, NOS | 37 (77%) | 17 (68%) | 42 (86%) |
| Hyperplasia, epithelial | 1 (2%) | | |
| CIRCULATORY SYSTEM | | | |
| #Brain | (50) | (20) | (49) |
| Periarteritis | 1 (2%) | | |
| *Mediastinum | (50) | (50) | (50) |
| Periarteritis | 2 (4%) | 1 (2%) | |
| #Bone marrow | (50) | (17) | (50) |
| Periarteritis | 1 (2%) | | |
| #Lung | (50) | (27) | (50) |
| Thrombosis, NOS | 1 (2%) | | |
| #Heart | (50) | (20) | (50) |
| Hemorrhage | 2 (4%) | | |
| Inflammation, chronic focal | 44 (88%) | 14 (70%) | 43 (86%) |
| Fibrosis | | | 1 (2%) |
| Hypertrophy, focal | | | 1 (2%) |
| #Heart/atrium | (50) | (20) | (50) |
| Thrombosis, NOS | 1 (2%) | 1 (5%) | 2 (4%) |
| Fibrosis, focal | | | 1 (2%) |
| *Pulmonary artery | (50) | (50) | (50) |
| Mineralization | 31 (62%) | 14 (28%) | 25 (50%) |
| *Mesentery | (50) | (50) | (50) |
| Periarteritis | | 1 (2%) | |

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| DIGESTIVE SYSTEM | | | |
| *Palate | (50) | (50) | (50) |
| Cyst, NOS | | 1 (2%) | |
| Inflammation, acute focal | 1 (2%) | | |
| Hyperplasia, epithelial | 2 (4%) | 1 (2%) | 1 (2%) |
| #Salivary gland | (47) | (18) | (47) |
| Mineralization | 1 (2%) | | |
| Cyst, NOS | | | 1 (2%) |
| Inflammation, chronic focal | 3 (6%) | 1 (6%) | 3 (6%) |
| Fibrosis, focal | 1 (2%) | | |
| Focal cellular change | 3 (6%) | | 3 (6%) |
| Atrophy, focal | | | 3 (6%) |
| Hyperplasia, epithelial | | | 1 (2%) |
| Hyperplasia, intraductal | 4 (9%) | | 3 (6%) |
| #Liver | (50) | (50) | (50) |
| Abnormal curvature | 2 (4%) | 2 (4%) | 4 (8%) |
| Congestion, NOS | 3 (6%) | | 2 (4%) |
| Hemorrhage | 2 (4%) | | 1 (2%) |
| Hemorrhagic cyst | 2 (4%) | | |
| Inflammation, chronic | 1 (2%) | | |
| Inflammation, chronic focal | 31 (62%) | 20 (40%) | 27 (54%) |
| Inflammation, chronic diffuse | | 1 (2%) | |
| Peliosis hepatis | 1 (2%) | 1 (2%) | 1 (2%) |
| Necrosis, focal | 2 (4%) | 8 (16%) | 6 (12%) |
| Pigmentation, NOS | | 1 (2%) | 1 (2%) |
| Angiectasis | | 1 (2%) | |
| #Liver/hepatocytes | (50) | (50) | (50) |
| Cytoplasmic vacuolization | 17 (34%) | 15 (30%) | 13 (26%) |
| Basophilic cyto change | 37 (74%) | 32 (64%) | 38 (76%) |
| Eosinophilic cyto change | 1 (2%) | | |
| Clear cell change | 8 (16%) | 7 (14%) | 6 (12%) |
| #Bile duct | (50) | (50) | (50) |
| Pigmentation, NOS | | | 1 (2%) |
| Hyperplasia, focal | 32 (64%) | 26 (52%) | 36 (72%) |
| #Pancreas | (50) | (17) | (50) |
| Edema, NOS | 1 (2%) | | |
| Hemorrhage | | | 1 (2%) |
| Inflammation, chronic focal | 7 (14%) | 3 (18%) | 6 (12%) |
| #Pancreatic acinus | (50) | (17) | (50) |
| Focal cellular change | | | 1 (2%) |
| Atrophy, focal | 19 (38%) | 6 (35%) | 23 (46%) |
| Hyperplasia, focal | | | 1 (2%) |
| #Glandular stomach | (50) | (18) | (50) |
| Ulcer, NOS | 3 (6%) | 4 (22%) | 7 (14%) |
| Inflammation, chronic focal | | 1 (6%) | 2 (4%) |
| #Forestomach | (50) | (18) | (50) |
| Cyst, NOS | | | 1 (2%) |
| Edema, NOS | | 4 (22%) | 1 (2%) |
| Ulcer, NOS | 2 (4%) | 5 (28%) | 1 (2%) |
| Inflammation, chronic focal | 2 (4%) | 1 (6%) | 1 (2%) |
| Fibrosis | | | 1 (2%) |
| Hyperplasia, epithelial | 31 (62%) | 8 (44%) | 25 (50%) |
| #Colon | (50) | (19) | (50) |
| Hemorrhage | 1 (2%) | | |
| Parasitism | 10 (20%) | 2 (11%) | 10 (20%) |
| #Cecum | (50) | (19) | (50) |
| Mineralization | | 1 (5%) | |
| Edema, NOS | 1 (2%) | 1 (5%) | |
| Hemorrhage | 1 (2%) | 2 (11%) | |
| *Rectum | (50) | (50) | (50) |
| Parasitism | 1 (2%) | | 4 (8%) |

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| URINARY SYSTEM | | | |
| #Kidney | (50) | (38) | (50) |
| Mineralization | 9 (18%) | 8 (21%) | 13 (26%) |
| Cyst, NOS | 2 (4%) | | 2 (4%) |
| Congestion, NOS | 1 (2%) | | 1 (2%) |
| Hemorrhage | | 1 (3%) | |
| Nephropathy | 47 (94%) | 36 (95%) | 49 (98%) |
| Pigmentation, NOS | 12 (24%) | 10 (26%) | 18 (36%) |
| Hypertrophy, compensatory | 1 (2%) | | |
| Hyperplasia, tubular cell | 1 (2%) | | |
| #Kidney/tubule | (50) | (38) | (50) |
| Degeneration, NOS | 1 (2%) | | |
| Necrosis, focal | 4 (8%) | 2 (5%) | 2 (4%) |
| #Kidney/pelvis | (50) | (38) | (50) |
| Dilatation, NOS | 3 (6%) | | 2 (4%) |
| Inflammation, suppurative | | 1 (3%) | |
| Hyperplasia, epithelial | 2 (4%) | | 2 (4%) |
| *Ureter | (50) | (50) | (50) |
| Dilatation, NOS | | 1 (2%) | |
| #Urinary bladder/submucosa | (47) | (17) | (49) |
| Inflammation, chronic focal | 2 (4%) | | |
| ENDOCRINE SYSTEM | | | |
| #Pituitary intermedia | (49) | (50) | (50) |
| Cyst, NOS | 3 (6%) | 1 (2%) | 4 (8%) |
| Hyperplasia, focal | | | 1 (2%) |
| Angiectasis | | 1 (2%) | |
| #Anterior pituitary | (49) | (50) | (50) |
| Cyst, NOS | 22 (45%) | 22 (44%) | 15 (30%) |
| Congestion, NOS | | 1 (2%) | |
| Hemorrhage | | | 2 (4%) |
| Pigmentation, NOS | | | 2 (4%) |
| Atrophy, NOS | | | 1 (2%) |
| Hyperplasia, focal | 19 (39%) | 23 (46%) | 23 (46%) |
| Angiectasis | | 1 (2%) | |
| #Adrenal cortex | (50) | (22) | (50) |
| Mineralization | | | 1 (2%) |
| Cyst, NOS | 1 (2%) | 1 (5%) | 4 (8%) |
| Congestion, NOS | 5 (10%) | 5 (23%) | 1 (2%) |
| Hemorrhage | 1 (2%) | | 2 (4%) |
| Degeneration, NOS | 6 (12%) | 4 (18%) | 8 (16%) |
| Necrosis, focal | | | 1 (2%) |
| Pigmentation, NOS | 1 (2%) | 1 (5%) | 1 (2%) |
| Focal cellular change | 11 (22%) | | 11 (22%) |
| Atrophy, diffuse | | | 1 (2%) |
| Hyperplasia, focal | 24 (48%) | 9 (41%) | 25 (50%) |
| Angiectasis | 2 (4%) | 1 (5%) | 4 (8%) |
| #Adrenal medulla | (50) | (22) | (50) |
| Hyperplasia, NOS | 5 (10%) | | 1 (2%) |
| Hyperplasia, focal | 2 (4%) | | 1 (2%) |
| #Thyroid | (50) | (50) | (50) |
| Cyst, NOS | 1 (2%) | 1 (2%) | 1 (2%) |
| Follicular cyst, NOS | 1 (2%) | 1 (2%) | 2 (4%) |
| Hemorrhage | 1 (2%) | | |
| Hyperplasia, C-cell | 29 (58%) | 24 (48%) | 26 (52%) |
| #Parathyroid | (46) | (28) | (46) |
| Hyperplasia, NOS | 1 (2%) | 1 (4%) | 3 (7%) |
| #Pancreatic islets | (50) | (17) | (50) |
| Hyperplasia, focal | 1 (2%) | | |

**TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (50) |
| Galactocele | | | 2 (4%) |
| Hyperplasia, cystic | 40 (80%) | 18 (36%) | 41 (82%) |
| #Clitoral gland | (48) | (45) | (49) |
| Cyst, NOS | 3 (6%) | | 1 (2%) |
| Inflammation, suppurative | 6 (13%) | 1 (2%) | 7 (14%) |
| Inflammation, chronic | 2 (4%) | | 3 (6%) |
| Inflammation, chronic focal | | 2 (4%) | |
| Atrophy, NOS | 43 (90%) | 42 (93%) | 48 (98%) |
| Hyperplasia, NOS | 1 (2%) | 5 (11%) | 6 (12%) |
| #Uterus | (49) | (28) | (49) |
| Hydrometra | 17 (35%) | 7 (25%) | 11 (22%) |
| Cyst, NOS | 4 (8%) | 1 (4%) | 3 (6%) |
| Hemorrhage | | | 1 (2%) |
| Inflammation, suppurative | 2 (4%) | | |
| Hypoplasia, NOS | | 1 (4%) | |
| Atrophy, focal | | | 1 (2%) |
| #Cervix uteri | (49) | (28) | (49) |
| Dilatation, NOS | | | 1 (2%) |
| Epidermal inclusion cyst | 3 (6%) | 2 (7%) | 3 (6%) |
| Inflammation, suppurative | 4 (8%) | 2 (7%) | 3 (6%) |
| #Uterus/endometrium | (49) | (28) | (49) |
| Hyperplasia, NOS | 1 (2%) | 1 (4%) | 1 (2%) |
| #Ovary | (48) | (22) | (49) |
| Cyst, NOS | 10 (21%) | 6 (27%) | 5 (10%) |
| Congestion, NOS | 2 (4%) | | 1 (2%) |
| Hyperplasia, focal | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| #Brain/meninges | (50) | (20) | (49) |
| Mineralization | 1 (2%) | | |
| Congestion, NOS | | 1 (5%) | |
| Hyperplasia, NOS | | | 1 (2%) |
| #Brain | (50) | (20) | (49) |
| Mineralization | 1 (2%) | | |
| Hydrocephalus, NOS | 6 (12%) | | 8 (16%) |
| Congestion, NOS | 1 (2%) | | |
| Hemorrhage | 2 (4%) | 1 (5%) | 4 (8%) |
| Gliosis | 1 (2%) | 1 (5%) | |
| *Optic nerve | (50) | (50) | (50) |
| Atrophy, NOS | 1 (2%) | | |
| SPECIAL SENSE ORGANS | | | |
| *Eye/anterior chamber | (50) | (50) | (50) |
| Hemorrhage | | 1 (2%) | |
| *Eye/sclera | (50) | (50) | (50) |
| Mineralization | 3 (6%) | 2 (4%) | |
| *Eye/cornea | (50) | (50) | (50) |
| Vascularization | 1 (2%) | | |
| *Eye/retina | (50) | (50) | (50) |
| Atrophy, focal | 2 (4%) | 2 (4%) | 2 (4%) |
| Atrophy, diffuse | 2 (4%) | 2 (4%) | 2 (4%) |
| *Eye/crystalline lens | (50) | (50) | (50) |
| Mineralization | 1 (2%) | | |
| Degeneration, NOS | 3 (6%) | 3 (6%) | 2 (4%) |
| *Eye/lacrimal gland | (50) | (50) | (50) |
| Pigmentation, NOS | 1 (2%) | 1 (2%) | |

**TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| SPECIAL SENSE ORGANS (Continued) | | | |
| *Nasolacrimal duct | (50) | (50) | (50) |
| Hemorrhage | | | 1 (2%) |
| Inflammation, acute focal | 3 (6%) | | 3 (6%) |
| Inflammation, chronic focal | | 1 (2%) | |
| *Harderian gland | (50) | (50) | (50) |
| Abscess, NOS | 1 (2%) | | |
| Inflammation, chronic focal | 2 (4%) | 3 (6%) | 1 (2%) |
| Pigmentation, NOS | | | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *Bone | (50) | (50) | (50) |
| Fibrous osteodystrophy | 1 (2%) | | |
| Osteosclerosis | 6 (12%) | | |
| *Sternum | (50) | (50) | (50) |
| Osteosclerosis | | | 1 (2%) |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | 1 (2%) | |
| *Abdominal cavity | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | 1 (2%) | |
| Inflammation, chronic focal | | 1 (2%) | 3 (6%) |
| Necrosis, fat | 10 (20%) | 4 (8%) | 10 (20%) |
| ALL OTHER SYSTEMS | | | |
| Tail | | | |
| Necrosis, diffuse | | 1 | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| None | | | |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

# APPENDIX C

# SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE


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TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Sarcoma, NOS | 11 (22%) | ‡7 (14%) | 5 (10%) |
| Sarcoma, NOS, metastatic | 1 (2%) | | |
| Fibroma | 1 (2%) | 1 (2%) | |
| Lipoma | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #Lung | (50) | (28) | (50) |
| Hepatocellular carcinoma, metastatic | | | 1 (2%) |
| Alveolar/bronchiolar adenoma | 3 (6%) | 2 (7%) | 3 (6%) |
| Alveolar/bronchiolar carcinoma | 2 (4%) | 1 (4%) | 1 (2%) |
| Sarcoma, NOS, metastatic | 1 (2%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Malignant lymphoma, undifferentiated type | | | 1 (2%) |
| Malignant lymphoma, lymphocytic type | 2 (4%) | 1 (2%) | 1 (2%) |
| Malignant lymphoma, mixed type | 5 (10%) | 6 (12%) | 3 (6%) |
| #Mesenteric lymph node | (48) | (41) | (50) |
| Malignant lymphoma, mixed type | 1 (2%) | 1 (2%) | |
| #Peyer's patch | (46) | (23) | (49) |
| Malignant lymphoma, mixed type | 1 (2%) | 1 (4%) | |
| CIRCULATORY SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Hemangiosarcoma | | 1 (2%) | |
| #Spleen | (49) | (50) | (50) |
| Hemangioma | | 1 (2%) | |
| #Heart | (50) | (23) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic | | | 1 (2%) |
| Sarcoma, NOS, metastatic | 1 (2%) | | |
| #Liver | (49) | (50) | (50) |
| Hemangioma | | 1 (2%) | |
| Hemangiosarcoma | | 1 (2%) | 2 (4%) |
| *Mesentery | (50) | (50) | (50) |
| Hemangioma | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| #Salivary gland | (48) | (23) | (49) |
| Sarcoma, NOS, invasive | | 1 (4%) | |
| #Liver | (49) | (50) | (50) |
| Hepatocellular adenoma | 9 (18%) | 8 (16%) | 10 (20%) |
| Hepatocellular carcinoma | 6 (12%) | 8 (16%) | 12 (24%) |
| Lipoma | | 1 (2%) | |
| #Bile duct | (49) | (50) | (50) |
| Adenocarcinoma, NOS | | | 1 (2%) |
| #Forestomach | (46) | (22) | (49) |
| Squamous cell papilloma | | | 1 (2%) |
| #Jejunum | (46) | (23) | (49) |
| Adenocarcinoma, NOS | 1 (2%) | | |

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| URINARY SYSTEM | | | |
| #Kidney | (50) | (50) | (50) |
| Tubular cell adenoma | | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #Adrenal/capsule | (48) | (49) | (50) |
| Adenoma, NOS | 1 (2%) | | |
| #Adrenal medulla | (48) | (49) | (50) |
| Pheochromocytoma | | 2 (4%) | |
| #Thyroid | (48) | (50) | (50) |
| Follicular cell adenoma | 2 (4%) | 1 (2%) | |
| C-cell adenoma | 1 (2%) | | |
| #Pancreatic islets | (48) | (23) | (50) |
| Islet cell adenoma | 2 (4%) | | |
| REPRODUCTIVE SYSTEM | | | |
| #Testis | (48) | (23) | (49) |
| Interstitial cell tumor | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| #Brain | (47) | (50) | (50) |
| Meningioma | | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| *Harderian gland | (50) | (50) | (50) |
| Adenoma, NOS | 1 (2%) | 2 (4%) | 4 (8%) |
| MUSCULOSKELETAL SYSTEM | | | |
| None | | | |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Sarcoma, NOS, metastatic | 1 (2%) | | |
| ALL OTHER SYSTEMS | | | |
| *Multiple organs | (50) | (50) | (50) |
| Adenocarcinoma, NOS, metastatic | | | 1 (2%) |
| Histiocytic sarcoma | | 2 (4%) | 2 (4%) |
| Tail | | | |
| Osteosarcoma | 1 | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural death | 8 | 11 | 8 |
| Moribund sacrifice | 12 | 15 | 16 |
| Terminal sacrifice | 30 | 24 | 26 |

**TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| Total animals with primary tumors** | 33 | 34 | 34 |
| Total primary tumors | 52 | 51 | 47 |
| Total animals with benign tumors | 20 | 15 | 17 |
| Total benign tumors | 22 | 20 | 19 |
| Total animals with malignant tumors | 24 | 28 | 25 |
| Total malignant tumors | 30 | 31 | 28 |
| Total animals with secondary tumors## | 2 | 1 | 3 |
| Total secondary tumors | 4 | 1 | 3 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
## Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ
‡ Multiple occurrence of morphology; tissue is counted only once.

## TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: UNTREATED CONTROL

| ANIMAL NUMBER | 02 36 | 03 36 | 03 37 | 03 30 | 03 31 | 00 02 | 03 35 | 04 40 | 02 24 | 02 22 | 02 28 | 00 05 | 00 06 | 00 09 | 03 33 | 02 27 | 04 43 | 00 08 | 01 17 | 01 11 | 00 01 | 00 03 | 00 04 | 00 07 | 01 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 01 11 | 01 19 | 07 70 | 07 74 | 07 79 | 08 87 | 08 87 | 08 87 | 08 88 | 08 89 | 08 89 | 09 91 | 09 91 | 09 93 | 09 93 | 09 97 | 09 97 | 10 03 | 10 44 | 10 55 | 10 66 | 10 66 | 10 66 | 10 66 | 10 66 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS | | | X | | X | | | X | | | X | | X | | | | | | X | X | X | | X | | |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Fibroma | | | | | | | | | | | | | | | | X | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | X | | X | | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | X | | | | | X | | |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | – | + | – | – | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + | + |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thymus | + | – | – | – | – | + | – | – | + | – | – | + | – | – | – | – | – | + | – | – | + | + | – | – | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | X | X | |
| Hepatocellular carcinoma | | | | X | | | X | | | | | | | X | | | | | | X | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | + | + | + | + | N | + | + | + | + | + | + | + | + | + | N | + | + | + | + | N | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | – | + | – | – | + | + | + | + | – | + | + | + | + | + |
| Small intestine | + | – | + | + | + | + | + | + | + | + | + | – | + | – | – | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Large intestine | + | – | + | + | + | + | + | + | + | + | + | – | + | – | – | + | + | + | + | – | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | – | + | – | – | – | + | + | + | + | – | + | + | + | + |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | | X | | | | | | | |
| C-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | + | + | + | + | – | + | + | + | + | – | + | + | – | + | + | – | + | – | + | – | + | + | – |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Islet cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | N | N | N | N | N | N | N |
| Testis | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Interstitial cell tumor | | | | | | | | X | | | | | | | | | | | | | | | | | |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | + | + | + |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | – | + | – | – | + | + | + | + | + | + | + | + | + | + |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mediastinum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Mesentery | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Hemangioma | | | | | | | | | | | | | | X | | | | | | | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | | | | X | X | | | | | | | | | | |
| Malignant lymphoma, mixed type | | | | | | | | | X | | | | | | | | | | | X | | | | | |
| Tail | | | | | | | | | | | | | | | | | | | | | | | | | |
| Osteosarcoma | | | | | | | | X | | | | | | | | | | | | | | | | | |

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

## TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: UNTREATED CONTROL (Continued)

| ANIMAL NUMBER | 012 | 013 | 014 | 015 | 016 | 018 | 019 | 020 | 021 | 023 | 025 | 029 | 032 | 034 | 038 | 039 | 041 | 042 | 044 | 045 | 046 | 047 | 048 | 049 | 050 | TOTAL: TISSUES TUMORS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Sarcoma, NOS | | | | | | | | | X | | | | | | X | | | | | | | | | | | 11 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar adenoma | | | | | X | | | | | | | | | | | | | | | | | | | | | 3 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | 48 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | X | | | 1 |
| Thymus | + | – | – | – | + | + | + | – | + | + | + | – | + | + | – | – | + | – | + | + | – | + | + | – | – | 22 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | X | | | | | | | | | | | 1 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Hepatocellular adenoma | | | | | | X | | | X | | X | | X | | | | | | | X | | X | | | | 9 |
| Hepatocellular carcinoma | | | | | X | | X | | | | | | | | | | | | | | | | | | | 6 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Gallbladder & common bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | + | N | + | + | + | + | + | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | X | | | | | | 1 |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | X | | | | | | | | 1 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 45 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | – | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 43 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | X | | | | | | | | 1 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | | | | X | | | | | | 2 |
| C-cell adenoma | | | | | | | | | | | | | | | | X | | | | | | | | | | 1 |
| Parathyroid | – | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | – | + | + | – | – | + | + | + | + | 38 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Islet cell adenoma | | | | | | X | | | | | | | | | | | X | | | | | | | | | 2 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Interstitial cell tumor | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | X | | | 1 |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mediastinum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Mesentery | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Hemangioma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Malignant lymphoma, lymphocytic type | | | | | | | X | | | | | X | | | | | | | | | | X | | | | 2 |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | | 5 |
| Tail | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Osteosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |

* Animals necropsied

## TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: LOW DOSE

| ANIMAL NUMBER | 0004 | 0449 | 0488 | 0443 | 0447 | 0005 | 0034 | 0115 | 0228 | 0003 | 0008 | 0226 | 0331 | 0335 | 0550 | 0224 | 0001 | 0229 | 0332 | 0339 | 0117 | 0338 | 0112 | 0114 | 0337 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 0255 | 0388 | 0579 | 0595 | 0611 | 0700 | 0730 | 0744 | 0755 | 0799 | 0844 | 0877 | 0888 | 0911 | 0911 | 0922 | 0933 | 0933 | 0933 | 0955 | 0966 | 0966 | 0977 | 0977 | 1033 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | + | N | N | + | + | + | N |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | X | | | | | X |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lipoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | X | | | | | | | | | | | | | | | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hemangioma | | | | | | | | | | | | | | X | | | | | | | | | | | |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thymus | + | − | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | + | + | + | − |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | + | + | − |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | + | + | − |
| Sarcoma, NOS, invasive | | | | | | | | | | | | | | | | | | | | | | | | | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular adenoma | | | | | | | | | | | | | | X | | | | | | | | | X | | |
| Hepatocellular carcinoma | | | | | | | X | | | | X | | X | | | | X | X | | | X | | | | |
| Lipoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hemangioma | | | | | | | | | | | | | | | | | | | | X | | | | | |
| Hemangiosarcoma | | | | | | | | | | | | | | | | X | | | | | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | + | + | + | + | + | + | N | + | + | + | + | + | N | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | + | + | − |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | − |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | − | + | + | + | − |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | − | + | + | − | − |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Large intestine | + | + | + | + | − | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | − | − |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | − | − | + | + | + | + | + | + | − | − | − | + | + | + | + | + | + | + | + |
| Adrenal | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | | | | | X | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Follicular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | − | + | + | + | − | + | + | − | + | − |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | N | + | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | N |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | + | + | − |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Meningioma | | | | | | | | X | | | | | | | | | | | | | | | | | |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Histiocytic sarcoma | | | | | | | | | | | | X | | | | | | | | | | | | | |
| Hemangiosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, mixed type | | | | | | | | X | | | X | | | | X | | | | | | | X | | |

## TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

| ANIMAL NUMBER | 0016 | 0002 | 0008 | 0007 | 0009 | 0010 | 0011 | 0013 | 0018 | 0019 | 0020 | 0021 | 0022 | 0023 | 0025 | 0027 | 0030 | 0033 | 0036 | 0040 | 0041 | 0042 | 0044 | 0045 | 0046 | TOTAL: |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 104 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | TISSUES TUMORS |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | N | + | + | + | N | N | N | N | N | N | N | + | N | N | N | N | N | N | + | N | N | N | N | N | *50 |
| Sarcoma, NOS | @X | | | | | | | | | X | X | X | | | | | | | | X | | | | | | 7 |
| Fibroma | | | | | | | | | | | X | | | | | | | | | | | | | | | 1 |
| Lipoma | | | | | | | | | | | X | | | | | | | | | | | | | | | 1 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | − | + | − | − | − | − | − | − | + | − | − | − | − | − | + | − | − | − | − | − | − | + | − | − | − | 28 |
| Alveolar/bronchiolar adenoma | | X | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | − | − | − | − | + | + | + | + | + | + | + | − | − | + | + | + | 45 |
| Nasal cavity | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 1 |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Hemangioma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | − | + | + | + | − | + | − | + | − | + | + | − | + | − | − | + | 41 |
| Malignant lymphoma, mixed type | | X | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Thymus | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | 9 |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | 23 |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 23 |
| Sarcoma, NOS, invasive | X | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Hepatocellular adenoma | | | | | | | X | X | | | | X | | | X | X | | | | | | | X | | | 8 |
| Hepatocellular carcinoma | | | X | X | | | | | | | | | | | | | | | | | | | | | | 8 |
| Lipoma | X | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Hemangioma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Hemangiosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Gallbladder & common bile duct | + | N | N | + | + | N | + | + | + | N | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | *50 |
| Pancreas | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | 23 |
| Esophagus | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 32 |
| Stomach | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 22 |
| Small intestine | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | 23 |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | X | | | 1 |
| Large intestine | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | 21 |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 45 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Pheochromocytoma | | | | | | | | | X | | | | | | | | | | | | | | | | | 2 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Follicular cell adenoma | | | | | | X | | | | | | | | | | | | | | | | | | | | 1 |
| Parathyroid | + | + | + | + | + | − | + | − | + | − | + | − | + | + | − | + | − | − | − | − | − | − | − | − | − | 31 |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Testis | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | 23 |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Meningioma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| SPECIAL SENSE ORGANS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Adenoma, NOS | | | | | | | X | | | | | | | | | X | | | | | | | | | | 2 |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | X | 2 |
| Hemangiosarcoma | | | | | | | | | | | | | X | | | | | | | | | | | | | 1 |
| Malignant lymphoma, lymphocytic type | | | | | | | | X | | | | | | | | | | | | | | | | | | 1 |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | X | | | X | | | | | | | | 6 |

* Animals necropsied

@ Multiple occurrence of morphology

## TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: HIGH DOSE

| ANIMAL NUMBER | 026 | 030 | 025 | 021 | 023 | 031 | 035 | 001 | 034 | 022 | 013 | 018 | 043 | 027 | 037 | 005 | 010 | 039 | 014 | 002 | 033 | 028 | 041 | 045 | 003 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 034 | 038 | 058 | 061 | 069 | 072 | 072 | 074 | 074 | 076 | 081 | 081 | 084 | 088 | 088 | 089 | 090 | 092 | 093 | 096 | 099 | 104 | 104 | 104 | 105 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS | | | | | | | | | | | | | | | | X | | | | | | | X | X | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular carcinoma, metastatic | | | | | | | | | | | X | | | | | | | | | | | | | | |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | X | | | | | | X | | X | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | X | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | − | − | + | − | − | + | + | + | − | − | − | − | − | + | + | − | − | − | − | − | − | − | − | − | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar carcinoma, metastatic | | | | | | | | | | | | | | | | | | X | | | | | | | |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular adenoma | | | | | | | | X | | X | | | | | | X | | | X | X | | | | | X |
| Hepatocellular carcinoma | | | | | | | | | | | X | X | | | X | | X | | | X | | | X | | |
| Hemangiosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Gallbladder & common bile duct | + | + | N | + | + | + | + | + | + | + | + | + | N | + | N | + | + | + | N | + | + | N | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + |
| Squamous cell papilloma | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + |
| Large intestine | − | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tubular cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | − | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | − | + | + | + | + | + | + |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Parathyroid | + | − | + | − | − | − | − | + | + | − | − | + | + | + | − | + | + | + | + | − | − | + | + | + | − |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Adenoma, NOS | | | | | | | | | | | | | | | | | X | | | | | | | X | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Adenocarcinoma, NOS, metastatic | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | X | | | |
| Malignant lymphoma, undifferentiated type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | |

## TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

| ANIMAL NUMBER | 0004 | 0006 | 0007 | 0008 | 0009 | 0111 | 0112 | 0115 | 0116 | 0117 | 0119 | 0120 | 0224 | 0229 | 0232 | 0236 | 0238 | 0240 | 0242 | 0244 | 0246 | 0247 | 0248 | 0249 | 0250 | TOTAL: TISSUES TUMORS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | *50 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | X | | | X | | | | 5 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Hepatocellular carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 3 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Thymus | + | + | + | − | − | − | + | + | + | − | − | − | + | + | − | + | + | − | + | − | + | − | − | + | − | 20 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar carcinoma, metasta | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Hepatocellular adenoma | | | | | | | | | | X | | | | | X | | | | | | | X | | X | 10 |
| Hepatocellular carcinoma | X | | | | | X | | | | | | | | X | | | | X | | X | | X | | | | 12 |
| Hemangiosarcoma | | | | | | | | | | | | X | | | | | | | | | X | | | | | 2 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Gallbladder & common bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Tubular cell adenoma | | | | | | | | X | | | | | | | | | | | | | | | | | | 1 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Parathyroid | + | + | + | + | + | + | + | − | + | + | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | 36 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Testis | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Adenoma, NOS | | | X | | X | | | | | | | | | | | | | | | | | | | | | 4 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Adenocarcinoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Histiocytic sarcoma | | | | | | X | | | | | | | | | | | | | | | | | | | | 2 |
| Malignant lymphoma, undifferentiated | | | | | | | | | | | | | | | X | | | | | | | | | | | 1 |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | X | | | | | | | | | | | | | | | 1 |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | X | | | | | | X | X | | 3 |

* Animals necropsied

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **Subcutaneous Tissue: Sarcoma** | | | |
| Overall Rates (a) | 11/50 (22%) | 7/50 (14%) | 5/50 (10%) |
| Adjusted Rates (b) | 27.7% | 25.6% | 16.5% |
| Terminal Rates (c) | 5/31 (16%) | 4/24 (17%) | 2/26 (8%) |
| Week of First Observation | 70 | 95 | 89 |
| Life Table Tests (d) | P=0.132N | P=0.385N | P=0.164N |
| Incidental Tumor Tests (d) | P=0.081N | P=0.221N | P=0.097N |
| Cochran-Armitage Trend Test (d) | P=0.063N | | |
| Fisher Exact Test (d) | | P=0.218N | P=0.086N |
| **Subcutaneous Tissue: Fibroma or Sarcoma** | | | |
| Overall Rates (a) | 12/50 (24%) | 7/50 (14%) | 5/50 (10%) |
| Adjusted Rates (b) | 29.7% | 25.6% | 16.5% |
| Terminal Rates (c) | 5/31 (16%) | 4/24 (17%) | 2/26 (8%) |
| Week of First Observation | 70 | 95 | 89 |
| Life Table Tests (d) | P=0.092N | P=0.309N | P=0.119N |
| Incidental Tumor Tests (d) | P=0.048N | P=0.140N | P=0.061N |
| Cochran-Armitage Trend Test (d) | P=0.038N | | |
| Fisher Exact Test (d) | | P=0.154N | P=0.054N |
| **Lung: Alveolar/Bronchiolar Adenoma** | | | |
| Overall Rates (a) | 3/50 (6%) | (e) 2/28 (7%) | 3/50 (6%) |
| Adjusted Rates (b) | 9.4% | | 9.6% |
| Terminal Rates (c) | 2/31 (6%) | | 0/26 (0%) |
| Week of First Observation | 104 | | 89 |
| Life Table Test (d) | | | P=0.598 |
| Incidental Tumor Test (d) | | | P=0.629 |
| Fisher Exact Test (d) | | | P=0.661 |
| **Lung: Alveolar/Bronchiolar Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 5/50 (10%) | (e) 3/28 (11%) | 4/50 (8%) |
| Adjusted Rates (b) | 15.2% | | 12.3% |
| Terminal Rates (c) | 3/31 (10%) | | 0/26 (0%) |
| Week of First Observation | 103 | | 89 |
| Life Table Test (d) | | | P=0.582N |
| Incidental Tumor Test (d) | | | P=0.555N |
| Fisher Exact Test (d) | | | P=0.500N |
| **Hematopoietic System: Malignant Lymphoma, Mixed Type** | | | |
| Overall Rates (a) | 7/50 (14%) | 8/50 (16%) | 3/50 (6%) |
| Adjusted Rates (b) | 21.3% | 25.4% | 11.5% |
| Terminal Rates (c) | 6/31 (19%) | 4/24 (17%) | 3/26 (12%) |
| Week of First Observation | 88 | 74 | 105 |
| Life Table Tests (d) | P=0.229N | P=0.337 | P=0.233N |
| Incidental Tumor Tests (d) | P=0.185N | P=0.405 | P=0.232N |
| Cochran-Armitage Trend Test (d) | P=0.141N | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.159N |
| **Hematopoietic System: Lymphoma, All Malignant** | | | |
| Overall Rates (a) | 9/50 (18%) | 9/50 (18%) | 5/50 (10%) |
| Adjusted Rates (b) | 25.5% | 29.1% | 19.2% |
| Terminal Rates (c) | 6/31 (19%) | 5/24 (21%) | 5/26 (19%) |
| Week of First Observation | 88 | 74 | 105 |
| Life Table Tests (d) | P=0.278N | P=0.426 | P=0.298N |
| Incidental Tumor Tests (d) | P=0.223N | P=0.555 | P=0.282N |
| Cochran-Armitage Trend Test (d) | P=0.166N | | |
| Fisher Exact Test (d) | | P=0.602 | P=0.194N |

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **Circulatory System: Hemangioma or Hemangiosarcoma** | | | |
| Overall Rates (a) | 1/50 (2%) | 4/50 (8%) | 2/50 (4%) |
| Adjusted Rates (b) | 2.7% | 12.3% | 7.7% |
| Terminal Rates (c) | 0/31 (0%) | 1/24 (4%) | 2/26 (8%) |
| Week of First Observation | 93 | 91 | 105 |
| Life Table Tests (d) | P=0.345 | P=0.153 | P=0.443 |
| Incidental Tumor Tests (d) | P=0.358 | P=0.190 | P=0.459 |
| Cochran-Armitage Trend Test (d) | P=0.406 | | |
| Fisher Exact Test (d) | | P=0.181 | P=0.500 |
| **Liver: Hepatocellular Adenoma** | | | |
| Overall Rates (a) | 9/49 (18%) | 8/50 (16%) | 10/50 (20%) |
| Adjusted Rates (b) | 29.0% | 29.6% | 29.9% |
| Terminal Rates (c) | 9/31 (29%) | 6/24 (25%) | 5/26 (19%) |
| Week of First Observation | 105 | 91 | 74 |
| Life Table Tests (d) | P=0.301 | P=0.495 | P=0.350 |
| Incidental Tumor Tests (d) | P=0.399 | P=0.533 | P=0.510 |
| Cochran-Armitage Trend Test (d) | P=0.467 | | |
| Fisher Exact Test (d) | | P=0.482N | P=0.520 |
| **Liver: Hepatocellular Carcinoma** | | | |
| Overall Rates (a) | 6/49 (12%) | 8/50 (16%) | 12/50 (24%) |
| Adjusted Rates (b) | 16.0% | 22.6% | 35.5% |
| Terminal Rates (c) | 3/31 (10%) | 2/24 (8%) | 6/26 (23%) |
| Week of First Observation | 74 | 70 | 81 |
| Life Table Tests (d) | P=0.049 | P=0.288 | P=0.060 |
| Incidental Tumor Tests (d) | P=0.069 | P=0.552 | P=0.085 |
| Cochran-Armitage Trend Test (d) | P=0.079 | | |
| Fisher Exact Test (d) | | P=0.403 | P=0.104 |
| **Liver: Hepatocellular Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 15/49 (31%) | 16/50 (32%) | 20/50 (40%) |
| Adjusted Rates (b) | 43.0% | 47.2% | 53.7% |
| Terminal Rates (c) | 12/31 (39%) | 8/24 (33%) | 10/26 (38%) |
| Week of First Observation | 74 | 70 | 74 |
| Life Table Tests (d) | P=0.083 | P=0.262 | P=0.096 |
| Incidental Tumor Tests (d) | P=0.145 | P=0.476 | P=0.190 |
| Cochran-Armitage Trend Test (d) | P=0.189 | | |
| Fisher Exact Test (d) | | P=0.527 | P=0.222 |
| **Harderian Gland: Adenoma** | | | |
| Overall Rates (a) | 1/50 (2%) | 2/50 (4%) | 4/50 (8%) |
| Adjusted Rates (b) | 3.2% | 8.3% | 13.5% |
| Terminal Rates (c) | 1/31 (3%) | 2/24 (8%) | 2/26 (8%) |
| Week of First Observation | 105 | 105 | 90 |
| Life Table Tests (d) | P=0.091 | P=0.410 | P=0.140 |
| Incidental Tumor Tests (d) | P=0.091 | P=0.410 | P=0.146 |
| Cochran-Armitage Trend Test (d) | P=0.118 | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.181 |
| **All Sites: Benign Tumors** | | | |
| Overall Rates (a) | 20/50 (40%) | 15/50 (30%) | 17/50 (34%) |
| Adjusted Rates (b) | 55.2% | 50.3% | 47.1% |
| Terminal Rates (c) | 15/31 (48%) | 10/24 (42%) | 8/26 (31%) |
| Week of First Observation | 87 | 74 | 74 |
| Life Table Tests (d) | P=0.530N | P=0.501N | P=0.571N |
| Incidental Tumor Tests (d) | P=0.388N | P=0.261N | P=0.418N |
| Cochran-Armitage Trend Test (d) | P=0.300N | | |
| Fisher Exact Test (d) | | P=0.201N | P=0.340N |

