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TOXICOLOGY AND CARCINOGENESIS STUDIES OF NALIDIXIC ACID

(CAS NO. 389-08-2)

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 NALIDIXIC ACID

(CAS NO. 389-08-2)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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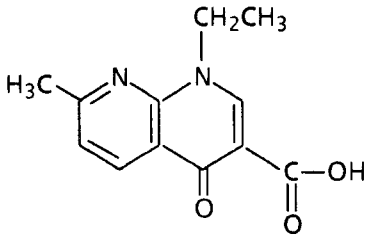

NALIDIXIC ACID

CAS No. 389-08-2

$C_{12}H_{12}N_2O_3$ Molecular weight 232.2

Synonyms: 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid

Trade Names: NegGram®; Dixiben®; Nalidixan®; Nalurin®; Nogram®; UroNeg®; Uralgin®; Urisal®

ABSTRACT

Nalidixic acid is an antimicrobial agent used to treat bacterial infections of the urinary tract. Toxicology and carcinogenesis studies were conducted by feeding diets containing nalidixic acid (approximately 99% pure) to groups of F344/N rats and B6C3F$_1$ mice of each sex for 13 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Thirteen-Week Studies: Nalidixic acid was administered at dietary concentrations ranging from 1,000 to 16,000 ppm. One female rat that received 16,000 ppm nalidixic acid died before the end of the studies; no other compound-related deaths occurred in rats or mice. The final mean body weights of rats that received 8,000 or 16,000 ppm were 23% or 49% lower than those of controls for males and 11% or 31% lower for females. Feed consumption by rats receiving 16,000 ppm was approximately two-thirds that by controls. Liver weight to body weight ratios for male rats that received 2,000 ppm or more and female rats that received 8,000 ppm or more were significantly greater than those for controls. Degeneration of the germinal epithelium in the seminiferous tubules of the testis was observed in 10/10 male rats that received 16,000 ppm; no other compound-related histopathologic effects were observed in rats. The final mean body weights of mice that received 8,000 or 16,000 ppm were 10%-20% lower than those of controls. Feed consumption by dosed mice was similar to that by controls. Liver weight to body weight ratios were significantly greater for male mice receiving 2,000, 8,000, or 16,000 ppm and for female mice receiving 4,000, 8,000, or 16,000 ppm than for the controls. No compound-related histopathologic effects were observed in mice.

Based on these results, 2-year studies of nalidixic acid were conducted by feeding diets containing 0, 2,000, or 4,000 ppm nalidixic acid to groups of 50 male and 50 female F344/N rats and 50 male and 50 female B6C3F$_1$ mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose rats were 7%-23% lower than those of controls, and those of low dose male rats were 6%-11% lower than those of controls. The average daily feed consumption by dosed rats ranged from 89% to 96% that by controls. The average amount of nalidixic acid consumed per day was approximately 80 or 175 mg/kg for low

dose or high dose rats. Mean body weights of high dose male mice were 1%-8% lower than those of controls throughout the study. Mean body weights of dosed female mice were 5%-17% lower than those of controls. Average daily feed consumption by dosed mice was within 3% of that by controls. The estimated average amount of nalidixic acid consumed per day was approximately 220 or 475 mg/kg for low dose or high dose mice. No significant differences in survival were seen between any groups of rats or mice of either sex after 2 years (male rats: control, 27/50; low dose, 28/50; high dose, 27/50; female rats: 22/50; 31/50; 29/50; male mice: 33/50; 34/50; 31/50; female mice: 40/50; 43/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidences of preputial gland neoplasms in dosed male rats and of clitoral gland neoplasms in dosed female rats were significantly greater than those in controls (male--preputial gland adenomas, papillomas, or carcinomas, combined: control, 3/49; low dose, 19/49; high dose, 20/47; female--clitoral gland adenomas, papillomas, or carcinomas, combined: 5/46; 15/46; 16/47).

A squamous cell carcinoma of the tongue was seen in two high dose male rats. The historical incidence of oral cavity neoplasms in untreated control male F344/N rats is 7/1,596 (0.4%).

There were decreased incidences of leukemia (20/50; 9/50; 7/50) and mammary gland neoplasms (10/50; 7/50; 2/50) in dosed female rats and of pituitary gland neoplasms (11/49; 2/50; 2/50) in dosed male rats.

Retinal degeneration and cataracts of the eye were observed at increased incidences in dosed rats (degeneration--male: 4/48; 41/48; 47/49; female: 2/47; 40/48; 46/50; cataracts--male: 11/48; 23/48; 38/49; female: 0/47; 18/48; 14/50). The cause of these cataracts and retinal degeneration is uncertain because cages were not rotated and low and high dose groups of rats may have been exposed to greater light intensity than were the controls.

Subcutaneous tissue fibrosarcomas and fibromas or fibrosarcomas (combined) were increased in dosed male mice (fibromas or fibrosarcomas, combined: 5/50; 9/50; 14/50). There were no increased incidences of neoplasms in dosed female mice.

Genetic Toxicology: Nalidixic acid was not mutagenic in any of several in vitro short-term tests. No gene reversion was observed in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 after exposure to nalidixic acid in either the presence or absence of exogenous metabolic activation. Results of tests for induction of trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells were negative with or without metabolic activation. In CHO cells, nalidixic acid did not induce sister chromatid exchanges or chromosomal aberrations in either the presence or absence of activation.

Conclusions: Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of nalidixic acid for F344/N rats, as indicated by increased incidences of preputial gland neoplasms in males and clitoral gland neoplasms in females. There was *equivocal evidence of carcinogenic activity* for male $B6C3F_1$ mice fed diets containing nalidixic acid, as indicated by marginally increased incidences of subcutaneous tissue neoplasms. There was *no evidence of carcinogenic activity* for female $B6C3F_1$ mice fed diets containing 2,000 or 4,000 ppm nalidixic acid for 2 years.

SUMMARY OF THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Dietary concentrations			
0, 2,000, or 4,000 ppm nalidixic acid	0, 2,000, or 4,000 ppm nalidixic acid	0, 2,000, or 4,000 ppm nalidixic acid	0, 2,000, or 4,000 ppm nalidixic acid
Body weights in the 2-year study			
Dosed groups lower than controls	High dose group lower than controls	High dose group lower than controls	Dosed groups lower than controls
Survival rates in the 2-year study			
27/50; 28/50; 27/50	22/50; 31/50; 29/50	33/50; 34/50; 31/50	40/50; 43/50; 32/50
Nonneoplastic effects			
None	None	None	None
Neoplastic effects			
Preputial gland adenomas, papillomas, or carcinomas (combined) (3/49; 19/49; 20/47)	Clitoral gland adenomas, papillomas, or carcinomas (combined) (5/46; 15/46; 16/47)	Subcutaneous tissue fibromas or fibrosarcomas (combined) (5/50; 9/50; 14/50)	None
Level of evidence of carcinogenic activity			
Clear evidence	Clear evidence	Equivocal evidence	No evidence
Other considerations			
Decreased incidences of anterior pituitary gland adenomas (11/49; 2/50; 2/50)	Decreased incidences of leukemia (20/50; 9/50; 7/50) and of mammary gland fibroadenomas or adenocarcinomas (combined) (10/50; 7/50; 2/50)		

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 9.

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 Nalidixic Acid is based on the 13-week studies that began in May 1980 and ended in August 1980 and on the 2-year studies that began in April 1981 and ended in May 1983 at Physiological Research Laboratories (Minneapolis, MN).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on nalidixic acid on March 13, 1989, 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 NALIDIXIC ACID

On March 13, 1989, the draft Technical Report on the toxicology and carcinogenesis studies of nalidixic acid 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, Research Triangle Park, NC.

Dr. R.E. Morrissey, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (clear evidence of carcinogenic activity for male and female rats, equivocal evidence of carcinogenic activity for male mice, no evidence of carcinogenic activity for female mice).

Dr. Klaassen, a principal reviewer, agreed with the conclusions. He pointed out that there was no dose response in tumor incidence in rats, indicating that emphasis could be given to the "anticarcinogenic" effects of nalidixic acid in female rats, and noted the greater survival in dosed female rats. Dr. Morrissey said that some of these negative effects may have been related to the decreased body weight gain observed in dosed female rats. Dr. Klaassen asked whether there are human equivalents of preputial and clitoral glands. Dr. Morrissey replied that they are not found as specific organs in humans, but prepuce and clitoris are components of other tissues.