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **All Sites: Malignant Tumors** | | | |
| Overall Rates (a) | 24/50 (48%) | 28/50 (56%) | 25/50 (50%) |
| Adjusted Rates (b) | 54.7% | 72.7% | 67.0% |
| Terminal Rates (c) | 12/31 (39%) | 14/24 (58%) | 14/26 (54%) |
| Week of First Observation | 70 | 70 | 81 |
| Life Table Tests (d) | P=0.231 | P=0.104 | P=0.266 |
| Incidental Tumor Tests (d) | P=0.311 | P=0.284 | P=0.346 |
| Cochran-Armitage Trend Test (d) | P=0.460 | | |
| Fisher Exact Test (d) | | P=0.274 | P=0.500 |
| **All Sites: All Tumors** | | | |
| Overall Rates (a) | 33/50 (66%) | 34/50 (68%) | 34/50 (68%) |
| Adjusted Rates (b) | 74.5% | 84.6% | 84.8% |
| Terminal Rates (c) | 20/31 (65%) | 18/24 (75%) | 20/26 (77%) |
| Week of First Observation | 70 | 70 | 74 |
| Life Table Tests (d) | P=0.172 | P=0.146 | P=0.198 |
| Incidental Tumor Tests (d) | P=0.260 | P=0.422 | P=0.333 |
| Cochran-Armitage Trend Test (d) | P=0.458 | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.500 |

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) Incomplete sampling of tissues

TABLE C4. HISTORICAL INCIDENCE OF HEPATOCELLULAR TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

| | Incidence in Controls | | |
|---|---|---|---|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| No 2-year studies by SRI International are included in the historical data base. | | | |
| **Overall Historical Incidence** | | | |
| TOTAL | 259/2,032 (12.7%) | 379/2,032 (18.7%) | 609/2,032 (30.0%) |
| SD (b) | 7.21% | 6.50% | 7.59% |
| Range (c) | | | |
| High | 22/50 | 15/50 | 29/50 |
| Low | 0/49 | 4/50 | 8/50 |

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (50) | (50) | (50) |
| Ulcer, NOS | 2 (4%) | | 1 (2%) |
| Inflammation, suppurative | | 1 (2%) | |
| Inflammation, acute focal | | 1 (2%) | |
| Inflammation, chronic focal | 1 (2%) | ‡4 (8%) | 2 (4%) |
| Necrosis, focal | 1 (2%) | | |
| Hyperplasia, epithelial | | 1 (2%) | 1 (2%) |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Mineralization | | | 2 (4%) |
| Cyst, NOS | 1 (2%) | 4 (8%) | 2 (4%) |
| Epidermal inclusion cyst | | | 1 (2%) |
| Edema, NOS | 1 (2%) | | |
| Inflammation, suppurative | 2 (4%) | 3 (6%) | 2 (4%) |
| Inflammation, chronic focal | ‡5 (10%) | 3 (6%) | 7 (14%) |
| Necrosis, focal | 1 (2%) | | |
| RESPIRATORY SYSTEM | | | |
| #Nasal cavity | (47) | (1) | (49) |
| Foreign body, NOS | 2 (4%) | | |
| Hemorrhage | 4 (9%) | | 2 (4%) |
| Inflammation, acute focal | | | 1 (2%) |
| #Lung | (50) | (28) | (50) |
| Congestion, NOS | 6 (12%) | 13 (46%) | 11 (22%) |
| Edema, NOS | | | 2 (4%) |
| Hemorrhage | 6 (12%) | 2 (7%) | 5 (10%) |
| Inflammation, interstitial | 5 (10%) | 4 (14%) | 2 (4%) |
| Inflammation, suppurative | 1 (2%) | | |
| Inflammation, acute focal | | | 2 (4%) |
| Inflammation, chronic focal | 9 (18%) | 3 (11%) | 5 (10%) |
| Necrosis, focal | 1 (2%) | | |
| Pigmentation, NOS | | 1 (4%) | |
| Alveolar macrophages | 3 (6%) | 1 (4%) | 2 (4%) |
| Hyperplasia, alveolar epithelium | 4 (8%) | 2 (7%) | 4 (8%) |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Hyperplasia, lymphoid | 1 (2%) | 1 (2%) | |
| Hematopoiesis | 1 (2%) | | |
| #Bone marrow | (49) | (50) | (50) |
| Hyperplasia, granulocytic | 12 (24%) | 20 (40%) | 19 (38%) |
| #Spleen | (49) | (50) | (50) |
| Cyst, NOS | | | 1 (2%) |
| Abscess, NOS | | | 1 (2%) |
| Inflammation, granulomatous focal | | | 1 (2%) |
| Necrosis, focal | | | 1 (2%) |
| Atrophy, diffuse | 1 (2%) | | |
| Depletion, lymphoid | | | 1 (2%) |
| Angiectasis | 1 (2%) | | |
| Hyperplasia, plasma cell | 1 (2%) | | |
| Hyperplasia, lymphoid | 2 (4%) | | 1 (2%) |
| Hematopoiesis | 15 (31%) | 22 (44%) | 27 (54%) |
| #Lymph node | (48) | (41) | (50) |
| Cyst, NOS | | | 1 (2%) |
| Pigmentation, NOS | 1 (2%) | 1 (2%) | |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| HEMATOPOIETIC SYSTEM | | | |
| #Lymph node (Continued) | (48) | (41) | (50) |
| Hyperplasia, plasma cell | | 1 (2%) | 3 (6%) |
| Hyperplasia, lymphoid | | 1 (2%) | 2 (4%) |
| #Mandibular lymph node | (48) | (41) | (50) |
| Pigmentation, NOS | 1 (2%) | | 2 (4%) |
| #Thoracic lymph node | (48) | (41) | (50) |
| Inflammation, acute focal | | | 1 (2%) |
| Pigmentation, NOS | | 1 (2%) | |
| Hyperplasia, plasma cell | 1 (2%) | 1 (2%) | 1 (2%) |
| Hematopoiesis | 1 (2%) | | |
| #Abdominal lymph node | (48) | (41) | (50) |
| Hyperplasia, plasma cell | | 1 (2%) | |
| #Hepatic lymph node | (48) | (41) | (50) |
| Hyperplasia, lymphoid | | 1 (2%) | |
| #Pancreatic lymph node | (48) | (41) | (50) |
| Angiectasis | | | 1 (2%) |
| Hyperplasia, plasma cell | | | 1 (2%) |
| Hyperplasia, lymphoid | | | 1 (2%) |
| #Lumbar lymph node | (48) | (41) | (50) |
| Hyperplasia, plasma cell | | 1 (2%) | 1 (2%) |
| #Mesenteric lymph node | (48) | (41) | (50) |
| Cyst, NOS | | 1 (2%) | |
| Congestion, NOS | 3 (6%) | 5 (12%) | 5 (10%) |
| Edema, NOS | | | 1 (2%) |
| Hemorrhage | 9 (19%) | 11 (27%) | 11 (22%) |
| Hemorrhagic cyst | 1 (2%) | | |
| Inflammation, suppurative | | | 1 (2%) |
| Inflammation, acute focal | 2 (4%) | 1 (2%) | 3 (6%) |
| Inflammation, chronic focal | | 1 (2%) | 2 (4%) |
| Necrosis, focal | 1 (2%) | | |
| Angiectasis | 7 (15%) | 9 (22%) | 7 (14%) |
| Hyperplasia, plasma cell | | 1 (2%) | |
| Hyperplasia, lymphoid | 2 (4%) | 5 (12%) | 2 (4%) |
| Hematopoiesis | 9 (19%) | 8 (20%) | 9 (18%) |
| #Renal lymph node | (48) | (41) | (50) |
| Hyperplasia, plasma cell | | 2 (5%) | |
| #Sacral lymph node | (48) | (41) | (50) |
| Hyperplasia, lymphoid | | | 1 (2%) |
| #Brachial lymph node | (48) | (41) | (50) |
| Hyperplasia, plasma cell | | 1 (2%) | |
| Hyperplasia, lymphoid | | 1 (2%) | 1 (2%) |
| #Inguinal lymph node | (48) | (41) | (50) |
| Edema, NOS | | 2 (5%) | |
| Histiocytosis | | 1 (2%) | |
| Hyperplasia, plasma cell | 1 (2%) | 5 (12%) | 1 (2%) |
| Hyperplasia, lymphoid | 1 (2%) | 2 (5%) | |
| #Lung | (50) | (28) | (50) |
| Hyperplasia, lymphoid | | | 2 (4%) |
| Hematopoiesis | | | 1 (2%) |
| #Liver | (49) | (50) | (50) |
| Mastocytosis | 1 (2%) | | |
| Hematopoiesis | 4 (8%) | 9 (18%) | 8 (16%) |
| #Peyer's patch | (46) | (23) | (49) |
| Hyperplasia, lymphoid | 1 (2%) | | 3 (6%) |
| #Adrenal cortex | (48) | (49) | (50) |
| Hematopoiesis | | 1 (2%) | |
| #Thymus | (22) | (9) | (20) |
| Cyst, NOS | 1 (5%) | | 2 (10%) |
| Necrosis, focal | | | 1 (5%) |
| Involution, NOS | 4 (18%) | 4 (44%) | 4 (20%) |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| CIRCULATORY SYSTEM | | | |
| *Lymphatics/mammary gland | (50) | (50) | (50) |
| Cyst, NOS | 1 (2%) | | |
| #Heart | (50) | (23) | (50) |
| Mineralization | | 1 (4%) | |
| Hemorrhage | | | 1 (2%) |
| Inflammation, chronic focal | 28 (56%) | 6 (26%) | 29 (58%) |
| Pigmentation, NOS | 1 (2%) | 3 (13%) | 2 (4%) |
| #Endocardium | (50) | (23) | (50) |
| Hyperplasia, focal | | 1 (4%) | |
| *Coronary artery | (50) | (50) | (50) |
| Hypertrophy, focal | | 1 (2%) | |
| *Pulmonary artery | (50) | (50) | (50) |
| Inflammation, chronic focal | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| *Palate | (50) | (50) | (50) |
| Inflammation, chronic focal | | | 1 (2%) |
| *Tooth | (50) | (50) | (50) |
| Dysplasia, NOS | 1 (2%) | | |
| #Salivary gland | (48) | (23) | (49) |
| Mineralization | | 1 (4%) | 1 (2%) |
| Hemorrhage | | 1 (4%) | |
| Inflammation, chronic focal | 14 (29%) | 6 (26%) | 8 (16%) |
| #Liver | (49) | (50) | (50) |
| Mineralization | | 2 (4%) | |
| Cyst, NOS | | 1 (2%) | |
| Congestion, NOS | | 1 (2%) | |
| Hemorrhage | 1 (2%) | | |
| Inflammation, suppurative | | 1 (2%) | |
| Inflammation, chronic focal | 14 (29%) | 11 (22%) | 14 (28%) |
| Necrosis, focal | 6 (12%) | 6 (12%) | 4 (8%) |
| Infarct, NOS | 2 (4%) | 1 (2%) | |
| Pigmentation, NOS | 2 (4%) | 2 (4%) | 1 (2%) |
| #Liver/hepatocytes | (49) | (50) | (50) |
| Nuclear shape alteration | 1 (2%) | | |
| Cytoplasmic vacuolization | 1 (2%) | | |
| Basophilic cyto change | | 1 (2%) | |
| Clear cell change | 1 (2%) | | |
| *Gallbladder | (50) | (50) | (50) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 4 (8%) | 1 (2%) | 1 (2%) |
| #Pancreas | (48) | (23) | (50) |
| Cyst, NOS | | 1 (4%) | |
| Hemorrhage | | | 1 (2%) |
| Inflammation, suppurative | | 1 (4%) | |
| Inflammation, chronic focal | 14 (29%) | 4 (17%) | 17 (34%) |
| #Pancreatic acinus | (48) | (23) | (50) |
| Basophilic cyto change | 2 (4%) | | 1 (2%) |
| Eosinophilic cyto change | | 1 (4%) | |
| Atrophy, focal | 1 (2%) | 1 (4%) | |
| #Esophagus | (49) | (32) | (50) |
| Hemorrhage | | 1 (3%) | |
| Inflammation, chronic focal | 1 (2%) | | |
| #Glandular stomach | (46) | (22) | (49) |
| Cyst, NOS | 2 (4%) | | |
| Ulcer, NOS | 1 (2%) | 1 (5%) | |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 1 (2%) | | |
| Necrosis, focal | | 1 (5%) | |
| Atrophy, focal | 4 (9%) | | 4 (8%) |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| DIGESTIVE SYSTEM (Continued) | | | |
| #Forestomach | (46) | (22) | (49) |
| Ulcer, NOS | | 1 (5%) | |
| Inflammation, acute focal | | 1 (5%) | |
| Inflammation, chronic focal | 1 (2%) | 1 (5%) | 2 (4%) |
| Hyperplasia, epithelial | 2 (4%) | 4 (18%) | 1 (2%) |
| #Small intestine | (46) | (23) | (49) |
| Congestion, NOS | | 1 (4%) | |
| #Peyer's patch | (46) | (23) | (49) |
| Necrosis, focal | | 1 (4%) | 1 (2%) |
| #Large intestine | (45) | (21) | (48) |
| Pigmentation, NOS | | 1 (5%) | |
| #Colon | (45) | (21) | (48) |
| Parasitism | 1 (2%) | | |
| #Cecum | (45) | (21) | (48) |
| Hemorrhage | | 1 (5%) | |
| URINARY SYSTEM | | | |
| #Urinary bladder/cavity | (48) | (50) | (50) |
| Calculus, gross observation only | | | 1 (2%) |
| Dilatation, NOS | | 5 (10%) | 16 (32%) |
| #Kidney | (50) | (50) | (50) |
| Mineralization | 9 (18%) | 2 (4%) | 7 (14%) |
| Cyst, NOS | 2 (4%) | 3 (6%) | 8 (16%) |
| Congestion, NOS | 1 (2%) | | |
| Inflammation, suppurative | | 11 (22%) | 14 (28%) |
| Abscess, NOS | | 1 (2%) | 4 (8%) |
| Inflammation, chronic focal | 20 (40%) | 17 (34%) | 15 (30%) |
| Fibrosis | | 1 (2%) | 2 (4%) |
| Nephropathy | 24 (48%) | 44 (88%) | 49 (98%) |
| Necrosis, focal | | 2 (4%) | 1 (2%) |
| Infarct, NOS | | 2 (4%) | |
| Infarct, focal | | 1 (2%) | 1 (2%) |
| Atrophy, diffuse | | 1 (2%) | |
| Hyperplasia, tubular cell | | | 2 (4%) |
| Metaplasia, osseous | 1 (2%) | | |
| #Kidney/tubule | (50) | (50) | (50) |
| Degeneration, NOS | 2 (4%) | | 2 (4%) |
| #Kidney/pelvis | (50) | (50) | (50) |
| Dilatation, NOS | 1 (2%) | 13 (26%) | 42 (84%) |
| Hyperplasia, epithelial | | 5 (10%) | 3 (6%) |
| *Ureter | (50) | (50) | (50) |
| Inflammation, suppurative | | 1 (2%) | |
| Inflammation, acute focal | | | 1 (2%) |
| Inflammation, chronic focal | | | 1 (2%) |
| Hyperplasia, epithelial | | | 1 (2%) |
| #Urinary bladder | (48) | (50) | (50) |
| Inflammation, chronic focal | | 1 (2%) | |
| #Urinary bladder/mucosa | (48) | (50) | (50) |
| Inflammation, acute focal | 1 (2%) | | 1 (2%) |
| Hyperplasia, epithelial | 1 (2%) | 7 (14%) | 8 (16%) |
| #Urinary bladder/submucosa | (48) | (50) | (50) |
| Edema, NOS | | 2 (4%) | |
| Inflammation, suppurative | 1 (2%) | 1 (2%) | |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 16 (33%) | 21 (42%) | 31 (62%) |
| *Urethra | (50) | (50) | (50) |
| Inflammation, suppurative | | 2 (4%) | |
| Hyperplasia, epithelial | | 1 (2%) | |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ENDOCRINE SYSTEM | | | |
| #Anterior pituitary | (43) | (45) | (47) |
| Cyst, NOS | 5 (12%) | 3 (7%) | 3 (6%) |
| Hyperplasia, focal | 6 (14%) | 2 (4%) | 2 (4%) |
| #Adrenal | (48) | (49) | (50) |
| Congestion, NOS | | | 1 (2%) |
| #Adrenal/capsule | (48) | (49) | (50) |
| Hyperplasia, focal | 41 (85%) | 38 (78%) | 38 (76%) |
| #Adrenal cortex | (48) | (49) | (50) |
| Degeneration, NOS | 1 (2%) | | 1 (2%) |
| Necrosis, focal | | | 1 (2%) |
| Pigmentation, NOS | 1 (2%) | | |
| Hyperplasia, focal | 12 (25%) | 11 (22%) | 7 (14%) |
| #Adrenal medulla | (48) | (49) | (50) |
| Hyperplasia, NOS | 6 (13%) | 7 (14%) | 7 (14%) |
| #Thyroid | (48) | (50) | (50) |
| Cyst, NOS | | | 1 (2%) |
| Follicular cyst, NOS | 14 (29%) | 8 (16%) | 15 (30%) |
| Inflammation, chronic focal | | 2 (4%) | 3 (6%) |
| Necrosis, focal | | | 1 (2%) |
| Hyperplasia, C-cell | | | 1 (2%) |
| #Parathyroid | (38) | (31) | (36) |
| Cyst, NOS | 4 (11%) | | |
| Crystals, NOS | 1 (3%) | | |
| Hyperplasia, NOS | 1 (3%) | | 1 (3%) |
| #Pancreatic islets | (48) | (23) | (50) |
| Eosinophilic cyto change | 1 (2%) | | |
| Hyperplasia, focal | 1 (2%) | | |
| REPRODUCTIVE SYSTEM | | | |
| *Penis | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| Inflammation, chronic focal | 2 (4%) | | |
| *Prepuce | (50) | (50) | (50) |
| Cyst, NOS | | | 1 (2%) |
| Inflammation, chronic focal | | 1 (2%) | |
| *Preputial gland | (50) | (50) | (50) |
| Cyst, NOS | 2 (4%) | 1 (2%) | |
| Inflammation, suppurative | 3 (6%) | | 4 (8%) |
| Inflammation, chronic focal | 3 (6%) | 1 (2%) | |
| Atrophy, NOS | 1 (2%) | | 3 (6%) |
| #Prostate | (48) | (50) | (50) |
| Cyst, NOS | | | 1 (2%) |
| Hemorrhage | | | 3 (6%) |
| Inflammation, suppurative | 2 (4%) | 4 (8%) | 9 (18%) |
| Inflammation, acute focal | | | 2 (4%) |
| Inflammation, chronic focal | 9 (19%) | 9 (18%) | 7 (14%) |
| Adhesion, NOS | | | 1 (2%) |
| Hyperplasia, epithelial | 3 (6%) | 3 (6%) | 4 (8%) |
| *Seminal vesicle | (50) | (50) | (50) |
| Dilatation, NOS | 5 (10%) | 6 (12%) | 4 (8%) |
| Congestion, NOS | | | 1 (2%) |
| Inflammation, suppurative | 1 (2%) | | 2 (4%) |
| Inflammation, chronic focal | | 2 (4%) | |
| Atrophy, NOS | | | 2 (4%) |
| #Testis | (48) | (23) | (49) |
| Mineralization | 5 (10%) | 1 (4%) | 2 (4%) |
| Spermatocele | | 1 (4%) | |
| Cyst, NOS | | | 1 (2%) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 1 (2%) | | |
| Fibrosis | 1 (2%) | | |

**TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| REPRODUCTIVE SYSTEM | | | |
| #Testis (Continued) | (48) | (23) | (49) |
| Necrosis, focal | 1 (2%) | | |
| Atrophy, focal | 3 (6%) | | |
| Atrophy, diffuse | 2 (4%) | | |
| *Epididymis | (50) | (50) | (50) |
| Spermatocele | | 2 (4%) | |
| Inflammation, suppurative | 1 (2%) | | 1 (2%) |
| Inflammation, chronic focal | 1 (2%) | 1 (2%) | |
| NERVOUS SYSTEM | | | |
| #Brain/meninges | (47) | (50) | (50) |
| Cyst, NOS | | 1 (2%) | |
| Hemorrhage | | | 1 (2%) |
| Inflammation, chronic focal | 1 (2%) | | 2 (4%) |
| Pigmentation, NOS | | | 1 (2%) |
| #Brain | (47) | (50) | (50) |
| Mineralization | 27 (57%) | 20 (40%) | 19 (38%) |
| Hydrocephalus, NOS | 3 (6%) | 2 (4%) | |
| Congestion, NOS | | 1 (2%) | |
| Hemorrhage | | 1 (2%) | 1 (2%) |
| Inflammation, chronic focal | 1 (2%) | | |
| SPECIAL SENSE ORGANS | | | |
| *Eye | (50) | (50) | (50) |
| Phthisis bulbi | | | 1 (2%) |
| *Eye/cornea | (50) | (50) | (50) |
| Inflammation, chronic focal | | 1 (2%) | |
| *Eye/retina | (50) | (50) | (50) |
| Atrophy, diffuse | | | 1 (2%) |
| *Nasolacrimal duct | (50) | (50) | (50) |
| Inflammation, acute focal | 2 (4%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| *Bone | (50) | (50) | (50) |
| Fibrous osteodystrophy | | | 2 (4%) |
| *Skeletal muscle | (50) | (50) | (50) |
| Hemorrhage | | | 1 (2%) |
| Inflammation, suppurative | 1 (2%) | | |
| Inflammation, chronic focal | | | 1 (2%) |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Hemorrhage | | | 2 (4%) |
| Inflammation, chronic focal | 1 (2%) | | |
| *Abdominal cavity | (50) | (50) | (50) |
| Cyst, NOS | 1 (2%) | | |
| Hemorrhage | | | 1 (2%) |
| Inflammation, suppurative | 2 (4%) | 3 (6%) | 4 (8%) |
| Inflammation, chronic focal | 2 (4%) | 1 (2%) | 1 (2%) |
| Necrosis, fat | 3 (6%) | 3 (6%) | 1 (2%) |
| *Pleural cavity | (50) | (50) | (50) |
| Inflammation, suppurative | | 1 (2%) | |
| Inflammation, chronic focal | 1 (2%) | | |
| *Pericardium | (50) | (50) | (50) |
| Inflammation, chronic suppurative | | 1 (2%) | |
| *Mesentery | (50) | (50) | (50) |
| Angiectasis | | 1 (2%) | |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| BODY CAVITIES (Continued) | | | |
| *Tunica vaginalis | (50) | (50) | (50) |
| Adhesion, NOS | | 1 (2%) | |
| Necrosis, fat | | | 1 (2%) |
| ALL OTHER SYSTEMS | | | |
| *Multiple organs | (50) | (50) | (50) |
| Inflammation, suppurative | | 2 (4%) | 2 (4%) |
| Tail | | | |
| Edema, NOS | | 1 | |
| Knee | | | |
| Osteosclerosis | | 1 | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| None | | | |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site
‡ Multiple occurrence of morphology in the same organ; tissue is counted only once.

# APPENDIX D

# SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE


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**TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 48 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 49 | 48 |
| INTEGUMENTARY SYSTEM | | | |
| *Subcutaneous tissue | (50) | (50) | (48) |
| Sarcoma, NOS | 1 (2%) | 3 (6%) | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #Lung | (50) | (47) | (47) |
| Adenocarcinoma, NOS, metastatic | | 1 (2%) | |
| Hepatocellular carcinoma, metastatic | | | 1 (2%) |
| Alveolar/bronchiolar adenoma | 1 (2%) | 2 (4%) | 3 (6%) |
| Alveolar/bronchiolar carcinoma | 2 (4%) | | |
| Acinar cell carcinoma, metastatic | | 1 (2%) | |
| Sarcoma, NOS, metastatic | | 1 (2%) | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (48) |
| Malignant lymphoma, NOS | | 1 (2%) | |
| Malignant lymphoma, undifferentiated type | | | 1 (2%) |
| Malignant lymphoma, lymphocytic type | 3 (6%) | 4 (8%) | 2 (4%) |
| Malignant lymphoma, mixed type | 13 (26%) | 6 (12%) | 16 (33%) |
| #Spleen | (50) | (49) | (47) |
| Malignant lymphoma, mixed type | | 1 (2%) | 1 (2%) |
| #Lymph node | (50) | (48) | (47) |
| Sarcoma, NOS, metastatic | | 1 (2%) | |
| #Thoracic lymph node | (50) | (48) | (47) |
| Adenocarcinoma, NOS, metastatic | | 1 (2%) | |
| #Peyer's patch | (47) | (46) | (45) |
| Malignant lymphoma, NOS | | 1 (2%) | |
| CIRCULATORY SYSTEM | | | |
| *Subcutaneous tissue | (50) | (50) | (48) |
| Hemangiosarcoma | | | 1 (2%) |
| #Spleen | (50) | (49) | (47) |
| Hemangioma | | 1 (2%) | |
| Hemangiosarcoma | | 1 (2%) | |
| #Heart | (50) | (48) | (47) |
| Sarcoma, NOS, metastatic | | 1 (2%) | |
| DIGESTIVE SYSTEM | | | |
| #Liver | (50) | (49) | (47) |
| Hepatocellular adenoma | 5 (10%) | 4 (8%) | 1 (2%) |
| Hepatocellular carcinoma | 1 (2%) | | 4 (9%) |
| #Forestomach | (47) | (46) | (46) |
| Squamous cell papilloma | | 2 (4%) | |
| Squamous cell carcinoma | 1 (2%) | | |
| #Duodenum | (47) | (46) | (45) |
| Adenocarcinoma, NOS | 1 (2%) | | |
| #Jejunum | (47) | (46) | (45) |
| Adenocarcinoma, NOS | | | 1 (2%) |

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| URINARY SYSTEM | | | |
| #Kidney | (50) | (49) | (47) |
| Sarcoma, NOS, metastatic | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #Anterior pituitary | (50) | (46) | (45) |
| Carcinoma, NOS | | | 1 (2%) |
| Adenoma, NOS | 10 (20%) | 5 (11%) | 7 (16%) |
| #Adrenal | (49) | (49) | (47) |
| Cortical adenoma | | | 1 (2%) |
| #Adrenal/capsule | (49) | (49) | (47) |
| Carcinoma, NOS | | | 1 (2%) |
| #Adrenal medulla | (49) | (49) | (47) |
| Pheochromocytoma | 2 (4%) | 2 (4%) | 2 (4%) |
| #Thyroid | (50) | (47) | (47) |
| Follicular cell adenoma | | 4 (9%) | 3 (6%) |
| Follicular cell carcinoma | 2 (4%) | | 1 (2%) |
| #Pancreatic islets | (49) | (48) | (46) |
| Islet cell adenoma | | | 1 (2%) |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (48) |
| Acinar cell carcinoma | | 1 (2%) | |
| Mixed tumor, malignant | | 1 (2%) | 5 (10%) |
| #Uterus | (50) | (47) | (47) |
| Endometrial stromal polyp | 4 (8%) | | |
| #Uterus/endometrium | (50) | (47) | (47) |
| Carcinoma, NOS | | | 1 (2%) |
| #Ovary | (50) | (47) | (47) |
| Granulosa cell tumor | 1 (2%) | | |
| Mixed tumor, benign | 1 (2%) | | |
| Teratoma, NOS | | 1 (2%) | |
| NERVOUS SYSTEM | | | |
| #Brain | (48) | (46) | (47) |
| Carcinoma, NOS, invasive | | | 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| *Harderian gland | (50) | (50) | (48) |
| Adenoma, NOS | 4 (8%) | 2 (4%) | 1 (2%) |
| Adenocarcinoma, NOS | | 1 (2%) | |
| MUSCULOSKELETAL SYSTEM | | | |
| *Bone | (50) | (50) | (48) |
| Sarcoma, NOS | | | 1 (2%) |
| *Rib | (50) | (50) | (48) |
| Osteoma | 1 (2%) | | |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (48) |
| Follicular cell carcinoma, metastatic | | | 1 (2%) |
| *Abdominal cavity | (50) | (50) | (48) |
| Sarcoma, NOS, metastatic | | 1 (2%) | |
| *Pelvis | (50) | (50) | (48) |
| Sarcoma, NOS | | | 1 (2%) |

**TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)**

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ALL OTHER SYSTEMS | | | |
| *Multiple organs | (50) | (50) | (48) |
| Alveolar/bronchiolar carcinoma, metastatic | 1 (2%) | | |
| Histiocytic sarcoma | 1 (2%) | 1 (2%) | |
| ANIMAL DISPOSITION SUMMARY | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural death | 7 | 9 | 19 |
| Moribund sacrifice | 11 | 12 | 13 |
| Terminal sacrifice | 32 | 29 | 17 |
| Accidentally killed, NOS | | | 1 |
| TUMOR SUMMARY | | | |
| Total animals with primary tumors** | 34 | 35 | 39 |
| Total primary tumors | 54 | 44 | 57 |
| Total animals with benign tumors | 23 | 19 | 16 |
| Total benign tumors | 28 | 22 | 19 |
| Total animals with malignant tumors | 21 | 19 | 34 |
| Total malignant tumors | 25 | 21 | 38 |
| Total animals with secondary tumors## | 1 | 4 | 4 |
| Total secondary tumors | 1 | 8 | 4 |
| Total animals with tumors uncertain--benign or malignant | 1 | 1 | |
| Total uncertain tumors | 1 | 1 | |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
## Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

## TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: UNTREATED CONTROL

| ANIMAL NUMBER | 0111 | 0116 | 0003 | 0008 | 0447 | 0550 | 0223 | 0119 | 0221 | 0339 | 0344 | 0449 | 0330 | 0440 | 0235 | 0001 | 0002 | 0004 | 0005 | 0006 | 0007 | 0009 | 0109 | 0112 | 0113 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 063 | 065 | 068 | 074 | 074 | 079 | 083 | 090 | 090 | 090 | 097 | 098 | 100 | 102 | 105 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | N | + | N | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS | | | | | | | | | | | | | X | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | X | | | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | − | − | − | + | + | + | − | + | + | + | − | + | − | + | + | − | + | − | + | + | + | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | X | | | | | | |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | X | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | − | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Squamous cell carcinoma | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Small intestine | + | + | + | + | − | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Large intestine | + | + | + | + | − | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | X | | | X | | | | | | X | X | | | | | | |
| Adrenal | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | | | X | | | | | | | | X | | | | | | | | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Follicular cell carcinoma | | | | | | | | | | | | | | | | | X | | | | | | | | |
| Parathyroid | − | − | + | + | − | + | + | − | + | + | + | + | + | + | − | + | + | − | + | − | + | + | + | + | − |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | + | + | + | N | + | N | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endometrial stromal polyp | | | | | | | | | | | | X | | | | | | | | | | | | | |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Granulosa cell tumor | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mixed tumor, benign | | | | | | | | | | | | | | | | | | | | | | | X | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | − | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Adenoma, NOS | | | | | | | | | | | X | | X | | | | | | | | X | | | | |
| **MUSCULOSKELETAL SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Osteoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Alveolar/bronchiolar carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | |
| Histiocytic sarcoma | | | | | | | X | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, lymphocytic type | X | | | | | | | | | | | | | | | | | | | X | | | | | |
| Malignant lymphoma, mixed type | | | | | | | | | X | | X | | | X | X | | X | | X | | X | | | | |

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

## TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: UNTREATED CONTROL (Continued)

| ANIMAL NUMBER | 0 1 4 | 0 1 5 | 0 1 7 | 0 1 8 | 0 2 0 | 0 2 2 | 0 2 4 | 0 2 5 | 0 2 6 | 0 2 7 | 0 2 8 | 0 2 9 | 0 3 1 | 0 3 2 | 0 3 3 | 0 3 6 | 0 3 7 | 0 3 8 | 0 4 3 | 0 4 1 | 0 4 2 | 0 4 5 | 0 4 6 | 0 4 8 | 0 4 9 | TOTAL: |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 6 | 1 0 7 | 1 0 7 | 1 0 7 | 1 0 7 | 1 0 7 | 1 0 7 | TISSUES TUMORS |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Alveolar/bronchiolar carcinoma | | | | | | | X | | | | | | | | | | | | X | | | | | | | 2 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Thymus | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | − | + | − | 38 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Hepatocellular adenoma | | | | | X | | | | | | | | X | | | | X | X | | | | | | | | 5 |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Gallbladder & common bile duct | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | 47 |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | 47 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | X | | | | | 1 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | 47 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | | | | | X | | X | | | X | | | | | | | | | X | | | | X | | X | 10 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Follicular cell carcinoma | | | | | | X | | | | | | | | | | | | | | | | | | | | 2 |
| Parathyroid | + | − | + | + | + | + | − | + | + | + | + | + | + | + | − | + | − | + | − | + | + | + | + | + | + | 37 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Endometrial stromal polyp | | | | | | | | | | X | X | | | | X | | | | | | | | | | | 4 |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Granulosa cell tumor | X | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Mixed tumor, benign | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Adenoma, NOS | | | | | | X | | | | | | | | | | | | | | | | | | | | 4 |
| **MUSCULOSKELETAL SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Osteoma | | | | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Alveolar/bronchiolar carcinoma, metast. | | | | | | | | | | | | | | | | | | | X | | | | | | | 1 |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | | | | | | | X | | | | | | | | | 3 |
| Malignant lymphoma, mixed type | | | X | X | | X | | X | | X | | | | | | X | | | | | | | | | | 13 |

* Animals necropsied

## TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: LOW DOSE

| ANIMAL NUMBER | 0048 | 0050 | 0267 | 0223 | 0110 | 0113 | 0331 | 0088 | 0840 | 0846 | 0118 | 0447 | 0826 | 0099 | 0299 | 0033 | 0116 | 0199 | 0411 | 0011 | 0220 | 0092 | 0004 | 0055 | 0006 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 037 | 066 | 068 | 071 | 074 | 081 | 081 | 086 | 086 | 086 | 089 | 090 | 091 | 093 | 096 | 097 | 099 | 099 | 102 | 104 | 104 | 106 | 106 | 106 | 106 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | N | + | N | + | + | + | N | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | X | | X | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS, metastatic | | | X | | | | | | | | | | | | | | | | | | | | | | |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acinar cell carcinoma, metastatic | | | | | | | | | | | | | | | | | X | | | | | | | | |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | X | | | | | | |
| Trachea | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Nasal cavity | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Spleen | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hemangioma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hemangiosarcoma | | | | | | | | | | | | | | | | X | | | | | | | | | |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lymph nodes | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS, metastatic | | | X | | | | | | | | | | | | | | | | | | | | | | |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | X | | | | | | |
| Thymus | + | − | − | A | + | + | + | + | − | − | − | − | − | + | + | + | + | − | ~ | + | + | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | A | + | + | + | + | − | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Liver | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular adenoma | | | | | | | X | | | | | X | | | | | | | | | | | | | |
| Bile duct | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | N | + | + | N | + | + | + | + | + | + | N | + | N | + | + | + | + | N | + | + | + | + | + | + | + |
| Pancreas | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Stomach | − | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Squamous cell papilloma | | | | | | | | | | | | | | | | X | | | | | | | | | |
| Small intestine | − | + | + | A | + | + | + | + | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Malignant lymphoma, NOS | | | | | | | | | | | | | | | | | | X | | | | | | | |
| Large intestine | − | − | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | X | | | | | | |
| Urinary bladder | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | A | + | + | + | + | − | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | X | | | | | | | | | X |
| Adrenal | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | | X | X | | | | |
| Thyroid | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Follicular cell adenoma | | | | | X | | | | | | X | | | | | | | | | | | | | X | |
| Parathyroid | + | + | + | A | − | − | + | + | + | − | + | + | + | + | + | + | + | − | − | + | + | + | − | + | + |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | N | + | + | + | + | N | N | N | + | + | + | + | + | + | N | + | + | + | + | + | + | + |
| Acinar cell carcinoma | | | | | | | | | | | | | | | | | X | | | | | | | | |
| Mixed tumor, malignant | | | | | | | | | | | | | | | | | | | | | X | | | | |
| Uterus | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Ovary | + | + | + | A | + | + | + | + | + | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| Teratoma, NOS | X | | | | | | | | | | | | | | | | | | | | | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | A | + | + | + | + | − | − | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adenocarcinoma, NOS | | | X | | | | | | | | | | | | | | | | | | | | | | |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Histiocytic sarcoma | | | | | | | | | | | X | | | | | | | | | | | | | | |
| Malignant lymphoma, NOS | | | | | | | | | | X | | | | | | | | | | | | | | | |
| Malignant lymphoma, lymphocytic type | | | | | | | | | X | | | | X | | | | | | | | | | X | | |
| Malignant lymphoma, mixed type | | | | | | X | | | | | | X | | | X | | | | | | | | | | |

## TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

| ANIMAL NUMBER | 007 | 011 | 012 | 014 | 015 | 017 | 021 | 022 | 024 | 025 | 028 | 030 | 032 | 033 | 034 | 035 | 036 | 037 | 038 | 039 | 042 | 043 | 044 | 045 | 049 | TOTAL: |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 108 | 108 | 106 | 108 | 106 | 106 | 106 | 106 | 106 | 106 | 108 | 106 | 106 | 108 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | TISSUES TUMORS |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Sarcoma, NOS | | | | | X | | | | | | | | | | | | | | | | | | | | | 3 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Adenocarcinoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Alveolar/bronchiolar adenoma | | | | X | | | | | | | | | | | | X | | | | | | | | | | 2 |
| Acinar cell carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Hemangioma | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| Hemangiosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Malignant lymphoma, mixed type | | | X | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Adenocarcinoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Thymus | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | – | + | + | + | + | – | | 37 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Sarcoma, NOS, metastatic | | | | | X | | | | | | | | | | | | | | | | | | | | | 1 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Hepatocellular adenoma | | X | | | | | | | X | | | | | | | | | | | | | | | | | 4 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Gallbladder & common bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Esophagus | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Squamous cell papilloma | | | | | | | | | | | | | | | X | | | | | | | | | | | 2 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Malignant lymphoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 45 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | X | X | | | | X | | | | 5 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Follicular cell adenoma | | X | | | | | | | | | | | | | | | | | | | | | | | | 4 |
| Parathyroid | + | + | – | + | + | – | + | + | + | + | + | + | – | – | + | + | – | + | + | – | + | + | + | + | + | 37 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Acinar cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Mixed tumor, malignant | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Teratoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Adenoma, NOS | | | | | | | | | | X | | | | | | | | | | | | | | X | | 2 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Sarcoma, NOS, metastatic | | | | | X | | | | | | | | | | | | | | | | | | | | | 1 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Malignant lymphoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Malignant lymphoma, lymphocytic type | X | | | | | | | | | | | | | | | | | | | | | | | | | 4 |
| Malignant lymphoma, mixed type | | | | | X | | | | | | | X | | | | | | | | | | | | | X | 6 |

* Animals necropsied

## TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE: HIGH DOSE

| ANIMAL NUMBER | 0 1 2 | 0 4 8 | 0 0 8 | 0 1 7 | 0 4 1 | 0 4 6 | 0 2 0 | 0 4 4 | 0 3 7 | 0 2 5 | 0 1 8 | 0 2 9 | 0 0 5 | 0 0 6 | 0 1 6 | 0 3 6 | 0 1 1 | 0 2 7 | 0 0 4 | 0 2 4 | 0 0 9 | 0 4 9 | 0 3 3 | 0 0 3 | 0 1 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 0 7 2 | 0 7 2 | 0 7 5 | 0 7 8 | 0 7 9 | 0 7 9 | 0 8 6 | 0 8 6 | 0 8 8 | 0 9 0 | 0 9 1 | 0 9 2 | 0 9 3 | 0 9 3 | 0 9 4 | 0 9 4 | 0 9 5 | 0 9 5 | 0 9 6 | 0 9 6 | 0 9 7 | 0 9 7 | 0 9 8 | 0 9 9 | 0 9 9 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | N | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Sarcoma, NOS | X | | | | | | | | | | | | | | | | | | | | | | | | |
| Hemangiosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Hepatocellular carcinoma, metastatic | | | | | | | | | | | X | | | | | | | | | | | | | | |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | X | X | | | | | | |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | X | | | | | | | | | | |
| Trachea | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Nasal cavity | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Spleen | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lymph nodes | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Thymus | – | – | – | + | A | + | – | + | – | – | + | – | – | – | + | – | + | + | – | – | A | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Liver | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular carcinoma | | | X | | | | | | | X | X | | | | | | | X | | | | | | | |
| Bile duct | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Gallbladder & common bile duct | + | N | N | + | A | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | A | + | + | + | + |
| Pancreas | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | A | + | + | + | + |
| Esophagus | + | – | + | + | A | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Stomach | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | A | + | + | + | + |
| Small intestine | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | A | + | – | + | + |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Large intestine | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | – | – | A | + | – | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Urinary bladder | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | A | + | – | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Adenoma, NOS | | | | | | | | | | X | | | | | | | | | | | | | | | X |
| Adrenal | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Carcinoma, NOS | | | | X | | | | | | | | | | | | | | | | | | | | | |
| Cortical adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Follicular cell adenoma | | | | | | | | | | | | | | | | X | | | | | | | | | |
| Follicular cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | – | + | + | A | – | + | + | + | + | + | + | + | + | – | + | + | + | + | + | A | + | + | + | + |
| Pancreatic islets | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | A | + | + | + | + |
| Islet cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Mixed tumor, malignant | | X | | | | | | | | | | | | | | | | | | | | X | | | |
| Uterus | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ovary | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | – | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Carcinoma, NOS, invasive | | | | | | | | | X | | | | | | | | | | | | | | | | |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | A | N | N | N | N |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| **MUSCULOSKELETAL SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | N | N | N | N | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | A | N | N | N | N |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mediastinum | N | N | N | N | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | A | N | N | N | N |
| Follicular cell carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | N | N | N | N | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | A | N | N | N | N |
| Sarcoma, NOS | | | | | | | | | | | | | | | X | | | | | | | | | | |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | A | N | N | N | N |
| Malignant lymphoma, undifferentiated type | | | | | | | | | | | | | | | | | | X | | | | | | | |
| Malignant lymphoma, lymphocytic type | | | | | | X | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, mixed type | | | | X | | | X | | | | | | X | | | | | X | X | | | X | | X | X |

## TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

| ANIMAL NUMBER | 032 | 021 | 030 | 034 | 038 | 035 | 042 | 001 | 002 | 007 | 010 | 013 | 015 | 019 | 022 | 023 | 026 | 028 | 031 | 039 | 040 | 043 | 045 | 047 | 050 | TOTAL TISSUES TUMORS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 099 | 100 | 102 | 102 | 103 | 104 | 104 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 | |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *48 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Hemangiosarcoma | | | | | | | | | X | | | | | | | | | | | | | | | | | 1 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Hepatocellular carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Alveolar/bronchiolar adenoma | | | | | | | | X | | | | | | | | | | | | | | | | | | 3 |
| Sarcoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Thymus | − | − | + | − | + | − | + | + | + | + | + | − | + | − | − | + | + | − | − | − | + | + | + | + | + | 26 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Hepatocellular adenoma | | | | | | | | X | | | | | | | | | | | | | | | | | | 1 |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 4 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Gallbladder & common bile duct | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + | + | + | + | + | *48 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 45 |
| Adenocarcinoma, NOS | | | | | | X | | | | | | | | | | | | | | | | | | | | 1 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 44 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Urinary bladder | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 44 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | 45 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Adenoma, NOS | | | | | | X | | | | | | | | | | | | | X | X | X | | X | | | 7 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Cortical adenoma | | | X | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Pheochromocytoma | | | | | | | | | | | | | | X | | | | | | | | | X | | | 2 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Follicular cell adenoma | | | | | | | | | | | | | X | | | | | | | | X | | | | | 3 |
| Follicular cell carcinoma | | | | | | | | | | | | | | | | | | | | | X | | | | | 1 |
| Parathyroid | − | + | − | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | 41 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Islet cell adenoma | | | | | | | | | | | | | | | X | | | | | | | | | | | 1 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *48 |
| Mixed tumor, malignant | | | X | | X | | | | | | | | X | | | | | | | | | | | | | 5 |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Carcinoma, NOS, invasive | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **SPECIAL SENSE ORGANS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *48 |
| Adenoma, NOS | | | X | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **MUSCULOSKELETAL SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *48 |
| Sarcoma, NOS | | | | | | | | X | | | | | | | | | | | | | | | | | | 1 |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mediastinum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *48 |
| Follicular cell carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | X | | | | | 1 |
| Peritoneum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *48 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **ALL OTHER SYSTEMS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *48 |
| Malignant lymphoma, undifferentiated | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | X | | | | | | | | | | | | | | | 2 |
| Malignant lymphoma, mixed type | | | | | | | X | | X | X | | X | X | | X | | | X | X | | | | | | | 16 |

* Animals necropsied

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **Subcutaneous Tissue: Sarcoma** | | | |
| Overall Rates (a) | 1/50 (2%) | 3/50 (6%) | 1/48 (2%) |
| Adjusted Rates (b) | 2.6% | 9.5% | 2.0% |
| Terminal Rates (c) | 0/36 (0%) | 1/29 (3%) | 0/18 (0%) |
| Week of First Observation | 100 | 102 | 72 |
| Life Table Tests (d) | P=0.459 | P=0.251 | P=0.718 |
| Incidental Tumor Tests (d) | P=0.345N | P=0.476 | P=0.643N |
| Cochran-Armitage Trend Test (d) | P=0.596 | | |
| Fisher Exact Test (d) | | P=0.309 | P=0.742 |
| **Lung: Alveolar/Bronchiolar Adenoma** | | | |
| Overall Rates (a) | 1/50 (2%) | 2/47 (4%) | 3/47 (6%) |
| Adjusted Rates (b) | 2.8% | 6.9% | 11.2% |
| Terminal Rates (c) | 1/36 (3%) | 2/29 (7%) | 1/18 (6%) |
| Week of First Observation | 105 | 105 | 95 |
| Life Table Tests (d) | P=0.096 | P=0.424 | P=0.168 |
| Incidental Tumor Tests (d) | P=0.227 | P=0.424 | P=0.415 |
| Cochran-Armitage Trend Test (d) | P=0.204 | | |
| Fisher Exact Test (d) | | P=0.477 | P=0.285 |
| **Lung: Alveolar/Bronchiolar Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 3/50 (6%) | 2/47 (4%) | 3/47 (6%) |
| Adjusted Rates (b) | 8.3% | 6.9% | 11.2% |
| Terminal Rates (c) | 3/36 (8%) | 2/29 (7%) | 1/18 (6%) |
| Week of First Observation | 105 | 105 | 95 |
| Life Table Tests (d) | P=0.335 | P=0.599N | P=0.410 |
| Incidental Tumor Tests (d) | P=0.538 | P=0.599N | P=0.677 |
| Cochran-Armitage Trend Test (d) | P=0.559 | | |
| Fisher Exact Test (d) | | P=0.530N | P=0.631 |
| **Hematopoietic System: Malignant Lymphoma, Lymphocytic Type** | | | |
| Overall Rates (a) | 3/50 (6%) | 4/50 (8%) | 2/48 (4%) |
| Adjusted Rates (b) | 7.4% | 11.5% | 7.7% |
| Terminal Rates (c) | 2/36 (6%) | 2/29 (7%) | 1/18 (6%) |
| Week of First Observation | 63 | 86 | 79 |
| Life Table Tests (d) | P=0.571 | P=0.429 | P=0.680 |
| Incidental Tumor Tests (d) | P=0.563N | P=0.487 | P=0.671N |
| Cochran-Armitage Trend Test (d) | P=0.436N | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.520N |
| **Hematopoietic System: Malignant Lymphoma, Mixed Type** | | | |
| Overall Rates (a) | 13/50 (26%) | 7/50 (14%) | 17/48 (35%) |
| Adjusted Rates (b) | 33.1% | 20.2% | 58.7% |
| Terminal Rates (c) | 10/36 (28%) | 4/29 (14%) | 8/18 (44%) |
| Week of First Observation | 90 | 81 | 78 |
| Life Table Tests (d) | P=0.018 | P=0.218N | P=0.014 |
| Incidental Tumor Tests (d) | P=0.219 | P=0.094N | P=0.240 |
| Cochran-Armitage Trend Test (d) | P=0.175 | | |
| Fisher Exact Test (d) | | P=0.106N | P=0.214 |
| **Hematopoietic System: Lymphoma, All Malignant** | | | |
| Overall Rates (a) | 16/50 (32%) | 13/50 (26%) | 20/48 (42%) |
| Adjusted Rates (b) | 39.5% | 33.8% | 64.7% |
| Terminal Rates (c) | 12/36 (33%) | 6/29 (21%) | 9/18 (50%) |
| Week of First Observation | 63 | 81 | 78 |
| Life Table Tests (d) | P=0.019 | P=0.525N | P=0.013 |
| Incidental Tumor Tests (d) | P=0.254 | P=0.275N | P=0.232 |
| Cochran-Armitage Trend Test (d) | P=0.186 | | |
| Fisher Exact Test (d) | | P=0.330N | P=0.217 |

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **Liver: Hepatocellular Adenoma** | | | |
| Overall Rates (a) | 5/50 (10%) | 4/49 (8%) | 1/47 (2%) |
| Adjusted Rates (b) | 13.9% | 11.3% | 5.6% |
| Terminal Rates (c) | 5/36 (14%) | 2/29 (7%) | 1/18 (6%) |
| Week of First Observation | 105 | 81 | 105 |
| Life Table Tests (d) | P=0.233N | P=0.608N | P=0.325N |
| Incidental Tumor Tests (d) | P=0.202N | P=0.541N | P=0.325N |
| Cochran-Armitage Trend Test (d) | P=0.093N | | |
| Fisher Exact Test (d) | | P=0.513N | P=0.117N |
| **Liver: Hepatocellular Carcinoma** | | | |
| Overall Rates (a) | 1/50 (2%) | 0/49 (0%) | 4/47 (9%) |
| Adjusted Rates (b) | 2.8% | 0.0% | 9.6% |
| Terminal Rates (c) | 1/36 (3%) | 0/29 (0%) | 0/18 (0%) |
| Week of First Observation | 105 | | 75 |
| Life Table Tests (d) | P=0.064 | P=0.543N | P=0.138 |
| Incidental Tumor Tests (d) | P=0.098 | P=0.543N | P=0.217 |
| Cochran-Armitage Trend Test (d) | P=0.073 | | |
| Fisher Exact Test (d) | | P=0.505N | P=0.162 |
| **Liver: Hepatocellular Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 6/50 (12%) | 4/49 (8%) | 5/47 (11%) |
| Adjusted Rates (b) | 16.7% | 11.3% | 14.6% |
| Terminal Rates (c) | 6/36 (17%) | 2/29 (7%) | 1/18 (6%) |
| Week of First Observation | 105 | 81 | 75 |
| Life Table Tests (d) | P=0.431 | P=0.487N | P=0.448 |
| Incidental Tumor Tests (d) | P=0.535 | P=0.420N | P=0.554 |
| Cochran-Armitage Trend Test (d) | P=0.474N | | |
| Fisher Exact Test (d) | | P=0.383N | P=0.544N |
| **Anterior Pituitary Gland: Adenoma** | | | |
| Overall Rates (a) | 10/50 (20%) | 5/46 (11%) | 7/45 (16%) |
| Adjusted Rates (b) | 25.9% | 16.3% | 31.7% |
| Terminal Rates (c) | 8/36 (22%) | 4/29 (14%) | 4/17 (24%) |
| Week of First Observation | 90 | 97 | 90 |
| Life Table Tests (d) | P=0.413 | P=0.240N | P=0.395 |
| Incidental Tumor Tests (d) | P=0.387N | P=0.172N | P=0.507N |
| Cochran-Armitage Trend Test (d) | P=0.313N | | |
| Fisher Exact Test (d) | | P=0.171N | P=0.385N |
| **Anterior Pituitary Gland: Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 10/50 (20%) | 5/46 (11%) | 8/45 (18%) |
| Adjusted Rates (b) | 25.9% | 16.3% | 33.4% |
| Terminal Rates (c) | 8/36 (22%) | 4/29 (14%) | 4/17 (24%) |
| Week of First Observation | 90 | 97 | 88 |
| Life Table Tests (d) | P=0.308 | P=0.240N | P=0.299 |
| Incidental Tumor Tests (d) | P=0.493N | P=0.172N | P=0.601N |
| Cochran-Armitage Trend Test (d) | P=0.425N | | |
| Fisher Exact Test (d) | | P=0.171N | P=0.496N |
| **Thyroid Gland: Follicular Cell Adenoma** | | | |
| Overall Rates (a) | 0/50 (0%) | 4/47 (9%) | 3/47 (6%) |
| Adjusted Rates (b) | 0.0% | 11.2% | 13.6% |
| Terminal Rates (c) | 0/36 (0%) | 2/29 (7%) | 2/18 (11%) |
| Week of First Observation | | 74 | 94 |
| Life Table Tests (d) | P=0.051 | P=0.050 | P=0.050 |
| Incidental Tumor Tests (d) | P=0.074 | P=0.046 | P=0.093 |
| Cochran-Armitage Trend Test (d) | P=0.106 | | |
| Fisher Exact Test (d) | | P=0.051 | P=0.110 |

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **Thyroid Gland: Follicular Cell Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 2/50 (4%) | 4/47 (9%) | 3/47 (6%) |
| Adjusted Rates (b) | 5.6% | 11.2% | 13.6% |
| Terminal Rates (c) | 2/36 (6%) | 2/29 (7%) | 2/18 (11%) |
| Week of First Observation | 105 | 74 | 94 |
| Life Table Tests (d) | P=0.214 | P=0.269 | P=0.253 |
| Incidental Tumor Tests (d) | P=0.273 | P=0.263 | P=0.366 |
| Cochran-Armitage Trend Test (d) | P=0.386 | | |
| Fisher Exact Test (d) | | P=0.310 | P=0.470 |
| **Mammary Gland: Mixed Tumor, Malignant** | | | |
| Overall Rates (a) | 0/50 (0%) | 1/50 (2%) | 5/48 (10%) |
| Adjusted Rates (b) | 0.0% | 3.2% | 18.6% |
| Terminal Rates (c) | 0/36 (0%) | 0/29 (0%) | 1/18 (6%) |
| Week of First Observation | | 104 | 72 |
| Life Table Tests (d) | P=0.003 | P=0.470 | P=0.011 |
| Incidental Tumor Tests (d) | P=0.053 | P=0.638 | P=0.090 |
| Cochran-Armitage Trend Test (d) | P=0.009 | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.025 |
| **Mammary Gland: Mixed Tumor, Malignant or Acinar Cell Carcinoma** | | | |
| Overall Rates (a) | 0/50 (0%) | 2/50 (4%) | 5/48 (10%) |
| Adjusted Rates (b) | 0.0% | 6.1% | 18.6% |
| Terminal Rates (c) | 0/36 (0%) | 0/29 (0%) | 1/18 (6%) |
| Week of First Observation | | 99 | 72 |
| Life Table Tests (d) | P=0.005 | P=0.212 | P=0.011 |
| Incidental Tumor Tests (d) | P=0.102 | P=0.397 | P=0.090 |
| Cochran-Armitage Trend Test (d) | P=0.014 | | |
| Fisher Exact Test (d) | | P=0.247 | P=0.025 |
| **Uterus: Endometrial Stromal Polyp** | | | |
| Overall Rates (a) | 4/50 (8%) | 0/47 (0%) | 0/47 (0%) |
| Adjusted Rates (b) | 10.7% | 0.0% | 0.0% |
| Terminal Rates (c) | 3/36 (8%) | 0/29 (0%) | 0/18 (0%) |
| Week of First Observation | 98 | | |
| Life Table Tests (d) | P=0.039N | P=0.092N | P=0.165N |
| Incidental Tumor Tests (d) | P=0.013N | P=0.065N | P=0.067N |
| Cochran-Armitage Trend Test (d) | P=0.017N | | |
| Fisher Exact Test (d) | | P=0.066N | P=0.066N |
| **Harderian Gland: Adenoma** | | | |
| Overall Rates (a) | 4/50 (8%) | 2/50 (4%) | 1/48 (2%) |
| Adjusted Rates (b) | 10.3% | 6.9% | 4.3% |
| Terminal Rates (c) | 2/36 (6%) | 2/29 (7%) | 0/18 (0%) |
| Week of First Observation | 97 | 105 | 102 |
| Life Table Tests (d) | P=0.276N | P=0.431N | P=0.370N |
| Incidental Tumor Tests (d) | P=0.056N | P=0.297N | P=0.035N |
| Cochran-Armitage Trend Test (d) | P=0.126N | | |
| Fisher Exact Test (d) | | P=0.339N | P=0.194N |
| **Harderian Gland: Adenoma or Adenocarcinoma** | | | |
| Overall Rates (a) | 4/50 (8%) | 3/50 (6%) | 1/48 (2%) |
| Adjusted Rates (b) | 10.3% | 8.8% | 4.3% |
| Terminal Rates (c) | 2/36 (6%) | 2/29 (7%) | 0/18 (0%) |
| Week of First Observation | 97 | 68 | 102 |
| Life Table Tests (d) | P=0.288N | P=0.589N | P=0.370N |
| Incidental Tumor Tests (d) | P=0.077N | P=0.480N | P=0.035N |
| Cochran-Armitage Trend Test (d) | P=0.143N | | |
| Fisher Exact Test (d) | | P=0.500N | P=0.194N |