Dr. Ashby, the second principal reviewer, agreed with the conclusions. He commented on observations from the NTP data base that of all the chemicals that induce preputial or clitoral neoplasms, nalidixic acid is the only one that is neither genotoxic nor a multiorgan carcinogen. He speculated that this was a classic case to pursue to examine secondary mechanisms of the chemical induction of cancer, in that the chemical might be inducing changes in homeostatic mechanisms resulting in either increases or decreases in tumor incidences. Dr. Gallo commented that the specific tumors that had an increased or decreased incidence in rats had estrogen receptors and wondered if nalidixic acid might be modifying estrogen metabolism in some manner.

Dr. Newberne, the third principal reviewer, agreed with the conclusions. He noted the lack of a dose response in male and female rats. He thought the discussion concerning possible mechanisms for severe degeneration of germinal epithelium of seminiferous tubules of the testis in high dose male rats in the 13-week studies to be speculative. He had similar comments concerning discussion of retinal degeneration and cataracts in dosed male and female rats in the 2-year studies. There ensued a discussion as to whether the eye lesions were associated with chemical administration or with light. Dr. J. Huff, NIEHS, pointed out that, in the animal rooms, the low dose animals were housed on racks above the high dose animals and the incidences of eye lesions did not appear to be associated with cage location.

Drs. Ashby and McKnight wondered why there was not some evidence of carcinogenic activity for male mice, based on the increases in subcutaneous tissue tumors. Dr. S. Eustis, NIEHS, explained that pairwise comparisons (for high dose vs. control) and the trend test showed only marginal statistical significance and, further, the incidence of tumors was within the historical control range for the laboratory.

Dr. Klaassen moved that the Technical Report on nalidixic acid be accepted with the conclusions as written for male and female rats, clear evidence of carcinogenic activity, for male mice, equivocal evidence of carcinogenic activity, and for female mice, no evidence of carcinogenic activity. Dr. Gold seconded the motion, which was accepted by nine votes to one (Dr. Klaassen).

I. INTRODUCTION

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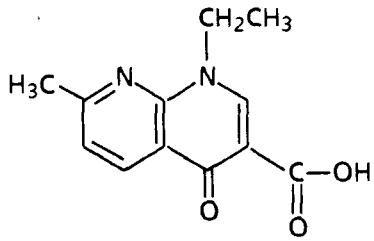

NALIDIXIC ACID

CAS No. 389-08-2

$C_{12}H_{12}N_2O_3$ Molecular weight 232.2

Synonyms: 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid

Trade Names: NegGram®; Dixiben®; Nalidixan®; Nalurin®; Nogram®; UroNeg®; Uralgin®; Urisal®

Physical Properties, Production, and Use

Nalidixic acid is an odorless, white to slightly yellow, crystalline powder (Remington's, 1980) that has a pK_a of 8.6 and a melting point of 229°-230° C and is practically insoluble in water (Merck, 1983). It is manufactured by the condensation of 2-amino-6-methylpyridine with the diethyl ester of (ethoxymethylene) malonic acid, cyclization to ethyl 1,4-dihydro-7-methyl-4-oxylate, and finally, ethylation with ethyl bromide and saponification (NIOSH, 1988).

Nalidixic acid, an oral antimicrobial agent effective against gram-negative bacteria, is used to treat infections of the urinary tract. It is effective against most Proteus strains, Klebsiella, Enterobacter, some Salmonella and Shigella strains, and *Escherichia coli* (Gleckman et al., 1979; Remington's, 1980; Merck, 1983). About 200,000 prescriptions were written in 1987 (IMS America Ltd., 1988). Nalidixic acid is available as nalidixic acid tablets, NF, containing 250, 500, or 1,000 mg of the drug and also as a pediatric suspension (250 µg/ml). In 1976, U.S production is estimated to have exceeded 1,000 lb (USITC, 1977); an equal or greater amount was imported (NIOSH, 1988). Nalidixic acid selectively and reversibly inhibits DNA synthesis in bacteria. It is an inhibitor of bacterial gyrase, an enzyme similar to topoisomerase II in eukaryotes (Crumplin, 1981). The enzyme requires ATP to catalyze supercoiling of duplex DNA.

Human Exposure and Health Effects

Nalidixic acid is usually well tolerated, but common adverse reactions include gastrointestinal disturbances (Ahlmen et al., 1983), skin lesions (Boisvert and Barbeau, 1981; Epstein and Wintroub, 1985), and neurologic reactions (reviewed by Gleckman et al., 1979). Nausea, vomiting, and abdominal pain are the most frequently reported gastrointestinal disturbances caused by nalidixic acid. Some patients experience allergic reactions, such as pruritus, urticaria, various rashes, and photosensitivity. Nalidixic acid is contraindicated for patients with a history of convulsive disorders. Central nervous system effects, including brief convulsions, increased intracranial pressure, and toxic psychosis, have been reported in a few individuals. These effects are rapidly reversible upon discontinuation of the drug. Increases in intracranial pressure and subjective visual disturbances have been reported mainly in children but also in adults (Anderson et al., 1971; Fraser and Harrower, 1977; Hecht, 1978; Jo et al., 1979; Gedroyc and Shorvon, 1982). Ocular side effects, including slight papilledema, may be due to increased intracranial pressure. Although no lesions have

been reported in children, toxicologic studies have shown that nalidixic acid and related drugs can cause erosion of cartilage at weight-bearing joints in some laboratory species (Ingham et al., 1977). Cholestasis, thrombocytopenia, leukopenia, and hemolytic anemia rarely occur (Mandal and Stevenson, 1970; Potasman and Bassan, 1980; Meyboom, 1984; Mandell and Sande, 1985).

No epidemiologic studies or case reports examining the relationship between exposure to nalidixic acid and human cancer incidences were found in the literature.

Distribution and Metabolism

After oral administration, nalidixic acid is rapidly absorbed from the gastrointestinal tract, partially metabolized in the liver, and rapidly excreted through the kidneys (reviewed by Gleckman et al., 1979). Absorption from the gastrointestinal tract is estimated to exceed 95%, with minimal fecal excretion. More than 90% of the nalidixic acid and 60% of its hydroxylated metabolite are protein bound in blood. A steady-state serum concentration is reached after 3 days of dosing. The recommended adult dose of 1 g (administered four times per day for 1 or 2 weeks, average daily dose of about 65 mg/kg) produces peak serum levels of active drug that average approximately 20-40 µg/ml (Moore et al., 1965; Mannisto, 1976). The plasma half-life of nalidixic acid in persons with normal renal function has been reported to be approximately 2 hours after administration of a 1-g dose.

Unchanged nalidixic acid (2%-3% of a dose) appears in the urine along with an active metabolite, hydroxynalidixic acid (13%), which also has antibacterial activity (McChesney et al., 1964; reviewed by Gleckman et al., 1979). Peak urine levels of the active drug are approximately 150-300 µg/ml, 3-4 hours after administration (Mannisto, 1976; Cuisinaud et al., 1982). Both nalidixic acid and its 7-hydroxy derivative were reported to have an apparent elimination half-life of 6-7 hours (Ferry et al., 1981). The daily urinary recovery of administered drug is approximately 80% at steady state (McChesney et al., 1964; Mannisto, 1976). More than 80% of a dose is excreted as biologically inactive glucuronide and dicarboxylic derivatives of the parent compound and the metabolite. Renal insufficiency did not affect the renal clearance of nalidixic acid, but it significantly decreased the elimination rate of hydroxynalidixic acid (Cuisinaud et al., 1982). Barbeau and Belanger (1982) reported that the plasma half-life of the active drug averaged 11.5 hours in an older volunteer group, a finding that the authors suggest may be due to diminished renal function.

In rats and mice, oral doses are rapidly absorbed; peak blood concentrations are detected about 1 hour later; elimination is via the kidneys, peaking at about 6 hours after dosing (Rossoff, 1974).

Short-Term Studies

The oral LD_{50} of nalidixic acid was reported by different investigators to be 1,350 mg/kg and 572 mg/kg for rats (NIOSH, 1979) and 3,300 mg/kg (Merck, 1983) or 1,120 mg/kg (Beliles, 1972) for mice. In mice, the subcutaneous LD_{50} was reported to be 500 mg/kg (NIOSH, 1979; Merck, 1983) and the intravenous LD_{50} to be 176 mg/kg (Merck, 1983). Negishi et al. (1985) reported that C57BL female mice given intraperitoneal injections at the LD_{50} for 2 consecutive days showed no adverse effects on hematocrit value or on the colony-forming ability of bone marrow cells. In vitro, nalidixic acid had no effect on the colony-forming ability at a concentration of 100 µg/ml, a clinically relevant level. Nalidixic acid was reported to induce phototoxicity in CF-1 mice (Ljunggren and Moller, 1978; Keane et al., 1984).