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Control | 700 ppm | 1,400 ppm |
|---|---|---|---|
| **All Sites: Benign Tumors** | | | |
| Overall Rates (a) | 23/50 (46%) | 19/50 (38%) | 16/48 (33%) |
| Adjusted Rates (b) | 55.8% | 51.6% | 61.0% |
| Terminal Rates (c) | 18/36 (50%) | 12/29 (41%) | 9/18 (50%) |
| Week of First Observation | 74 | 74 | 90 |
| Life Table Tests (d) | P=0.311 | P=0.561N | P=0.303 |
| Incidental Tumor Tests (d) | P=0.175N | P=0.306N | P=0.235N |
| Cochran-Armitage Trend Test (d) | P=0.118N | | |
| Fisher Exact Test (d) | | P=0.272N | P=0.141N |
| **All Sites: Malignant Tumors** | | | |
| Overall Rates (a) | 21/50 (42%) | 19/50 (38%) | 34/48 (71%) |
| Adjusted Rates (b) | 49.6% | 44.2% | 85.7% |
| Terminal Rates (c) | 15/36 (42%) | 6/29 (21%) | 13/18 (72%) |
| Week of First Observation | 63 | 68 | 72 |
| Life Table Tests (d) | P<0.001 | P=0.498 | P<0.001 |
| Incidental Tumor Tests (d) | P=0.027 | P=0.233N | P=0.008 |
| Cochran-Armitage Trend Test (d) | P=0.003 | | |
| Fisher Exact Test (d) | | P=0.419N | P=0.004 |
| **All Sites: All Tumors** | | | |
| Overall Rates (a) | 34/50 (68%) | 35/50 (70%) | 39/48 (81%) |
| Adjusted Rates (b) | 75.4% | 75.8% | 97.2% |
| Terminal Rates (c) | 25/36 (69%) | 18/29 (62%) | 17/18 (94%) |
| Week of First Observation | 63 | 37 | 72 |
| Life Table Tests (d) | P<0.001 | P=0.189 | P<0.001 |
| Incidental Tumor Tests (d) | P=0.116 | P=0.509N | P=0.060 |
| Cochran-Armitage Trend Test (d) | P=0.088 | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.101 |

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

**TABLE D4a. HISTORICAL INCIDENCE OF MAMMARY GLAND TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)**

| | Incidence in Controls | | |
|---|---|---|---|
| | Malignant Mixed Tumor | Adenocarcinoma | Malignant Mixed Tumor or Adenocarcinoma |
| No 2-year studies by SRI International are included in the historical data base. | | | |
| **Overall Historical Incidence** | | | |
| TOTAL | 9/2,040 (0.4%) | (d) 31/2,040 (1.5%) | (d) 40/2,040 (2.0%) |
| SD (b) | 1.33% | 1.85% | 2.34% |
| Range (c) | | | |
| High | 3/48 | 3/49 | 4/48 |
| Low | 0/50 | 0/50 | 0/50 |

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Includes one papillary adenocarcinoma

**TABLE D4b. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)**

| | Incidence in Controls | |
|---|---|---|
| | Lymphoma | Lymphoma or Leukemia |
| No 2-year studies by SRI International are included in the historical data base. | | |
| **Overall Historical Incidence** | | |
| TOTAL | 617/2,040 (30.2%) | 636/2,040 (31.2%) |
| SD (b) | 13.32% | 12.83% |
| Range (c) | | |
| High | 37/50 | 38/50 |
| Low | 5/50 | 6/50 |

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

**TABLE D4c. HISTORICAL INCIDENCE OF THYROID GLAND FOLLICULAR CELL TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)**

| | Incidence in Controls | | |
|---|---|---|---|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| No 2-year studies by SRI International are included in the historical data base. | | | |
| **Overall Historical Incidence** | | | |
| TOTAL | (b) 41/1,937 (2.1%) | 8/1,937 (0.4%) | (b) 49/1,937 (2.5%) |
| SD (c) | 2.58% | 1.17% | 3.22% |
| Range (d) | | | |
| High | 4/48 | 3/48 | 7/48 |
| Low | 0/50 | 0/50 | 0/50 |

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes 39 follicular cell adenomas, 2 cystadenomas, NOS, and 1 papillary cystadenoma, NOS
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 48 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 49 | 48 |
| INTEGUMENTARY SYSTEM | | | |
| None | | | |
| RESPIRATORY SYSTEM | | | |
| #Nasal cavity | (50) | (47) | (47) |
| Foreign body, NOS | | 1 (2%) | |
| Hemorrhage | 2 (4%) | 1 (2%) | |
| Inflammation, acute focal | 2 (4%) | 1 (2%) | 1 (2%) |
| Inflammation, chronic focal | | | 1 (2%) |
| #Lung | (50) | (47) | (47) |
| Mineralization | 1 (2%) | | 1 (2%) |
| Congestion, NOS | 6 (12%) | 3 (6%) | 6 (13%) |
| Edema, NOS | | 1 (2%) | |
| Hemorrhage | 2 (4%) | 7 (15%) | 2 (4%) |
| Inflammation, interstitial | 1 (2%) | 1 (2%) | 6 (13%) |
| Inflammation, suppurative | 2 (4%) | | 2 (4%) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 3 (6%) | 6 (13%) | 4 (9%) |
| Alveolar macrophages | 1 (2%) | | 2 (4%) |
| Hyperplasia, alveolar epithelium | 1 (2%) | 2 (4%) | |
| HEMATOPOIETIC SYSTEM | | | |
| *Harderian gland | (50) | (50) | (48) |
| Hyperplasia, lymphoid | | 1 (2%) | |
| *Multiple organs | (50) | (50) | (48) |
| Hyperplasia, lymphoid | 5 (10%) | 4 (8%) | 3 (6%) |
| #Bone marrow | (49) | (47) | (47) |
| Pigmentation, NOS | 1 (2%) | 3 (6%) | |
| Hyperplasia, granulocytic | 4 (8%) | 5 (11%) | 11 (23%) |
| #Spleen | (50) | (49) | (47) |
| Necrosis, focal | | | 1 (2%) |
| Amyloidosis | | | 1 (2%) |
| Angiectasis | | | 2 (4%) |
| Hyperplasia, lymphoid | 2 (4%) | 3 (6%) | |
| Hematopoiesis | 8 (16%) | 14 (29%) | 23 (49%) |
| #Lymph node | (50) | (48) | (47) |
| Pigmentation, NOS | 1 (2%) | | |
| Hyperplasia, plasma cell | 1 (2%) | 1 (2%) | 1 (2%) |
| Hyperplasia, lymphoid | 2 (4%) | 1 (2%) | |
| #Mandibular lymph node | (50) | (48) | (47) |
| Edema, NOS | | 1 (2%) | |
| Pigmentation, NOS | | 1 (2%) | |
| Hyperplasia, plasma cell | | | 1 (2%) |
| Hyperplasia, lymphoid | 2 (4%) | | 1 (2%) |
| Mastocytosis | | | 1 (2%) |
| #Thoracic lymph node | (50) | (48) | (47) |
| Hyperplasia, plasma cell | 3 (6%) | 1 (2%) | 5 (11%) |
| Hyperplasia, lymphoid | 4 (8%) | 4 (8%) | 1 (2%) |
| #Abdominal lymph node | (50) | (48) | (47) |
| Hyperplasia, plasma cell | | | 1 (2%) |
| #Hepatic lymph node | (50) | (48) | (47) |
| Hyperplasia, plasma cell | | | 1 (2%) |
| #Pancreatic lymph node | (50) | (48) | (47) |
| Inflammation, chronic focal | | | 1 (2%) |
| Hyperplasia, lymphoid | | 1 (2%) | |

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #Lumbar lymph node | (50) | (48) | (47) |
| Pigmentation, NOS | | | 1 (2%) |
| Hyperplasia, plasma cell | | | 2 (4%) |
| Hyperplasia, lymphoid | | | 1 (2%) |
| #Mesenteric lymph node | (50) | (48) | (47) |
| Cyst, NOS | | 1 (2%) | |
| Congestion, NOS | 2 (4%) | | 2 (4%) |
| Edema, NOS | 1 (2%) | | |
| Hemorrhage | | 2 (4%) | 2 (4%) |
| Inflammation, chronic focal | | | 1 (2%) |
| Angiectasis | 2 (4%) | 9 (19%) | 6 (13%) |
| Hyperplasia, plasma cell | | 1 (2%) | 1 (2%) |
| Hyperplasia, reticulum cell | | | 1 (2%) |
| Hyperplasia, lymphoid | 6 (12%) | 4 (8%) | 2 (4%) |
| Hematopoiesis | 2 (4%) | | 4 (9%) |
| #Renal lymph node | (50) | (48) | (47) |
| Inflammation, suppurative | | | 1 (2%) |
| Hyperplasia, plasma cell | 1 (2%) | 1 (2%) | 3 (6%) |
| Hyperplasia, lymphoid | | 1 (2%) | 1 (2%) |
| #Sacral lymph node | (50) | (48) | (47) |
| Hyperplasia, plasma cell | | | 1 (2%) |
| #Inguinal lymph node | (50) | (48) | (47) |
| Inflammation, suppurative | | | 1 (2%) |
| Hyperplasia, plasma cell | | | 4 (9%) |
| #Lung | (50) | (47) | (47) |
| Hyperplasia, lymphoid | 2 (4%) | 2 (4%) | |
| #Liver | (50) | (49) | (47) |
| Hematopoiesis | 4 (8%) | 5 (10%) | 14 (30%) |
| #Pancreas | (49) | (48) | (46) |
| Hematopoiesis | | | 1 (2%) |
| #Peyer's patch | (47) | (46) | (45) |
| Hyperplasia, lymphoid | | 3 (7%) | 1 (2%) |
| #Kidney | (50) | (49) | (47) |
| Hyperplasia, lymphoid | 1 (2%) | | |
| Hematopoiesis | | | 1 (2%) |
| #Adrenal cortex | (49) | (49) | (47) |
| Hematopoiesis | 2 (4%) | 1 (2%) | 8 (17%) |
| #Thymus | (38) | (37) | (26) |
| Cyst, NOS | 2 (5%) | 1 (3%) | |
| Involution, NOS | 7 (18%) | 6 (16%) | 4 (15%) |
| Hyperplasia, epithelial | | | 1 (4%) |
| Angiectasis | | 2 (5%) | |
| Hyperplasia, lymphoid | 1 (3%) | | |
| CIRCULATORY SYSTEM | | | |
| #Heart | (50) | (48) | (47) |
| Mineralization | 1 (2%) | | 1 (2%) |
| Endocarditis, bacterial | | | 1 (2%) |
| Inflammation, suppurative | | | 2 (4%) |
| Inflammation, acute focal | | | 1 (2%) |
| Inflammation, chronic focal | 21 (42%) | 24 (50%) | 25 (53%) |
| *Aortic arch | (50) | (50) | (48) |
| Mineralization | | | 2 (4%) |
| Metaplasia, cartilaginous | | | 1 (2%) |
| *Vena cava | (50) | (50) | (48) |
| Mineralization | | | 2 (4%) |

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| DIGESTIVE SYSTEM | | | |
| #Salivary gland | (49) | (46) | (47) |
| Inflammation, chronic focal | 17 (35%) | 18 (39%) | 11 (23%) |
| Hyperplasia, epithelial | 1 (2%) | | |
| #Liver | (50) | (49) | (47) |
| Mineralization | | | 1 (2%) |
| Cyst, NOS | 1 (2%) | | |
| Congestion, NOS | | 1 (2%) | |
| Hemorrhage | | 1 (2%) | |
| Hemorrhagic cyst | 1 (2%) | | |
| Inflammation, suppurative | | | 1 (2%) |
| Inflammation, acute focal | 2 (4%) | | |
| Inflammation, chronic focal | 20 (40%) | 24 (49%) | 13 (28%) |
| Necrosis, focal | 3 (6%) | 4 (8%) | 3 (6%) |
| Infarct, NOS | 1 (2%) | | |
| Amyloidosis | | 1 (2%) | |
| Pigmentation, NOS | 1 (2%) | | |
| #Liver/centrilobular | (50) | (49) | (47) |
| Degeneration, NOS | | 1 (2%) | |
| #Liver/hepatocytes | (50) | (49) | (47) |
| Cytoplasmic vacuolization | 5 (10%) | 2 (4%) | 2 (4%) |
| Basophilic cyto change | 2 (4%) | 1 (2%) | |
| Eosinophilic cyto change | 3 (6%) | | 1 (2%) |
| Clear cell change | 1 (2%) | 1 (2%) | 1 (2%) |
| Atrophy, NOS | | | 1 (2%) |
| *Gallbladder | (50) | (50) | (48) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 2 (4%) | 2 (4%) | 3 (6%) |
| #Bile duct | (50) | (49) | (47) |
| Hyperplasia, focal | | | 1 (2%) |
| #Pancreas | (49) | (48) | (46) |
| Inflammation, suppurative | | | 1 (2%) |
| Inflammation, chronic focal | 15 (31%) | 17 (35%) | 5 (11%) |
| #Pancreatic acinus | (49) | (48) | (46) |
| Amyloidosis, focal | | | 1 (2%) |
| Basophilic cyto change | | | 2 (4%) |
| Atrophy, focal | 2 (4%) | | |
| #Esophagus | (50) | (46) | (46) |
| Hyperplasia, epithelial | | 1 (2%) | |
| #Glandular stomach | (47) | (46) | (46) |
| Mineralization | 1 (2%) | | 2 (4%) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | 1 (2%) | 2 (4%) | 2 (4%) |
| Atrophy, focal | 2 (4%) | 1 (2%) | 1 (2%) |
| Hyperplasia, epithelial | | | 1 (2%) |
| #Forestomach | (47) | (46) | (46) |
| Cyst, NOS | 1 (2%) | | |
| Ulcer, NOS | 1 (2%) | 2 (4%) | |
| Inflammation, acute focal | 2 (4%) | 1 (2%) | |
| Inflammation, chronic focal | 1 (2%) | 2 (4%) | |
| Hyperplasia, epithelial | 1 (2%) | 8 (17%) | 1 (2%) |
| Hyperkeratosis | | 1 (2%) | |
| #Small intestine | (47) | (46) | (45) |
| Amyloidosis | | 4 (9%) | 9 (20%) |
| URINARY SYSTEM | | | |
| #Urinary bladder/cavity | (50) | (47) | (44) |
| Dilatation, NOS | | 2 (4%) | 1 (2%) |
| #Kidney | (50) | (49) | (47) |
| Mineralization | | 1 (2%) | 4 (9%) |
| Cyst, NOS | | | 1 (2%) |
| Inflammation, suppurative | | | 1 (2%) |

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| URINARY SYSTEM | | | |
| #Kidney (Continued) | (50) | (49) | (47) |
| Inflammation, chronic focal | 23 (46%) | 15 (31%) | 10 (21%) |
| Nephropathy | 11 (22%) | 46 (94%) | 47 (100%) |
| Glomerulosclerosis, NOS | 1 (2%) | | |
| Necrosis, focal | | 1 (2%) | 1 (2%) |
| Infarct, NOS | 4 (8%) | 1 (2%) | 1 (2%) |
| Amyloidosis | | 3 (6%) | |
| Hyperplasia, tubular cell | | | 2 (4%) |
| Metaplasia, osseous | 3 (6%) | 1 (2%) | 4 (9%) |
| #Kidney/tubule | (50) | (49) | (47) |
| Degeneration, NOS | | 4 (8%) | |
| #Kidney/pelvis | (50) | (49) | (47) |
| Dilatation, NOS | 1 (2%) | 3 (6%) | 17 (36%) |
| Hyperplasia, epithelial | | | 1 (2%) |
| #Urinary bladder/submucosa | (50) | (47) | (44) |
| Hemorrhage | | 1 (2%) | |
| Inflammation, chronic focal | 23 (46%) | 25 (53%) | 19 (43%) |
| ENDOCRINE SYSTEM | | | |
| #Pituitary intermedia | (50) | (46) | (45) |
| Hyperplasia, focal | 1 (2%) | 1 (2%) | |
| #Anterior pituitary | (50) | (46) | (45) |
| Cyst, NOS | 1 (2%) | 2 (4%) | 2 (4%) |
| Congestion, NOS | 1 (2%) | | 1 (2%) |
| Hyperplasia, focal | 23 (46%) | 21 (46%) | 18 (40%) |
| Angiectasis | 3 (6%) | 1 (2%) | |
| #Adrenal/capsule | (49) | (49) | (47) |
| Cyst, NOS | | | 1 (2%) |
| Hyperplasia, focal | 45 (92%) | 42 (86%) | 45 (96%) |
| #Adrenal cortex | (49) | (49) | (47) |
| Congestion, NOS | 2 (4%) | 1 (2%) | 2 (4%) |
| Hemorrhage | 1 (2%) | 2 (4%) | 1 (2%) |
| Inflammation, suppurative | | | 1 (2%) |
| Inflammation, chronic focal | 1 (2%) | | |
| Degeneration, NOS | 2 (4%) | 1 (2%) | 1 (2%) |
| Necrosis, focal | 1 (2%) | | |
| Pigmentation, NOS | | | 1 (2%) |
| Focal cellular change | | | 1 (2%) |
| Atrophy, NOS | | | 1 (2%) |
| Hyperplasia, focal | 7 (14%) | | 2 (4%) |
| Angiectasis | | | 1 (2%) |
| #Adrenal medulla | (49) | (49) | (47) |
| Hyperplasia, NOS | 3 (6%) | | 3 (6%) |
| #Thyroid | (50) | (47) | (47) |
| Cyst, NOS | | | 1 (2%) |
| Follicular cyst, NOS | 9 (18%) | 6 (13%) | 3 (6%) |
| Inflammation, acute focal | | | 1 (2%) |
| Inflammation, chronic focal | 6 (12%) | 2 (4%) | 1 (2%) |
| Hyperplasia, follicular cell | 2 (4%) | 7 (15%) | 5 (11%) |
| #Parathyroid | (37) | (37) | (41) |
| Cyst, NOS | 1 (3%) | | |
| Inflammation, chronic focal | 1 (3%) | | |
| Hyperplasia, NOS | | | 1 (2%) |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (48) |
| Hyperplasia, cystic | 10 (20%) | 10 (20%) | 12 (25%) |
| *Vaginal canal | (50) | (50) | (48) |
| Inflammation, suppurative | 1 (2%) | | |