A study performed to characterize the potential for nalidixic acid to chelate cupric and zinc ions showed that both ions complex with the chemical (G.S. Goldstein, Sterling Drug Inc., personal communication to Bureau of Drugs, 1977). Bailey et al. (1984) suggested that drug-metal complexes may play a role in the mechanism of action of nalidixic acid.

Genetic Toxicology

Nalidixic acid is a known inhibitor of DNA gyrase, exerting its effect by complexing with the A subunit of the enzyme (Cozzarelli, 1980;

McCoy et al., 1980; Nishio and Uyeki, 1982), thus interfering with DNA replication/repair by disruption of the supercoiling processes. The positive responses recorded in bacterial assays for induction of DNA damage after exposure to nalidixic acid (Kada et al., 1972; Boyle and Simpson, 1980; McCoy et al., 1980; Suter and Jaeger, 1982) are probably the effects of the secondary influences on replication and repair processes rather than a direct interaction with the DNA. These assays use, as the measure of damage, inhibition of DNA synthesis or differential growth inhibition between DNA repair-competent and repair-deficient strains. Nalidixic acid was also shown to induce amplification of the recA protein in *Salmonella typhimurium* (Pierre et al., 1982) and *E. coli* (Salles et al., 1987), a protein reported to be released during the initial processes of the SOS bacterial DNA repair network following strand breakage. Induction of a DNA repair-associated enzyme in yeast has also been reported following treatment with nalidixic acid (Robinson et al., 1986). The effects of nalidixic acid interference with DNA metabolism were also expressed in specific gene mutation assays with bacteriophage and bacteria. DeMarini and Lawrence (1988) observed a weakly mutagenic response in bacteriophage T4 rFC11 treated with highly toxic doses (100-400 µM) of nalidixic acid. In the *Bacillus subtilis* multigene sporulation test, a bacterial assay that has not been widely used, nalidixic acid treatment produced a significant increase in asporogenic mutant colonies (determined by loss of the normal dark pigmentation characteristic of sporulating bacteria); this increase in asporogenic colonies was most pronounced at nalidixic acid concentrations of 100 and 200 µg/ml, whereas at higher concentrations of 300 and 500 µg/ml, the proportion of mutant colonies recovered was not significantly increased (Sacks and MacGregor, 1982). Levin et al. (1984) observed a significant increase in mutant colonies of Salmonella strain TA102 after exposure to nalidixic acid; the authors attributed this mutagenic response to the secondary effects of DNA polymerase inhibition and the resultant single-strand breaks that ultimately produced small deletions in susceptible DNA regions of an incorporated plasmid. Other tests for gene mutation induction in *S. typhimurium*, however, were uniformly negative, with and without S9 (Sugimura et al., 1976; McCoy et al., 1980; Podger and Grigg, 1986; Zeiger et al., 1988; Table I1); results obtained in gene mutation studies with yeast were also negative (Sarachek, 1979). In contrast, induction of sex-linked recessive lethal mutations was reported in adult male Drosophila fed nutritional media coated with 10 or 20 mg nalidixic acid for 6 or 3 days, respectively (Filippova and Efremova, 1974).

In mammalian cell cultures, nalidixic acid exhibited weak inhibition of DNA synthesis in an in vitro preparation of Chinese hamster ovary cells after ultraviolet radiation (Clarkson and Mitchell, 1983). Two studies of chromosomal aberration induction in cultured human lymphocytes have been reported. In one study, a weak induction of chromosomal aberrations was observed at the high dose of 340 µg/ml nalidixic acid ($P<0.05$) (Shiratori and Takase, 1980); in the second, in which nalidixic acid concentrations up to 100 µg/ml were tested, no induction of aberrations was noted (Stenchever et al., 1970). In human epidermal keratinocytes cultured in 500 µg/ml nalidixic acid, DNA damage was not detected, but a strong inhibitory effect on replication was observed (Bohr et al., 1986).

In vivo tests for induction of dominant lethal mutation in C3H or DDY mice administered nalidixic acid orally at doses of 800 mg/kg once or 1,000 mg/kg per day for 5 days, respectively, were negative (Tokunaga et al., 1979; Shimada et al., 1980). No increase in the incidence of chromosomal aberrations was observed in the bone marrow cells of JCI-ICR mice administered 1,700 mg/kg nalidixic acid orally for 5 days (Shiratori and Takase, 1980).

Peripheral blood lymphocytes from children treated clinically with 50 mg/kg nalidixic acid for 10 days were reported to exhibit a significant increase in sister chromatid exchanges (SCEs) compared with cells from these children examined before initiation of treatment (Kowalczyk, 1980). The interpretation of results from this study, however, is complicated because the number of cells scored from each patient was much lower than normal in such a study, control levels of SCEs were quite high, and the individual SCE rates of group members (both exposed and control) differed significantly, thereby increasing the difficulty of assessing the pooled data.

Toxicity Studies

A series of toxicity studies was conducted by the Sterling-Winthrop Research Institute (1962a, b,c, 1965) to evaluate the potential toxicity of nalidixic acid in rats, dogs, and monkeys.

Nalidixic acid was administered by gavage at daily doses of 4, 40, and 400 mg/kg in 1% aqueous gum tragacanth for approximately 1 year to groups of 20 male and 20 female Charles River CD® rats (Sterling-Winthrop, 1962a). Additional groups of 10 rats of each sex received 800 mg/kg. Rats in the 4 and 40 mg/kg groups appeared normal throughout the studies; no pharmacologic effects or changes in feed consumption or stools were observed. At doses of 400 and 800 mg/kg, respiratory depression, dyspnea, ataxia, and prostration were observed. After 1 week of compound administration, rats in the 400 mg/kg groups showed more tolerance to the effects of nalidixic acid. All rats in the 800 mg/kg groups died within 23 weeks of the start of dosing. Male rats in the two lower dose groups gained weight at the same rate as control rats; female rats gained weight at similar rates for the first 15 weeks and then had lower growth rates during the last 40 weeks. Moderate-to-severe depression of the growth rate was observed in each sex in the 400 and 800 mg/kg groups. (Final body weights of the 4, 40, and 400 mg/kg groups were 109%, 100%, and 77% that of controls for males and 90%, 86%, and 79% for females.) Hematologic values (erythrocyte and leukocyte counts, hemoglobin concentration, hematocrit, and differential counts) for rats in the 40, 400, and 800 mg/kg groups were within normal limits at each determination. Significant effects on the blood pressure of rats in the 800 mg/kg group were not seen after 14 weeks of drug administration. A relatively large number of deaths occurred in all dosed groups (12/20 males and 7/20 females in the control group); only in the two highest dose groups were deaths attributed to the drug. No gross or pathologic changes were attributed to drug administration.

Pharmacologic activity related to nalidixic acid administration in dogs was observed at doses of 50, 100, 200, and 400 mg/kg in aqueous gum tragacanth given by gavage five times over a 6-day period (Sterling-Winthrop, 1962b). The most common effects of drug administration were salivation and emesis. In the 200 and 400 mg/kg groups, there were convulsions, prostration, and death of some dogs. This study was followed by a 13-month study in which nalidixic acid was given to three groups of three beagle dogs at doses of 12.5, 25, and 50 mg/kg (once per day, 6 days per week) by gavage. All dosed groups salivated after drug adminstration during the first 3 weeks but only sporadically thereafter. No drug-related deaths occurred. No peripheral blood or histopathologic changes were attributed to nalidixic acid. The urine was normal in all groups. Yasuda et al. (1983), in similar studies, dosed beagle dogs with 50 mg/kg nalidixic acid for 35 days and did not find any significant lesions by light microscopy. At the electron microscopic level, however, these investigators found focal hyperplasia of the parathyroid.

Adult rhesus monkeys tolerated gavage doses of 25, 75, or 225 mg/kg once per day for 1 year (Sterling-Winthrop, 1962c). A slightly lower growth rate, compared with controls, was the only effect observed. In a range-finding study, monkeys were normal after receiving doses of 450 or 900 mg/kg. At the doses used in the 1-year study, normal values were obtained in routine hematologic and serum biochemical analyses and urinalyses, and no pathologic tissue changes were observed. A 1-year study conducted with juvenile monkeys administered 100 mg/kg had similar findings (Sterling-Winthrop, 1965). Three of these monkeys were the offspring of rhesus monkeys given 200 mg/kg during pregnancy and for 2 weeks post partum while nursing. All dosed monkeys gained weight more rapidly than did the controls. Results of hematologic and serum biochemical analyses and urinalyses were normal at all times. Abnormalities of the pelvis and long bones of the legs were not detected by X-ray at any time in monkeys dosed with nalidixic acid for 12 months.

Nalidixic acid and related drugs have been shown to cause arthropathy in juvenile animals of some species (Ingham et al., 1977; Compendium, 1988). At high doses, hydroxynalidixic acid, the principal metabolite of nalidixic acid, has been shown to have oculotoxic potential in dogs and cats. However, nalidixic acid administered

to dogs and cats did not produce the same effects. Followup studies were limited by the sensitivity of dogs and cats to central nervous system side effects. Therefore, the doses of nalidixic acid that could be used were limited; this factor, together with a low rate of conversion to the hydroxy metabolite in these species, may explain the absence of these effects when nalidixic acid is administered (PDR, 1986).

Carcinogenicity

A study reported by Kanisawa et al. (1974) may not have been adequate for evaluation of carcinogenicity of nalidixic acid. Twenty mice were fed the chemical at dietary concentrations of 500 ppm. One animal developed a hepatic adenoma, but the duration of the study was only 36 weeks. Kurokawa et al. (1986) exposed groups of 51 CFD_1 mice of each sex to nalidixic acid at dietary concentrations of 800 or 1,600 ppm for 76 weeks. All surviving animals were killed at week 85, after 9 weeks on the basal diet. Body weights were slightly lower in the high dose groups and in the low dose males. No statistically significant differences in the incidences of neoplasms in any organ were observed between dosed and control mice of either sex.

Reproductive and Developmental Toxicology

Back et al. (1975) reported that nalidixic acid had no detrimental effect on stallion sperm motility. No lesions in reproductive organs were observed in the Sterling-Winthrop studies (1962a,b,c) cited above. In lactating women, only 0.003% of a 2-g nalidixic acid dose was excreted as unchanged nalidixic acid in milk within 24 hours (Traeger and Peiker, 1980).

Stenchever et al. (1970) found that nalidixic acid in vitro did not induce chromosomal damage in human leukocytes, but they suggested that it would be good practice to avoid the use of nalidixic acid during pregnancy because the developing embryo/fetus could possibly be damaged. Clinical experience does not support their view. Sixty-three women treated at various periods during pregnancy with nalidixic acid did not have any offspring with congenital malformations or intracranial hypertension (Murray, 1981). Kalter and Warkany (1983) concluded that nalidixic acid was unlikely to cause congenital malformations.

Diverse results of nalidixic acid on embryo/fetal development have been reported in animal studies. No standard Food and Drug Administration (FDA) tests (Segment I, II, or III) were found in the literature. Courtney et al. (1967) reported no apparent effects of nalidixic acid on the developing monkey fetus. In sea urchin embryos, nalidixic acid at concentrations of 500 µg/ml interfered with early in vitro development (Czinn et al., 1981). A summary of the study by Nishimura et al. (1971) states that nalidixic acid was not teratogenic at oral doses of 73, 220, or 660 mg/kg per day to mice and at 73 or 220 mg/kg per day to rats.

Study Rationale

This nomination resulted from a review of a large number of prescribed drugs by the FDA, which was concerned because nalidixic acid exerts its antibacterial effect through DNA synthesis inhibition and because of the lack of carcinogenicity data on this compound. The estimated human exposure to this drug is moderate to high and is long term in some persons. The drug was administered in feed because nalidixic acid is given orally to humans.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF NALIDIXIC ACID

CHARACTERIZATION OF FORMULATED DIETS

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Study Design

Source and Specifications of Animals

Animal Maintenance

Clinical Examinations and Pathology

Statistical Methods

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF NALIDIXIC ACID

Nalidixic acid was obtained in one lot (lot no. H120779) from Hilton-Davis Chemical Company (Cincinnati, OH). Purity and identity analyses were conducted at Midwest Research Institute (Kansas City, MO) (Appendix H). The study chemical was identified as nalidixic acid by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy.

Nalidixic acid lot no. H120779 was found to be approximately 99% pure, as determined by elemental analysis, Karl Fischer water analysis, potentiometric titration in pyridine solution of the carboxylic acid group with 0.1 N tetrabutylammonium hydroxide (in methanol:2-propanol, 1:9), thin-layer chromatography, and high-performance liquid chromatography. The identity of the chemical was verified at the study laboratory by infrared spectroscopy.

Stability of the bulk chemical during the course of the studies was monitored by nonaqueous titration of the carboxylic acid group and high-performance liquid chromatography. No notable degradation was observed during the studies.

CHARACTERIZATION OF FORMULATED DIETS

The homogeneity of formulated diets containing 8,000 ppm nalidixic acid was demonstrated to be within 1% of the target concentration by the analytical chemistry laboratory and within 3% by the study laboratory in samples taken from three locations in the blender. The chemical in feed at a concentration of 8,000 ppm was shown to be stable for at least 2 weeks in the dark at temperatures up to 25° C. A loss of approximately 5% was demonstrated after 2 weeks' storage at 45° C. In the 13-week studies, the formulated diets were stored protected from light at room temperature and used within 11 days of formulation. In the 2-year studies, the formulated diets were stored protected from light at room temperature for no longer than 2 weeks.

During the 2-year studies, the formulated diets were analyzed at approximately 8-week intervals. For the nalidixic acid studies, it was estimated that the mixtures were formulated within ±10% of the target concentrations approximately 96% of the time throughout the studies (Table H3). Results of referee analysis periodically performed by the analytical chemistry laboratory indicated generally good agreement with the results from the study laboratory (Table H4).

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to nalidixic acid and to determine the concentrations to be used in the 2-year studies. Dietary concentrations for the 13-week studies were based on prior studies by the manufacturer (Sterling-Winthrop, 1962a).

Four- to five-week-old male and female F344/N rats and 5- to 6-week-old male and female B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and were observed for 20 days. Animals were distributed to weight classes and assigned to cages and groups according to a standard operating procedure that was not fully described in the study report.

Groups of 10 rats and 10 mice of each sex were given diets containing 0, 1,000, 2,000, 4,000, 8,000, or 16,000 ppm nalidixic acid for 13 weeks. Control diets consisted of NIH 07 Rat and Mouse Ration. Formulated or control diets and water were available ad libitum.

Animals were observed twice per day; moribund animals were killed. Feed consumption was measured once per week by cage. Individual animal weights were recorded once per week.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals. Tissues and groups examined are listed in Table 1.

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF NALIDIXIC ACID

Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN	
Size of Study Groups	
10 males and 10 females of each species	50 males and 50 females of each species
Doses	
0, 1,000, 2,000, 4,000, 8,000, or 16,000 ppm nalidixic acid in feed	0, 2,000, or 4,000 ppm nalidixic acid in feed
Date of First Dose	
5/26/80	Rats--4/30/81; mice--male: 4/14/81; female: 5/7/81
Date of Last Dose	
8/24/80	Protocol called for dosing up to necropsy
Duration of Dosing	
13 wk	103 wk
Type and Frequency of Observation	
Observed 2 × d; weighed initially and 1 × wk thereafter	Observed 2 × d; weighed initially, 1 × wk for 12 (rats), 14 (male mice) or 13 (female mice) wk, and then 1 × mo; feed consumption measured 1 × mo
Necropsy and Histologic Examinations	
Necropsy performed on all animals; the tissues were examined histologically for control and high dose groups and animals with gross lesions	Necropsy performed on all animals. The following tissues examined histologically for control and high dose rats and mice and low dose female mice: adrenal glands, brain, cecum, colon, costochondral junction, duodenum, esophagus, eyes, gallbladder (mice), gross lesions, heart and aorta, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary gland, sciatic nerve, seminal vesicles/prostate/testes/tunica vaginalis/scrotal sac or ovaries/uterus, skin, spinal cord, spleen, sternebrae or vertebrae or femur including marrow, stomach, thigh muscle, thymus, thyroid gland, tissue masses and suspect tumors with regional lymph nodes, tongue, trachea, and urinary bladder; low dose male rats: adrenal glands, gross lesions, heart, nasal cavity, pancreas, pituitary gland, preputial gland, and skin; low dose female rats: adrenal glands, clitoral gland, eyes, gross lesions, liver, nasal cavity, spleen, thymus, and uterus; low dose male mice: adrenal glands, kidneys, liver, and lungs
ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	
F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source	
Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Kingston, NY)
Study Laboratory	
Physiological Research Laboratories	Physiological Research Laboratories
Method of Animal Identification	
Toe clip	Toe and ear clip
Time Held Before Study	
20 d	Rats--14 d; mice--male: 14 d; female: 11-14 d