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| REPRODUCTIVE SYSTEM (Continued) | | | |
| *Vagina | (50) | (50) | (48) |
| Hyperplasia, epithelial | 1 (2%) | | |
| #Uterus | (50) | (47) | (47) |
| Dilatation, NOS | 11 (22%) | 13 (28%) | 10 (21%) |
| Hydrometra | 1 (2%) | | 1 (2%) |
| Cyst, NOS | 1 (2%) | | |
| Inflammation, suppurative | 2 (4%) | | 8 (17%) |
| Pigmentation, NOS | | | 1 (2%) |
| Angiectasis | 2 (4%) | 1 (2%) | 2 (4%) |
| #Uterus/endometrium | (50) | (47) | (47) |
| Hyperplasia, NOS | 44 (88%) | 39 (83%) | 36 (77%) |
| Angiectasis | 1 (2%) | | |
| #Ovary | (50) | (47) | (47) |
| Mineralization | 1 (2%) | 1 (2%) | |
| Cyst, NOS | 16 (32%) | 18 (38%) | 10 (21%) |
| Hemorrhage | 2 (4%) | | |
| Hemorrhagic cyst | 2 (4%) | 3 (6%) | 2 (4%) |
| Inflammation, suppurative | 2 (4%) | 4 (9%) | 10 (21%) |
| Abscess, NOS | | 1 (2%) | 1 (2%) |
| Inflammation, chronic focal | | 1 (2%) | 1 (2%) |
| Pigmentation, NOS | | 2 (4%) | |
| Angiectasis | | 1 (2%) | 3 (6%) |
| NERVOUS SYSTEM | | | |
| #Brain/meninges | (48) | (46) | (47) |
| Inflammation, suppurative | | 1 (2%) | |
| Inflammation, chronic focal | 4 (8%) | 1 (2%) | 1 (2%) |
| Pigmentation, NOS | 1 (2%) | | |
| #Brain | (48) | (46) | (47) |
| Mineralization | 13 (27%) | 22 (48%) | 34 (72%) |
| Hydrocephalus, NOS | | | 1 (2%) |
| Cyst, NOS | | 1 (2%) | |
| Hemorrhage | | | 1 (2%) |
| Inflammation, acute focal | | | 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| *Eye/cornea | (50) | (50) | (48) |
| Inflammation, chronic focal | 1 (2%) | | |
| *Eye/retina | (50) | (50) | (48) |
| Atrophy, diffuse | 1 (2%) | | |
| *Eye/crystalline lens | (50) | (50) | (48) |
| Degeneration, NOS | 1 (2%) | | |
| *Nasolacrimal duct | (50) | (50) | (48) |
| Hemorrhage | | 2 (4%) | |
| Inflammation, acute focal | 1 (2%) | | 2 (4%) |
| *Harderian gland | (50) | (50) | (48) |
| Inflammation, chronic focal | | 1 (2%) | |
| Hyperplasia, NOS | 1 (2%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| *Bone | (50) | (50) | (48) |
| Fibrous osteodystrophy | 38 (76%) | 41 (82%) | 41 (85%) |
| *Muscle of trunk | (50) | (50) | (48) |
| Degeneration, NOS | 1 (2%) | | |

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE (Continued)

| | Untreated Control | Low Dose | High Dose |
|---|---|---|---|
| BODY CAVITIES | | | |
| *Thoracic cavity | (50) | (50) | (48) |
| Inflammation, suppurative | 2 (4%) | | |
| *Mediastinum | (50) | (50) | (48) |
| Hemorrhage | 1 (2%) | | |
| Inflammation, suppurative | | | 5 (10%) |
| *Abdominal cavity | (50) | (50) | (48) |
| Cyst, NOS | 1 (2%) | | |
| Hemorrhage | 1 (2%) | | |
| Inflammation, suppurative | 3 (6%) | 2 (4%) | 10 (21%) |
| Inflammation, chronic focal | | 3 (6%) | 1 (2%) |
| Necrosis, fat | 5 (10%) | 2 (4%) | |
| ALL OTHER SYSTEMS | | | |
| *Multiple organs | (50) | (50) | (48) |
| Inflammation, suppurative | | 1 (2%) | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| Auto/necropsy/no histo | | 1 | |
| Autolysis/no necropsy | | | 2 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

# APPENDIX E

# SENTINEL ANIMAL PROGRAM


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# APPENDIX E. SENTINEL ANIMAL PROGRAM

## I. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Fifteen B6C3F$_1$ mice and 15 F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Data from animals surviving 24 months were collected from 5/50 randomly selected control animals of each sex and species. The blood from each animal was collected and clotted, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the antibody titers. The following tests were performed:

| | Hemagglutination Inhibition | Complement Fixation | ELISA |
|---|---|---|---|
| Mice | PVM (pneumonia virus of mice)<br>Reo 3 (reovirus type 3)<br>GDVII (Theiler's encephalomyelitis virus)<br>Poly (polyoma virus)<br>MVM (minute virus of mice)<br>Ectro (infectious ectromelia)<br>Sendai (6,12,24 mo) | M. Ad. (mouse adenovirus)<br>LCM (lymphocytic choriomeningitis virus)<br>MHV (6 mo)<br>Sendai (18 mo) | MHV (mouse hepatitis virus) (12,18,24 mo)<br>*M. pul.* (*Mycoplasma pulmonis*) (24 mo) |
| Rats | PVM<br>KRV (Kilham rat virus)<br>H-1 (Toolan's H-1 virus)<br>Sendai (6,12,24 mo) | RCV (rat coronavirus) (6,12,18 mo)<br>Sendai (18 mo) | RCV/SDA (sialodacryoadenitis virus) (24 mo)<br>*M. pul.* (24 mo) |

## II. Results

Results are presented in Table E1.

TABLE E1. MURINE ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE (a)

| Interval (months) | Number of Animals | Positive Serologic Reaction for |
|---|---|---|
| **RATS** | | |
| 6 | -- | None positive |
| 12 | -- | None positive |
| 18 | -- | None positive |
| 24 | 8/10 | *M. pul.* (b) |
| **MICE** | | |
| 6 | -- | None positive |
| 12 | -- | None positive |
| 18 | 1/10 | MHV |
| 24 | 1/10 | *M. pul.* (b) |

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the control animals just before they were killed; samples were sent to Microbiological Associates (Bethesda, MD) for determination of antibody titers.
(b) Further evaluation of this assay indicated that it was not specific for *M. pulmonis*, and this result was considered to be a false positive.

# APPENDIX F

# FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF FUROSEMIDE


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TABLE F1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | | Low Dose | | | | High Dose | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Week | Grams Feed/ Day (a) | Body Weight (grams) | Grams Feed/ Day (a) | Body Weight (grams) | Low/ Control (b) | Dose/ Day (c) | Grams Feed/ Day (a) | Body Weight (grams) | High/ Control (b) | Dose/ Day (c) |
| 2 | 20 | 217 | 19 | 216 | 1.0 | 31 | 19 | 211 | 1.0 | 63 |
| 6 | 20 | 301 | 19 | 302 | 1.0 | 22 | 19 | 298 | 1.0 | 45 |
| 11 | 19 | 360 | 19 | 364 | 1.0 | 18 | 18 | 359 | 0.9 | 35 |
| 16 | 16 | 393 | 18 | 402 | 1.1 | 16 | 18 | 394 | 1.1 | 32 |
| 20 | 17 | 413 | 17 | 421 | 1.0 | 14 | 17 | 411 | 1.0 | 29 |
| 24 | 18 | 433 | 18 | 444 | 1.0 | 14 | 18 | 433 | 1.0 | 29 |
| 28 | 17 | 446 | 17 | 455 | 1.0 | 13 | 18 | 445 | 1.1 | 28 |
| 32 | 18 | 463 | 18 | 472 | 1.0 | 13 | 18 | 461 | 1.0 | 27 |
| 37 | 18 | 472 | 19 | 485 | 1.1 | 14 | 18 | 473 | 1.0 | 27 |
| 42 | 18 | 490 | 18 | 501 | 1.0 | 13 | 18 | 490 | 1.0 | 26 |
| 46 | 17 | 490 | 17 | 502 | 1.0 | 12 | 18 | 490 | 1.1 | 26 |
| 50 | 17 | 495 | 18 | 509 | 1.1 | 12 | 17 | 499 | 1.0 | 24 |
| 54 | 18 | 502 | 18 | 516 | 1.0 | 12 | 18 | 504 | 1.0 | 25 |
| 58 | 17 | 503 | 18 | 516 | 1.1 | 12 | 18 | 504 | 1.1 | 25 |
| 62 | 17 | 506 | 17 | 516 | 1.0 | 12 | 18 | 500 | 1.1 | 25 |
| 67 | 16 | 499 | 16 | 510 | 1.0 | 11 | 17 | 496 | 1.1 | 24 |
| 71 | 16 | 499 | 16 | 512 | 1.0 | 11 | 16 | 491 | 1.0 | 23 |
| 75 | 17 | 500 | 17 | 513 | 1.0 | 12 | 17 | 492 | 1.0 | 24 |
| 79 | 17 | 496 | 16 | 505 | 0.9 | 11 | 17 | 488 | 1.0 | 24 |
| 83 | 16 | 493 | 15 | 496 | 0.9 | 11 | 16 | 480 | 1.0 | 23 |
| 90 | 16 | 485 | 16 | 487 | 1.0 | 11 | 17 | 471 | 1.1 | 25 |
| 93 | 17 | 475 | 17 | 486 | 1.0 | 12 | 16 | 463 | 0.9 | 24 |
| 98 | 16 | 449 | 17 | 467 | 1.1 | 13 | 17 | 454 | 1.1 | 26 |
| 103 | 19 | 443 | 18 | 462 | 0.9 | 14 | 17 | 430 | 0.9 | 28 |
| Mean | 17.4 | 451 | 17.4 | 461 | 1.0 | 14 | 17.5 | 447 | 1.0 | 29 |
| SD (d) | 1.2 | | 1.1 | | 0.1 | 4 | 0.8 | | 0.1 | 9 |
| CV (e) | 6.9 | | 6.3 | | 10.0 | 28.6 | 4.6 | | 10.0 | 31.0 |

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of furosemide consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE F2. FEED AND COMPOUND CONSUMPTION BY FEMALE RATS IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| Week | Control | | Low Dose | | | | High Dose | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Grams Feed/ Day (a) | Body Weight (grams) | Grams Feed/ Day (a) | Body Weight (grams) | Low/ Control (b) | Dose/ Day (c) | Grams Feed/ Day (a) | Body Weight (grams) | High/ Control (b) | Dose/ Day (c) |
| 2 | 14 | 153 | 13 | 150 | 0.9 | 30 | 13 | 147 | 0.9 | 62 |
| 6 | 13 | 189 | 13 | 188 | 1.0 | 24 | 13 | 185 | 1.0 | 49 |
| 11 | 12 | 211 | 12 | 211 | 1.0 | 20 | 12 | 210 | 1.0 | 40 |
| 16 | 11 | 224 | 11 | 223 | 1.0 | 17 | 11 | 221 | 1.0 | 35 |
| 20 | 10 | 229 | 11 | 228 | 1.1 | 17 | 11 | 225 | 1.1 | 34 |
| 24 | 11 | 237 | 11 | 236 | 1.0 | 16 | 11 | 235 | 1.0 | 33 |
| 28 | 11 | 241 | 11 | 242 | 1.0 | 16 | 12 | 241 | 1.1 | 35 |
| 32 | 11 | 250 | 11 | 252 | 1.0 | 15 | 11 | 251 | 1.0 | 31 |
| 37 | 11 | 257 | 11 | 258 | 1.0 | 15 | 11 | 258 | 1.0 | 30 |
| 42 | 11 | 269 | 12 | 272 | 1.1 | 15 | 12 | 271 | 1.1 | 31 |
| 46 | 11 | 269 | 11 | 271 | 1.0 | 14 | 11 | 269 | 1.0 | 29 |
| 50 | 11 | 279 | 12 | 282 | 1.1 | 15 | 12 | 280 | 1.1 | 30 |
| 54 | 12 | 294 | 13 | 294 | 1.1 | 15 | 13 | 294 | 1.1 | 31 |
| 58 | 12 | 305 | 12 | 304 | 1.0 | 14 | 13 | 307 | 1.1 | 30 |
| 62 | 12 | 317 | 13 | 317 | 1.1 | 14 | 13 | 318 | 1.1 | 29 |
| 67 | 12 | 327 | 12 | 325 | 1.0 | 13 | 12 | 331 | 1.0 | 25 |
| 71 | 12 | 339 | 13 | 336 | 1.1 | 14 | 12 | 338 | 1.0 | 25 |
| 75 | 12 | 341 | 13 | 341 | 1.1 | 13 | 13 | 341 | 1.1 | 27 |
| 79 | 12 | 349 | 12 | 344 | 1.0 | 12 | 12 | 344 | 1.0 | 24 |
| 83 | 13 | 358 | 13 | 352 | 1.0 | 13 | 13 | 349 | 1.0 | 26 |
| 90 | 13 | 369 | 13 | 362 | 1.0 | 13 | 13 | 359 | 1.0 | 25 |
| 93 | 13 | 369 | 13 | 362 | 1.0 | 13 | 12 | 358 | 0.9 | 23 |
| 98 | 13 | 371 | 12 | 364 | 0.9 | 12 | 12 | 364 | 0.9 | 23 |
| 103 | 14 | 365 | 14 | 369 | 1.0 | 13 | 13 | 350 | 0.9 | 26 |
| Mean | 12.0 | 288 | 12.2 | 287 | 1.0 | 16 | 12.1 | 285 | 1.0 | 31 |
| SD (d) | 1.0 | | 0.9 | | 0.1 | 4 | 0.8 | | 0.1 | 9 |
| CV (e) | 8.3 | | 7.4 | | 10.0 | 25.0 | 6.6 | | 10.0 | 29.0 |

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of furosemide consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE F3. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| Week | Control | | Low Dose | | | | High Dose | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Grams Feed/ Day (a) | Body Weight (grams) | Grams Feed/ Day (a) | Body Weight (grams) | Low/ Control (b) | Dose/ Day (c) | Grams Feed/ Day (a) | Body Weight (grams) | High/ Control (b) | Dose/ Day (c) |
| 1 | 3.3 | 22.8 | 3.9 | 23.2 | 1.2 | 118 | 3.9 | 23.2 | 1.2 | 235 |
| 6 | 4.0 | 30.0 | 4.3 | 29.5 | 1.1 | 102 | 4.0 | 27.8 | 1.0 | 201 |
| 11 | 4.2 | 32.9 | 4.1 | 31.0 | 1.0 | 93 | 4.2 | 30.6 | 1.0 | 192 |
| 15 | 5.2 | 33.4 | 5.5 | 32.5 | 1.1 | 118 | 5.8 | 32.5 | 1.1 | 250 |
| 19 | 4.3 | 34.7 | 4.3 | 33.8 | 1.0 | 89 | 4.2 | 32.5 | 1.0 | 181 |
| 23 | 4.4 | 35.8 | 4.7 | 33.9 | 1.1 | 97 | 4.7 | 32.7 | 1.1 | 201 |
| 27 | 4.3 | 37.3 | 4.6 | 36.4 | 1.1 | 88 | 4.5 | 33.3 | 1.0 | 189 |
| 31 | 4.2 | 38.8 | 4.6 | 36.5 | 1.1 | 88 | 4.5 | 34.0 | 1.1 | 185 |
| 36 | 4.8 | 38.7 | 5.1 | 36.9 | 1.1 | 97 | 5.0 | 34.4 | 1.0 | 203 |
| 41 | 4.5 | 40.6 | 5.0 | 38.5 | 1.1 | 91 | 4.8 | 35.9 | 1.1 | 187 |
| 45 | 4.6 | 39.7 | 4.7 | 37.1 | 1.0 | 89 | 4.5 | 34.4 | 1.0 | 183 |
| 49 | 4.3 | 40.6 | 4.6 | 38.7 | 1.1 | 83 | 4.5 | 35.4 | 1.0 | 178 |
| 53 | 4.4 | 40.9 | 4.6 | 38.7 | 1.0 | 83 | 4.5 | 34.6 | 1.0 | 182 |
| 57 | 4.0 | 40.8 | 4.3 | 37.8 | 1.1 | 80 | 4.5 | 34.2 | 1.1 | 184 |
| 61 | 4.2 | 40.3 | 4.5 | 36.9 | 1.1 | 85 | 4.6 | 34.4 | 1.1 | 187 |
| 66 | 4.2 | 41.3 | 4.6 | 38.6 | 1.1 | 83 | 4.3 | 34.7 | 1.0 | 173 |
| 70 | 4.0 | 40.6 | 4.4 | 38.4 | 1.1 | 80 | 4.2 | 34.6 | 1.1 | 170 |
| 74 | 4.3 | 40.6 | 4.6 | 38.5 | 1.1 | 84 | 4.6 | 34.9 | 1.1 | 185 |
| 78 | 4.2 | 39.8 | 5.1 | 38.8 | 1.2 | 92 | 4.7 | 34.8 | 1.1 | 189 |
| 82 | 4.4 | 41.3 | 4.5 | 38.3 | 1.0 | 82 | 4.2 | 34.8 | 1.0 | 169 |
| 89 | 4.4 | 39.8 | 4.2 | 37.5 | 1.0 | 78 | 4.5 | 33.2 | 1.0 | 190 |
| 92 | 4.6 | 40.7 | 4.9 | 38.5 | 1.1 | 89 | 4.6 | 34.5 | 1.0 | 187 |
| 97 | 4.2 | 39.9 | 4.8 | 36.1 | 1.1 | 93 | 4.2 | 33.1 | 1.0 | 178 |
| 101 | 4.6 | 38.4 | 4.8 | 36.3 | 1.0 | 93 | 4.1 | 32.2 | 0.9 | 178 |
| 104 | 4.8 | 36.7 | 5.2 | 34.6 | 1.1 | 105 | 5.0 | 31.0 | 1.0 | 226 |
| Mean | 4.3 | 37.9 | 4.6 | 35.9 | 1.1 | 91 | 4.5 | 33.1 | 1.0 | 191 |
| SD (d) | 0.4 | | 0.4 | | 0.1 | 11 | 0.4 | | 0.1 | 20 |
| CV (e) | 9.3 | | 8.7 | | 9.1 | 12.1 | 8.9 | | 10.0 | 10.5 |