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF NALIDIXIC ACID (Continued)

Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)	
Age When Placed on Study Rats--7-8 wk; mice--8-9 wk	Rats--6-7 wk; mice--7-8 wk
Age When Killed Rats--20-21 wk; mice--21-22 wk	Rats--110-111 wk; mice--111-112 wk
Necropsy Dates Rats--8/25/80 and 8/27/80; mice--8/26/80-8/27/80	Rats--4/26/83-4/29/83; mice--male: 4/13/83-4/14/83; female: 5/2/83-5/3/83
Method of Animal Distribution Animals distributed to weight classes and then assigned to cages by one table of random numbers and to groups by a standard operating procedure not fully described in the protocol	Animals assigned to dosed and control groups according to tables of random numbers
Diet NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 13-wk studies
Bedding Aspen wood chips	Heat-treated aspen wood shavings (Minnesota Sawdust and Shavings Co., Anoka, MN)
Water Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 13-wk studies; softened to < 1 grain/gal through sodium zeolite
Cages Polycarbonate (Hazleton Systems, Inc., Aberdeen, MD)	Same as 13-wk studies
Cage Filters Cages placed on filtered Enviro Rack Systems (Mouse H1370, Rat H1430) (Hazleton Systems, Inc., Aberdeen, MD)	Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)
Animals per Cage 5	5
Other Chemicals on Study in the Same Room Not specified	None
Animal Room Environment Temp--20.0°-25.5° C; hum--40%-60%; fluorescent light 12 h/d	Temp--21.1°-26.7° C; hum--30%-74%; fluorescent light 12 h/d; 0-37 room air changes/h

TWO-YEAR STUDIES

Study Design

Diets containing 0, 2,000, or 4,000 ppm nalidixic acid were fed to groups of 50 male and 50 female rats and 50 male and 50 female mice for 103 weeks.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories. 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. Animals were shipped to the study laboratory at 4-6 weeks of age. The animals were

quarantined at the study facility for 11-14 days. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rodents were placed on study at 6-8 weeks of age. The health of the animals was monitored during the course of the study 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 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 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 (Appendix G) and water were available ad libitum. Cages were not rotated during the studies. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

All animals were observed two times per day. Body weights were recorded one time per week for the first 3 months of the studies and one time 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, except for tissues that were excessively autolyzed 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 the "inverse pyramid" design (McConnell, 1983a,b). Complete histopathologic examinations (Table 1) were performed on high dose and control animals and on low dose animals dying through month 21 of the studies. 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 groups were examined histopathologically (Table 1).

When the pathology evaluation was completed by the laboratory pathologist and the pathology data entered into the Carcinogenesis Bioassay Data System, the slides, paraffin blocks, and residual formalin-fixed tissues were sent to the NTP Archives. The slides, blocks, and residual wet tissues were audited for accuracy of labeling and animal identification and for thoroughness of tissue trimming. The slides, individual animal necropsy records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tissues with a tumor diagnosis, all potential target tissues, and all tissues from a randomly selected 10% of the animals were re-evaluated microscopically by a quality assessment pathologist. Potential target organs selected for review were the thymus and

preputial gland for male rats, the thymus and clitoral and mammary glands for female rats, and the lung and adrenal gland for male and female mice. Nonneoplastic lesions were evaluated for accuracy and consistency of diagnosis only in the potential target organs, in the randomly selected 10% of animals, and in tissues with unusual incidence patterns or trends. Tissues are generally not evaluated in a "blinded" fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle.

The quality assessment report and slides were submitted to a Pathology Working Group (PWG) Chairperson, who reviewed microscopically all potential target tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative examples of potential chemical-related nonneoplastic lesions and neoplasms and examples of disagreements in diagnosis between the laboratory and quality assessment pathologists were shown to the PWG. The PWG, which included the laboratory pathologist, the quality assessment pathologist, and other pathologists experienced in rodent toxicology, examined the tissues without knowledge of dose group or previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the diagnosis was changed to reflect the opinion of the PWG. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final pathology data 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).

Statistical Methods

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: The majority of tumors in this study were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was an incidental tumor analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In 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 to obtain a single overall result.

In addition to incidental tumor analysis, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart et al., 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance include pairwise comparisons of each dosed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. (For further discussion of these statistical methods, see Haseman, 1984.)

Analysis of Continuous Variables: Liver weights and liver weight to body weight ratios in the 13-week studies were analyzed using Dunnett's test (Dunnett, 1955).

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.

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

THIRTEEN-WEEK STUDIES

One female rat that received 16,000 ppm nalidixic acid died before the end of the studies (Table 2). No other compound-related deaths occurred. The final mean body weights of rats that received 8,000 or 16,000 ppm were 23% or 49% lower than that of the controls for males and 11% or 31% lower for females. Feed consumption at 16,000 ppm was approximately two-thirds that by controls. Liver weight to body weight ratios for males that received 2,000 ppm or more and females that received 8,000 ppm or more were significantly greater than those for controls (Table 3). Degeneration of the germinal epithelium in the seminiferous tubules of the testis was severe in all 10 male rats that received 16,000 ppm but was not present in male rats that received 8,000 ppm.

Dose Selection Rationale: Because of lower weight gain and lower feed consumption at higher doses, dietary concentrations of nalidixic acid selected for rats for the 2-year studies were 2,000 ppm and 4,000 ppm.

TABLE 2. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF NALIDIXIC ACID

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 6	Week 13
MALE							
0	10/10	198 ± 5	336 ± 5	+138 ± 3		14.3	12.7
1,000	10/10	196 ± 5	323 ± 5	+127 ± 4	96	13.6	13.2
2,000	10/10	183 ± 6	328 ± 4	+145 ± 5	98	14.1	14.2
4,000	10/10	195 ± 4	314 ± 4	+119 ± 5	93	13.7	14.2
8,000	10/10	185 ± 5	259 ± 5	+74 ± 4	77	9.4	12.1
16,000	10/10	186 ± 5	172 ± 6	−14 ± 4	51	9.1	9.9
FEMALE							
0	10/10	141 ± 2	194 ± 3	+53 ± 3		9.9	8.3
1,000	10/10	139 ± 3	196 ± 4	+57 ± 4	101	9.4	10.1
2,000	10/10	136 ± 1	194 ± 3	+58 ± 3	100	9.9	10.8
4,000	10/10	132 ± 2	184 ± 2	+52 ± 3	95	10.4	10.3
8,000	10/10	131 ± 1	173 ± 3	+42 ± 3	89	8.9	11.0
16,000	(e) 9/10	129 ± 2	133 ± 3	+3 ± 2	69	6.1	6.1

(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) Grams per animal per day; not corrected for scatter.
(e) Week of death: 3

TABLE 3. LIVER WEIGHTS FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF NALIDIXIC ACID (a)

Concentration (ppm)	Number Weighed	Final Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Final Body Weight (mg/g)
MALE				
0	10	335.9 ± 5.29	12,620 ± 650	37.5 ± 1.79
1,000	10	323.3 ± 4.89	13,370 ± 400	41.4 ± 1.16
2,000	10	327.6 ± 4.24	**14,630 ± 430	**44.6 ± 0.94
4,000	10	*314.2 ± 4.49	**14,980 ± 350	**47.7 ± 0.83
8,000	10	**259.0 ± 5.23	**14,550 ± 240	**56.3 ± 1.17
16,000	10	**172.3 ± 6.13	12,380 ± 330	**72.1 ± 1.24
FEMALE				
0	10	193.7 ± 3.36	7,220 ± 300	37.4 ± 1.75
1,000	10	196.1 ± 4.30	7,760 ± 340	39.6 ± 1.50
2,000	10	194.0 ± 3.20	8,120 ± 210	42.0 ± 1.39
4,000	10	183.6 ± 2.09	8,100 ± 240	44.1 ± 1.11
8,000	10	**172.6 ± 2.86	*8,570 ± 320	**49.6 ± 1.47
16,000	9	**133.0 ± 2.57	8,030 ± 500	**60.5 ± 3.89

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

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of high dose male rats were 7%-11% lower than those of controls from week 0 to week 10 and 11%-23% lower thereafter (Table 4 and Figure 1). Mean body weights of low dose male rats were 6%-11% lower than those of controls from week 24 to the end of the study. Mean body weights of high dose female rats were 7%-12% lower than those of controls from week 0 to week 20 and 12%-19% lower thereafter. Mean body weights of low dose and control female rats were similar. The average daily feed consumption per rat by low dose or high dose rats was 95% or 90% that by controls (Tables F1 and F2). The average amount of nalidixic acid consumed per day was approximately 80 or 165 mg/kg for low dose or high dose male rats and 85 or 185 mg/kg for low dose or high dose female rats. No compound-related clinical signs were observed.

TABLE 4. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

Weeks on Study	Control		2,000 ppm			4,000 ppm		
	Av. Wt. (grams)	Number of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	Number of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	Number of Survivors
MALE								
0	136	50	131	96	50	124	91	50
1	167	50	161	96	50	148	89	50
2	203	50	194	96	50	180	89	50
3	230	50	223	97	50	209	91	50
4	248	50	240	97	50	221	89	50
5	258	50	256	99	50	241	93	50
6	275	50	271	99	50	252	92	50
7	298	50	290	97	50	273	92	50
8	311	50	300	96	50	281	90	50
9	319	50	309	97	50	289	91	50
10	331	50	319	96	50	298	90	50
11	339	50	325	96	50	303	89	50
12	349	50	332	95	50	309	89	50
16	371	50	359	97	50	330	89	50
20	399	50	382	96	50	352	88	50
24	409	50	386	94	50	356	87	50
28	423	50	397	94	50	365	86	50
32	438	50	411	94	50	377	86	50
37	449	50	420	94	48	381	85	50
40	454	50	426	94	48	384	85	50
44	459	50	431	94	48	389	85	50
49	453	50	420	93	47	380	84	50
54	466	50	430	92	47	392	84	50
58	461	48	423	92	47	383	83	49
63	456	48	417	91	47	376	82	49
67	454	48	415	91	47	373	82	48
72	453	48	415	92	47	373	82	47
76	449	48	410	91	45	369	82	47
80	451	47	410	91	42	366	81	47
85	456	46	410	90	41	362	79	46
89	453	44	409	90	38	362	80	46
94	439	41	403	92	36	349	79	40
97	432	37	392	91	35	344	80	35
101	439	28	390	89	30	338	77	30
FEMALE								
0	111	50	108	97	50	102	92	50
1	129	50	125	97	50	118	91	50
2	145	50	139	96	50	133	92	50
3	156	50	152	97	50	145	93	50
4	164	50	159	97	50	148	90	50
5	173	50	168	97	50	157	91	50
6	177	50	172	97	50	161	91	50
7	188	50	182	97	50	171	91	50
8	192	50	185	96	50	172	90	50
9	198	50	190	96	50	174	88	50
10	201	50	196	98	50	180	90	50
11	205	50	199	97	50	182	89	50
12	205	50	202	99	50	183	89	50
16	214	50	211	99	50	191	89	50
20	224	50	220	98	50	201	90	50
24	229	50	223	97	50	201	88	50
28	236	50	228	97	50	206	87	50
32	245	50	237	97	50	212	87	50
37	254	49	243	96	50	218	86	50
40	256	49	247	96	50	220	86	50
44	260	49	254	98	50	225	87	50
49	259	49	249	96	49	219	85	50
54	276	49	261	95	48	231	84	50
58	286	48	269	94	48	235	82	50
63	293	47	276	94	48	236	81	49
67	293	47	280	96	45	237	81	49
72	300	45	291	97	45	249	83	48
76	307	43	299	97	44	255	83	48
80	313	43	305	97	43	258	82	44
85	322	42	314	98	40	266	83	43
89	324	41	320	99	39	272	84	42
94	326	32	317	97	38	269	83	37
97	322	32	314	98	36	271	84	35
101	321	28	317	99	33	271	84	32

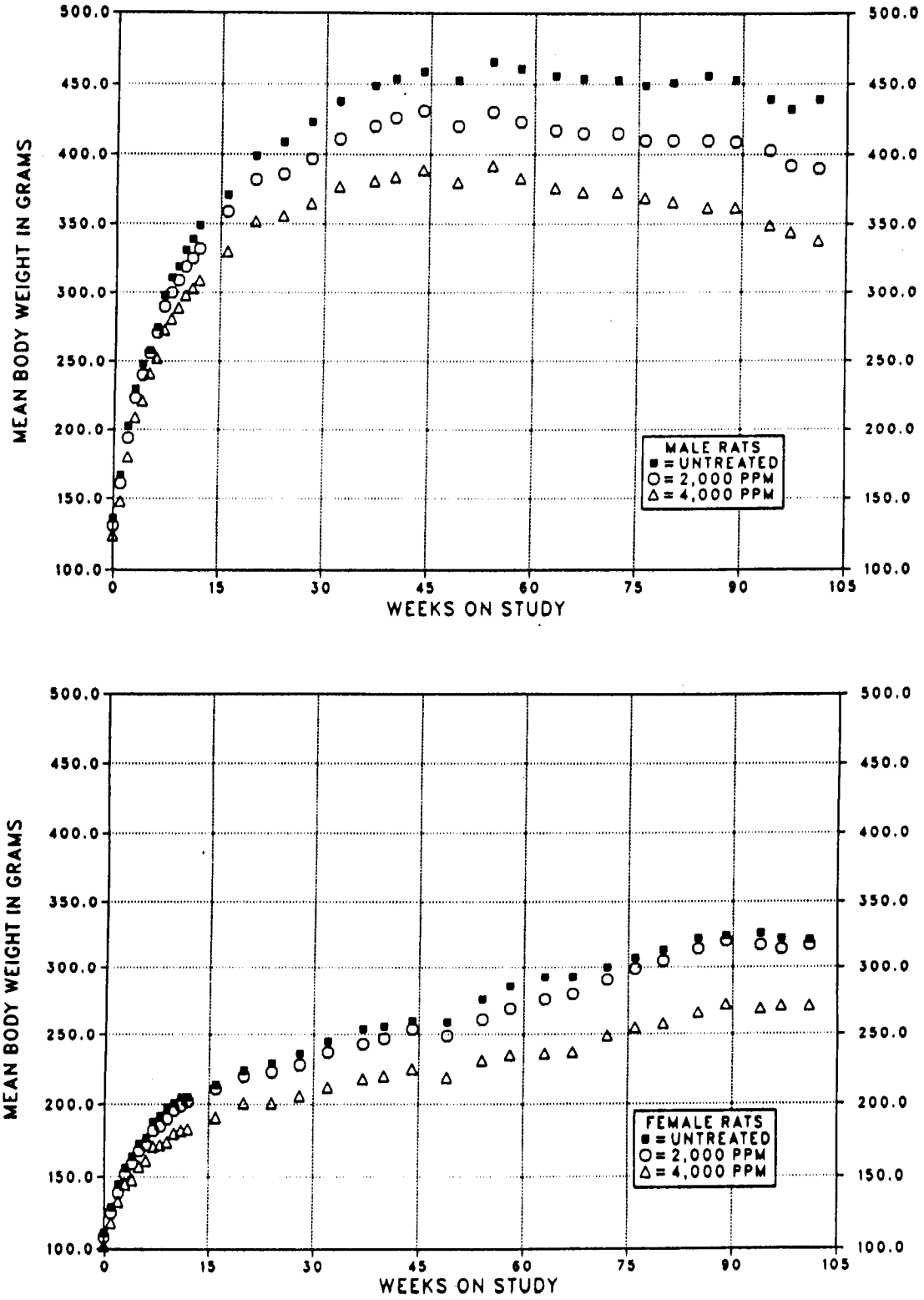

FIGURE 1. GROWTH CURVES FOR RATS FED DIETS CONTAINING NALIDIXIC ACID FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats fed diets containing nalidixic acid at the concentrations used in these studies and for controls are shown in Table 5 and in the Kaplan and Meier curves in Figure 2. No significant differences in survival were observed between any groups of either sex.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the preputial gland, clitoral gland, tongue, skin, eye, hematopoietic system, anterior pituitary gland, and mammary 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 5. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

	Control	2,000 ppm	4,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Natural deaths	4	1	1
Moribund kills	19	21	22
Animals surviving until study termination	27	28	27
Survival P values (b)	1.000	0.937	0.965
FEMALE (a)			
Animals initially in study	50	50	50
Natural deaths	6	1	0
Moribund kills	22	18	21
Animals surviving until study termination	22	31	29
Survival P values (b)	0.208	0.153	0.240

(a) Termination period: week 104
(b) 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.