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of furosemide consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE F4. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF FUROSEMIDE

| | Control | | Low Dose | | | | High Dose | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Week | Grams Feed/ Day (a) | Body Weight (grams) | Grams Feed/ Day (a) | Body Weight (grams) | Low/ Control (b) | Dose/ Day (c) | Grams Feed/ Day (a) | Body Weight (grams) | High/ Control (b) | Dose/ Day (c) |
| 1 | 3.7 | 19.6 | 3.6 | 19.1 | 1.0 | 132 | 3.7 | 18.6 | 1.0 | 278 |
| 6 | 3.6 | 23.5 | 3.7 | 23.2 | 1.0 | 112 | 3.7 | 22.8 | 1.0 | 227 |
| 11 | 3.7 | 25.0 | 3.8 | 24.8 | 1.0 | 107 | 3.9 | 24.9 | 1.1 | 219 |
| 15 | 4.9 | 26.4 | 5.1 | 26.5 | 1.0 | 135 | 5.2 | 26.6 | 1.1 | 274 |
| 19 | 4.3 | 27.6 | 3.7 | 27.4 | 0.9 | 95 | 3.9 | 27.1 | 0.9 | 201 |
| 23 | 4.6 | 28.6 | 4.5 | 27.9 | 1.0 | 113 | 4.7 | 27.8 | 1.0 | 237 |
| 27 | 4.3 | 29.6 | 4.6 | 29.1 | 1.1 | 111 | 4.4 | 28.1 | 1.0 | 219 |
| 31 | 4.3 | 31.0 | 4.5 | 30.3 | 1.0 | 104 | 3.9 | 29.5 | 0.9 | 185 |
| 36 | 4.9 | 32.5 | 4.9 | 30.9 | 1.0 | 111 | 5.1 | 30.7 | 1.0 | 233 |
| 41 | 4.6 | 33.7 | 5.0 | 33.0 | 1.1 | 106 | 4.5 | 32.4 | 1.0 | 194 |
| 45 | 4.6 | 34.6 | 4.5 | 33.3 | 1.0 | 95 | 4.4 | 32.1 | 1.0 | 192 |
| 49 | 4.5 | 36.1 | 4.6 | 35.4 | 1.0 | 91 | 4.7 | 33.7 | 1.0 | 195 |
| 53 | 4.2 | 36.3 | 4.4 | 36.2 | 1.0 | 85 | 4.5 | 34.3 | 1.1 | 184 |
| 57 | 4.1 | 36.6 | 4.3 | 36.3 | 1.0 | 83 | 4.5 | 34.6 | 1.1 | 182 |
| 61 | 4.3 | 36.8 | 4.3 | 36.0 | 1.0 | 84 | 4.5 | 34.2 | 1.0 | 184 |
| 66 | 4.4 | 39.1 | 4.6 | 37.5 | 1.0 | 86 | 4.5 | 34.8 | 1.0 | 181 |
| 70 | 4.2 | 39.6 | 4.3 | 37.9 | 1.0 | 79 | 4.2 | 34.7 | 1.0 | 169 |
| 74 | 4.6 | 39.6 | 4.8 | 38.6 | 1.0 | 87 | 4.9 | 35.2 | 1.1 | 195 |
| 78 | 4.4 | 40.0 | 4.7 | 38.2 | 1.1 | 86 | 5.0 | 35.1 | 1.1 | 199 |
| 82 | 4.5 | 40.5 | 4.6 | 38.4 | 1.0 | 84 | 4.5 | 35.3 | 1.0 | 178 |
| 89 | 4.5 | 40.7 | 5.1 | 38.4 | 1.1 | 93 | 4.9 | 33.4 | 1.1 | 205 |
| 92 | 5.0 | 42.6 | 5.2 | 38.7 | 1.0 | 94 | 5.2 | 33.6 | 1.0 | 217 |
| 97 | 4.5 | 41.5 | 4.9 | 36.9 | 1.1 | 93 | 5.7 | 33.0 | 1.3 | 242 |
| 101 | 5.0 | 41.7 | 5.1 | 36.9 | 1.0 | 97 | 6.4 | 32.5 | 1.3 | 276 |
| 104 | 5.1 | 39.9 | 5.3 | 34.8 | 1.0 | 107 | 6.1 | 31.4 | 1.2 | 272 |
| Mean | 4.4 | 34.5 | 4.6 | 33.0 | 1.0 | 99 | 4.7 | 31.1 | 1.1 | 214 |
| SD (d) | 0.4 | | 0.5 | | 0.1 | 15 | 0.7 | | 0.1 | 34 |
| CV (e) | 9.1 | | 10.9 | | 10.0 | 15.2 | 14.9 | | 9.1 | 15.9 |

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of furosemide consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

# APPENDIX G

# INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

## Meal Diet: April 1981 to April 1983

**(Manufactured by Zeigler Bros., Inc., Gardners, PA)**


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TABLE G1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

| Ingredients (b) | Percent by Weight |
|---|---|
| Ground #2 yellow shelled corn | 24.50 |
| Ground hard winter wheat | 23.00 |
| Soybean meal (49% protein) | 12.00 |
| Fish meal (60% protein) | 10.00 |
| Wheat middlings | 10.00 |
| Dried skim milk | 5.00 |
| Alfalfa meal (dehydrated, 17% protein) | 4.00 |
| Corn gluten meal (60% protein) | 3.00 |
| Soy oil | 2.50 |
| Dried brewer's yeast | 2.00 |
| Dry molasses | 1.50 |
| Dicalcium phosphate | 1.25 |
| Ground limestone | 0.50 |
| Salt | 0.50 |
| Premixes (vitamin and mineral) | 0.25 |

(a) NIH, 1978; NCI, 1976
(b) Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE G2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

| | Amount | Source |
|---|---|---|
| **Vitamins** | | |
| A | 5,500,000 IU | Stabilized vitamin A palmitate or acetate |
| $D_3$ | 4,600,000 IU | D-activated animal sterol |
| $K_3$ | 2.8 g | Menadione |
| *d*-α-Tocopheryl acetate | 20,000 IU | |
| Choline | 560.0 g | Choline chloride |
| Folic acid | 2.2 g | |
| Niacin | 30.0 g | |
| *d*-Pantothenic acid | 18.0 g | *d*-Calcium pantothenate |
| Riboflavin | 3.4 g | |
| Thiamine | 10.0 g | Thiamine mononitrate |
| $B_{12}$ | 4,000 µg | |
| Pyridoxine | 1.7 g | Pyridoxine hydrochloride |
| Biotin | 140.0 mg | *d*-Biotin |
| **Minerals** | | |
| Iron | 120.0 g | Iron sulfate |
| Manganese | 60.0 g | Manganous oxide |
| Zinc | 16.0 g | Zinc oxide |
| Copper | 4.0 g | Copper sulfate |
| Iodine | 1.4 g | Calcium iodate |
| Cobalt | 0.4 g | Cobalt carbonate |

(a) Per ton (2,000 lb) of finished product

TABLE G3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

| Nutrients | Mean ± Standard Deviation | Range | Number of Samples |
|---|---|---|---|
| Crude protein (percent by weight) | 24.19 ± 1.07 | 22.4-26.3 | 25 |
| Crude fat (percent by weight) | 5.02 ± 0.47 | 4.2-6.0 | 25 |
| Crude fiber (percent by weight) | 3.37 ± 0.37 | 2.4-4.2 | 25 |
| Ash (percent by weight) | 6.54 ± 0.26 | 5.97-7.03 | 25 |
| **Amino Acids (percent of total diet) (a)** | | | |
| Arginine | 1.300 | 1.21-1.38 | 3 |
| Cystine | 0.340 | 0.23-0.40 | 3 |
| Glycine | 1.137 | 1.06-1.20 | 3 |
| Histidine | 0.561 | 0.530-0.578 | 3 |
| Isoleucine | 0.899 | 0.881-0.934 | 3 |
| Leucine | 1.930 | 1.85-1.98 | 3 |
| Lysine | 1.243 | 1.20-1.30 | 3 |
| Methionine | 0.329 | 0.306-0.368 | 3 |
| Phenylalanine | 0.991 | 0.960-1.04 | 3 |
| Threonine | 0.851 | 0.827-0.886 | 3 |
| Tryptophan | 0.187 | 0.171-0.211 | 3 |
| Tyrosine | 0.647 | 0.566-0.769 | 3 |
| Valine | 1.090 | 1.05-1.12 | 3 |
| **Essential Fatty Acids (percent of total diet) (a)** | | | |
| Linoleic | 2.40 | 2.37-2.44 | 2 |
| Linolenic | 0.284 | 0.259-0.308 | 2 |
| **Vitamins (a)** | | | |
| Vitamin A (IU/kg) | 11,936 ± 2,547 | 8,900-22,000 | 25 |
| Vitamin D (IU/kg) | 5,220 | 4,140-6,300 | 2 |
| α-Tocopherol (ppm) | 39.1 | 31.1-44.0 | 3 |
| Thiamine (ppm) | 18.7 ± 3.20 | 14.0-26.0 | (b) 24 |
| Riboflavin (ppm) | 7.3 | 6.1-8.1 | 3 |
| Niacin (ppm) | 82 | 65-97 | 3 |
| Pantothenic acid (ppm) | 30.2 | 23.0-30.5 | 3 |
| Pyridoxine (ppm) | 7.7 | 5.6-8.8 | 3 |
| Folic acid (ppm) | 2.5 | 1.8-3.4 | 3 |
| Biotin (ppm) | 0.27 | 0.21-0.32 | 3 |
| Vitamin $B_{12}$ (ppb) | 21.2 | 10.6-38.0 | 3 |
| Choline (ppm) | 3,337 | 3,200-3,430 | 3 |
| **Minerals (a)** | | | |
| Calcium (percent) | 1.22 ± 0.10 | 1.10-1.45 | 25 |
| Phosphorus (percent) | 0.96 ± 0.05 | 0.84-1.10 | 25 |
| Potassium (percent) | 0.809 | 0.772-0.846 | 2 |
| Chloride (percent) | 0.581 | 0.479-0.635 | 3 |
| Sodium (percent) | 0.307 | 0.258-0.349 | 3 |
| Magnesium (percent) | 0.165 | 0.151-0.177 | 3 |
| Sulfur (percent) | 0.292 | 0.270-0.290 | 3 |
| Iron (ppm) | 420 | 409-431 | 3 |
| Manganese (ppm) | 87.7 | 81.7-95.5 | 3 |
| Zinc (ppm) | 52.1 | 46.1-56.0 | 3 |
| Copper (ppm) | 11.15 | 8.09-15.70 | 3 |
| Iodine (ppm) | 2.66 | 1.52-3.64 | 3 |
| Chromium (ppm) | 1.72 | 1.44-1.93 | 3 |
| Cobalt (ppm) | 0.64 | 0.49-0.78 | 3 |

(a) Two or three batches of feed analyzed for nutrients reported in this table were manufactured in 1983 or 1984.
(b) One batch (7/22/81) not analyzed for thiamine

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

| Contaminants | Mean ± Standard Deviation | Range | Number of Samples |
|---|---|---|---|
| Arsenic (ppm) | 0.45 ± 0.11 | 0.21-0.65 | 25 |
| Cadmium (ppm) (a) | <0.1 | | 25 |
| Lead (ppm) | 0.95 ± 0.78 | 0.27-2.93 | 25 |
| Mercury (ppm) (a) | <0.05 | | 25 |
| Selenium (ppm) | 0.28 ± 0.06 | 0.16-0.40 | 25 |
| Aflatoxins (ppb) (a,b) | <10 | <5.0-<10.0 | 25 |
| Nitrate nitrogen (ppm) (c) | 9.85 ± 4.55 | 0.6-19.0 | 25 |
| Nitrite nitrogen (ppm) (c) | 1.92 ± 1.28 | 0.4-5.3 | 25 |
| BHA (ppm) (d) | 5.67 ± 5.07 | 1.5-20.0 | 25 |
| BHT (ppm) (d) | 3.35 ± 2.55 | <1.0-13.0 | 25 |
| Aerobic plate count (CFU/g) (e) | 121,420 ± 94,844 | 7,000-420,000 | 25 |
| Coliform (MPN/g) (f) | 965 ± 991 | <3-2,400 | 25 |
| *E. coli* (MPN/g) (g) | 6.76 ± 7.06 | <3-23 | 24 |
| *E. coli* (MPN/g) (h) | 12.64 ± 29.46 | <3-150 | 25 |
| Total nitrosamines (ppb) (i, j) | 4.40 ± 3.16 | <1.2-12.9 | 24 |
| Total nitrosamines (ppb) (i,k) | 8.29 ± 19.41 | 1.2-100.3 | 25 |
| *N*-Nitrosodimethylamine (ppb) (i,l) | 3.05 ± 3.05 | 0.6-12.0 | 24 |
| *N*-Nitrosodimethylamine (ppb) (i,m) | 6.89 ± 19.42 | 0.6-99.0 | 25 |
| *N*-Nitrosopyrrolidine (ppb) | 1.20 ± 0.62 | <0.3-2.4 | 25 |
| **Pesticides (ppm)** | | | |
| α-BHC (a,n) | <0.01 | | 25 |
| β-BHC (a) | <0.02 | | 25 |
| γ-BHC-Lindane (a) | <0.01 | | 25 |
| δ-BHC (a) | <0.01 | | 25 |
| Heptachlor (a) | <0.01 | | 25 |
| Aldrin (a) | <0.01 | | 25 |
| Heptachlor epoxide (a) | <0.01 | | 25 |
| DDE (o) | <0.01 | 0.05 (7/14/81) | 25 |
| DDD (a) | <0.01 | | 25 |
| DDT (a) | <0.01 | | 25 |
| HCB (a) | <0.01 | | 25 |
| Mirex (a) | <0.01 | | 25 |
| Methoxychlor (p) | <0.05 | 0.13 (8/25/81); 0.6 (6/29/82) | 25 |
| Dieldrin (a) | <0.01 | | 25 |
| Endrin (a) | <0.01 | | 25 |
| Telodrin (a) | <0.01 | | 25 |
| Chlordane (a) | <0.05 | | 25 |
| Toxaphene (a) | <0.1 | | 25 |
| Estimated PCBs (a) | <0.2 | | 25 |
| Ronnel (a) | <0.01 | | 25 |
| Ethion (a) | <0.02 | | 25 |
| Trithion (a) | <0.05 | | 25 |
| Diazinon (a) | <0.1 | | 25 |
| Methyl parathion (a) | <0.02 | | 25 |
| Ethyl parathion (a) | <0.02 | | 25 |
| Malathion (q) | 0.08 ± 0.05 | <0.05-0.25 | 25 |
| Endosulfan I (a,r) | <0.01 | | 17 |
| Endosulfan II (a,r) | <0.01 | | 17 |
| Endosulfan sulfate (a,r) | <0.03 | | 17 |

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) CFU = colony-forming unit
(f) MPN = most probable number
(g) Mean, standard deviation, and range exclude one high value of 150 obtained for the batch produced on 8/26/82.
(h) Mean, standard deviation, and range include the high value given in footnote (g).
(i) All values were corrected for percent recovery.
(j) Mean, standard deviation, and range exclude one value of 100.3 obtained for the batch produced on 4/27/81.
(k) Mean, standard deviation, and range include the high value given in footnote (j).
(l) Mean, standard deviation, and range exclude one high value of 99.0 obtained for the batch produced on 4/27/81.
(m) Mean, standard deviation, and range include the high value given in footnote (l).
(n) BHC = hexachlorocyclohexane or benzene hexachloride
(o) One observation was above the detection limit. The value and the date it was obtained are listed under the range.
(p) Two observations were above the detection limit. The values and the dates they were obtained are given under the range.
(q) Ten batches contained more than 0.05 ppm.
(r) Analysis for endosulfan I, endosulfan II, and endosulfan sulfate was started on 12/23/81.

# APPENDIX H

# AUDIT SUMMARY

# APPENDIX H. AUDIT SUMMARY

The pathology specimens, experimental data, study documents, and the October 1987 NTP Technical Report on the 2-year studies of furosemide in rats and mice were audited for the National Institute of Environmental Health Sciences (NIEHS) at the National Toxicology Program (NTP) Archives in December 1987 by Argus Research Laboratories. Complete reports are on file at the NIEHS. The audit included review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) All inlife records including protocol, correspondence, dosing, environmental conditions, masses, mortality, animal identification, and serology.
(3) Body weight and clinical observation data for a random 10% sample of animals in each study group.
(4) All chemistry records.
(5) All postmortem records for individual animals concerning disposition codes, condition codes, tissue accountability, correlation of masses or clinical signs recorded at the last inlife observation with gross observations and microscopic diagnoses, and correlations between gross observations and microscopic diagnoses.
(6) All wet tissue bags for inventory and wet tissues from a random 20% sample of animals from each study group plus other relevant cases to verify animal identity and to examine for untrimmed potential lesions.
(7) Blocks and slides of tissues from a random 20% sample of animals from each study group to examine for proper match and inventory.
(8) Correlation between original microscopic observations and tabulated pathology diagnoses for a random 10% sample of study animals to verify computer data entry.
(9) Correlation between the data, results, and procedures for the 2-year studies presented in the Technical Report and the records available at the NTP Archives.

The audit showed that inlife procedures and events were documented by the archival records with minor exceptions. Dose mixtures were prepared and administered to animals properly except for two times when mixtures were prepared using half the feed required to prepare the 350-ppm mixtures and the records did not document what happened to the resulting 700-ppm mixtures. The feed consumption values presented in the Technical Report were verified by comparison with archival data for all but 5/9 values in male mice. Of the tissue masses noted among the inlife records, 143/154 in rats and 85/96 in mice were correlated with necropsy observations. The documentation for analysis of formulated diets throughout the studies was reviewed and found to be complete and accurate.

The audit of the pathology specimens showed that identifiers (punched ears) were present and correct for 48/68 rats and 27/67 mice examined. Review of residual lesions in the wet tissues for animals where the ears were absent or mutilated showed reasonable correspondence with the lesions described at necropsy; thus, the evidence suggests proper animal identification. The audit identified untrimmed potential lesions in nontarget organs for one rat and one mouse and revealed that the small intestine or colon had not been completely opened in most of the 68 rats and 67 mice examined.

Full details about these and other audit findings are presented in the audit report. In conclusion, the data and results presented in the draft Technical Report for the 2-year studies of furosemide are supported by the records at the NTP Archives.

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