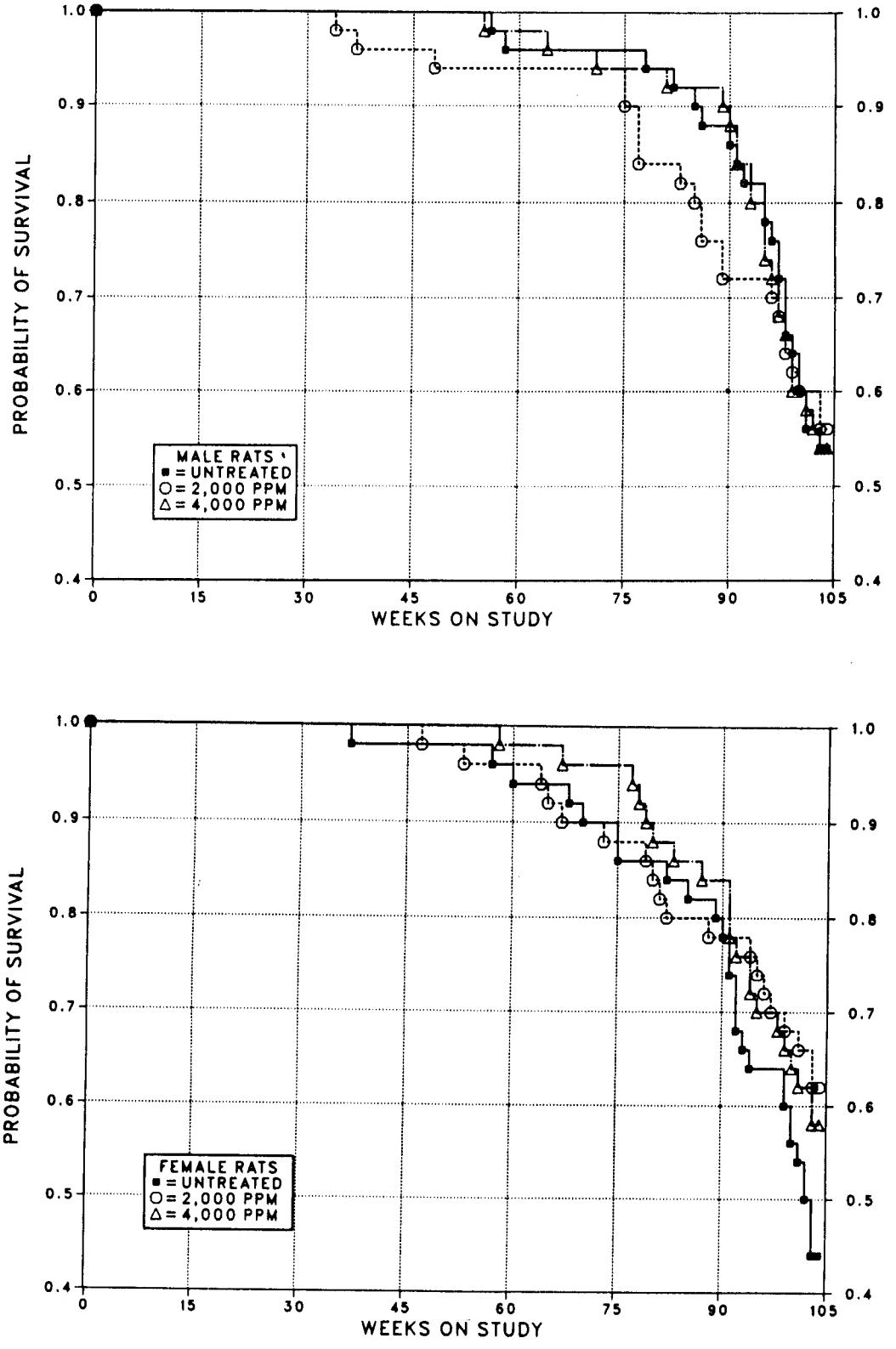

FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING NALIDIXIC ACID FOR TWO YEARS

Preputial Gland: Benign and malignant neoplasms of the preputial gland in male rats occurred with significant positive trends; the incidences in the dosed groups were significantly greater than those in controls (Table 6).

These neoplasms were classified as adenomas, papillomas, or carcinomas. The papilloma was an exophytic papillary mass protruding into the lumen of a dilated duct and consisted primarily of stratified squamous epithelium. Adenomas and carcinomas are part of a morphologic continuum. The adenomas were circumscribed masses consisting of glandular epithelium that retained some semblance of acinar structure. The carcinomas were generally not well circumscribed, were larger than the adenomas, and had irregular borders with infiltration of adjacent connective tissue by clusters and lobules of neoplastic cells. Cystic areas or duct-like channels lined by stratified squamous epithelium were present in both adenomas and carcinomas.

TABLE 6. PREPUTIAL GLAND LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID (a)

	Control	2,000 ppm (b)	4,000 ppm (b)
Epithelial Hyperplasia			
Overall Rates	1/49 (2%)	5/49 (10%)	1/47 (2%)
Adenoma			
Overall Rates	2/49 (4%)	10/49 (20%)	10/47 (21%)
Terminal Rates	0/27 (0%)	10/28 (36%)	5/26 (19%)
Week of First Observation	56	104	91
Incidental Tumor Tests	P=0.015	P=0.017	P=0.014
Papilloma			
Overall Rates	1/49 (2%)	0/49 (0%)	0/47 (0%)
Adenoma or Papilloma			
Overall Rates	3/49 (6%)	10/49 (20%)	10/47 (21%)
Terminal Rates	0/27 (0%)	10/28 (36%)	5/26 (19%)
Week of First Observation	56	104	91
Incidental Tumor Tests	P=0.033	P=0.035	P=0.034
Carcinoma			
Overall Rates	0/49 (0%)	10/49 (20%)	12/47 (26%)
Terminal Rates	0/27 (0%)	5/28 (18%)	7/26 (27%)
Week of First Observation		77	90
Incidental Tumor Tests	P<0.001	P=0.001	P<0.001
Adenoma, Papilloma, or Carcinoma (c)			
Overall Rates	3/49 (6%)	19/49 (39%)	20/47 (43%)
Terminal Rates	0/27 (0%)	14/28 (50%)	10/26 (38%)
Week of First Observation	56	77	90
Incidental Tumor Tests	P<0.001	P<0.001	P<0.001

(a) For a complete explanation of the entries in this table, see Table A3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods); denominator is the number of rats examined microscropically.
(b) The estimated dose in milligrams per kilogram per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.
(c) Historical incidence at study laboratory (mean ± SD): 31/399 (8% ± 6%); historical incidence in NTP studies: 118/1,596 (7% ± 5%)

Clitoral Gland: Benign and malignant neoplasms (combined) in female rats occurred with a significant positive trend; the incidences in the dosed groups were significantly greater than that in the controls (Table 7). The clitoral gland of female rats is analogous and morphologically similar to the preputial gland of male rats, and the clitoral gland neoplasms had the same morphologic features as their counterparts in the preputial gland. The spectrum of hyperplasia, papilloma, and carcinoma in the preputial and clitoral glands is illustrated in Figures 3 to 10.

Tongue: Squamous cell carcinomas were seen in two high dose male rats. The historical incidence of oral cavity neoplasms in untreated control male F344/N rats in NTP studies is 7/1,596 (0.4%).

Skin: Epithelial hyperplasia and inflammation with fibrosis were observed at increased incidences ($P<0.05$) in high dose male rats (epithelial hyperplasia: control, 0/50; low dose, 1/50; high dose, 6/50; inflammation with fibrosis: 0/50; 1/50; 5/50).

TABLE 7. CLITORAL GLAND LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID (a)

	Control	2,000 ppm	4,000 ppm
Epithelial Hyperplasia			
Overall Rates	3/46 (7%)	7/46 (15%)	5/47 (11%)
Papilloma			
Overall Rates	1/46 (2%)	1/46 (2%)	0/47(0%)
Adenoma			
Overall Rates	4/46 (9%)	10/46 (22%)	11/47 (23%)
Terminal Rates	4/21 (19%)	8/31 (26%)	5/27 (19%)
Week of First Observation	104	88	58
Incidental Tumor Tests	P=0.059	P=0.153	P=0.067
Adenoma or Papilloma			
Overall Rates	4/46 (9%)	11/46 (24%)	11/47 (23%)
Terminal Rates	4/21 (19%)	8/31 (26%)	5/27 (19%)
Week of First Observation	104	88	58
Incidental Tumor Tests	P=0.060	P=0.086	P=0.067
Papillary Carcinoma			
Overall Rates	0/46 (0%)	0/46 (0%)	1/47 (2%)
Carcinoma			
Overall Rates	1/46 (2%)	4/46 (9%)	4/47 (9%)
Carcinoma or Papillary Carcinoma			
Overall Rates	1/46 (2%)	4/46 (9%)	5/47 (11%)
Terminal Rates	0/21 (0%)	1/31 (3%)	2/27 (7%)
Week of First Observation	100	73	67
Incidental Tumor Tests	P=0.074	P=0.105	P=0.105
Adenoma, Papilloma, Carcinoma, or Papillary Carcinoma (b)			
Overall Rates	5/46 (11%)	15/46 (33%)	16/47 (34%)
Terminal Rates	4/21 (19%)	9/31 (29%)	7/27 (26%)
Week of First Observation	100	73	58
Incidental Tumor Tests	P=0.008	P=0.016	P=0.010

(a) For a complete explanation of the entries in this table, see Table B3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods); denominator is the number of rats examined microscropically.
(b) Historical incidence at study laboratory (mean ± SD): 44/397 (11% ± 6%); historical incidence in NTP studies: 116/1,643 (7% ± 5%)

Eye: The study protocol required the microscopic examination of eyes showing gross lesions at necropsy. The results are shown in Tables A5 and B5. Because the results suggested possible increased incidences of eye lesions in dosed female rats, the eyes from all remaining male and female rats were subsequently examined microscopically. The results of that complete evaluation are shown in Table 8. The incidences of retinal degeneration and cataracts were increased in dosed males and females. There appears to be a dose response in the incidences of cataracts in male rats. The incidences and mean severity of these eye lesions were generally similar in both dose groups of female rats. The cages containing the low dose groups of rats were on the top two tiers of the rack, the high dose groups were in the middle two tiers, and the controls were on the bottom two tiers. Diagrams with the incidences of these lesions by cage position on the racks are presented in Appendix J.

The cataracts were subcapsular and usually located near the equator of the lens. The larger, more extensive cataracts sometimes covered the entire anterior surface. The lens fibers in the affected area had lost their concentric laminated structure and dense eosinophilic staining to become pale and granular or foamy in appearance. Disintegration of the lens fibers resulted in the formation of vacuoles and amorphous debris. The retinal atrophy was characterized primarily by diminished cellularity of the outer nuclear layer, which is composed of the photoreceptor cells. The inner nuclear layer and the ganglion cell layer were affected to a much lesser extent.

Hematopoietic System: Mononuclear cell leukemia occurred with a significant negative trend in female rats; the incidences in the dosed groups were significantly lower than that in the controls (Table 9).

TABLE 8. OCULAR LESIONS IN RATS IN THE TWO-YEAR STUDIES OF NALIDIXIC ACID (a)

Site/Lesion	Control	2,000 ppm	4,000 ppm
MALE			
Retinal degeneration	4/48 (3.2)	41/48 (2.5)	47/49 (2.7)
Crystalline lens cataract	11/48 (1.6)	23/48 (1.3)	38/49 (1.4)
FEMALE			
Retinal degeneration	2/47 (1.5)	40/48 (2.6)	46/50 (2.6)
Crystalline lens cataract	0/47	18/48 (1.4)	14/50 (2.1)

(a) Number of animals affected/number of tissues examined; number in parentheses is average severity of affected tissues: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

TABLE 9. HEMATOPOIETIC SYSTEM TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID (a)

	Control	2,000 ppm	4,000 ppm
Mononuclear Cell Leukemia (b)			
Overall Rates	20/50 (40%)	9/50 (18%)	7/50 (14%)
Terminal Rates	5/22 (23%)	5/31 (16%)	0/29 (0%)
Week of First Observation	75	80	79
Life Table Tests	P=0.001N	P=0.008N	P=0.004N
Incidental Tumor Tests	P=0.002N	P=0.040N	P=0.004N

(a) For a complete explanation of the entries in this table, see Table B3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods).
(b) Historical incidence of leukemia at study laboratory (mean ± SD): 113/397 (28% ± 5%); historical incidence in NTP studies: 324/1,643 (20% ± 8%)

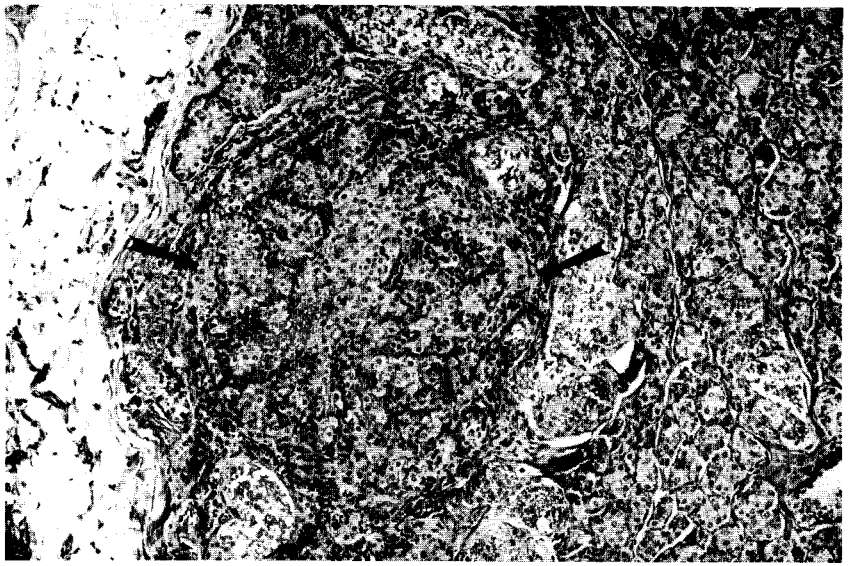

Figure 3. Focal hyperplasia (arrows) in the preputial gland of high dose male rat no. 73.

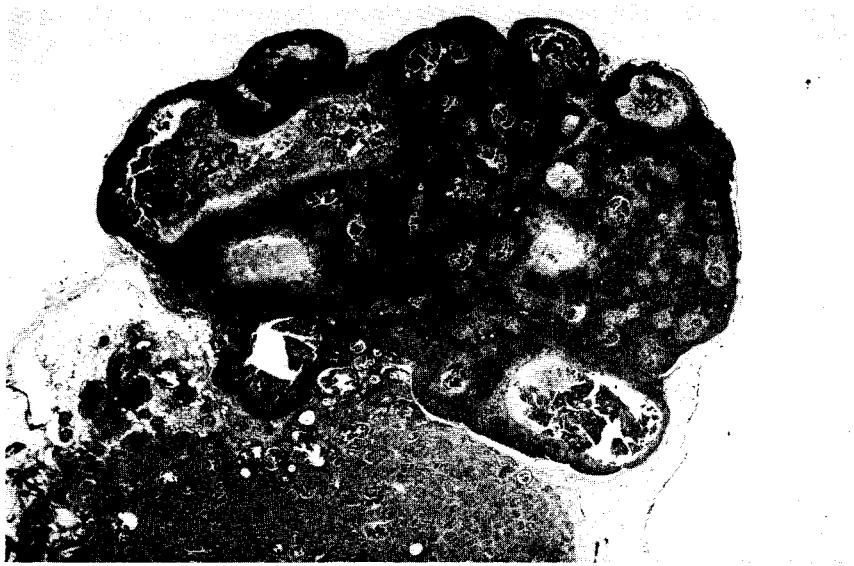

Figure 4. Preputial gland adenoma in high dose male rat no. 73. The neoplasm is well-demarcated without invasion of adjacent tissue. Note the channels containing secretory-like material resulting from the degeneration and necrosis of the neoplastic cells (the preputial gland has a holocrine type of secretion whereby the entire cell contributes to the secretory product).

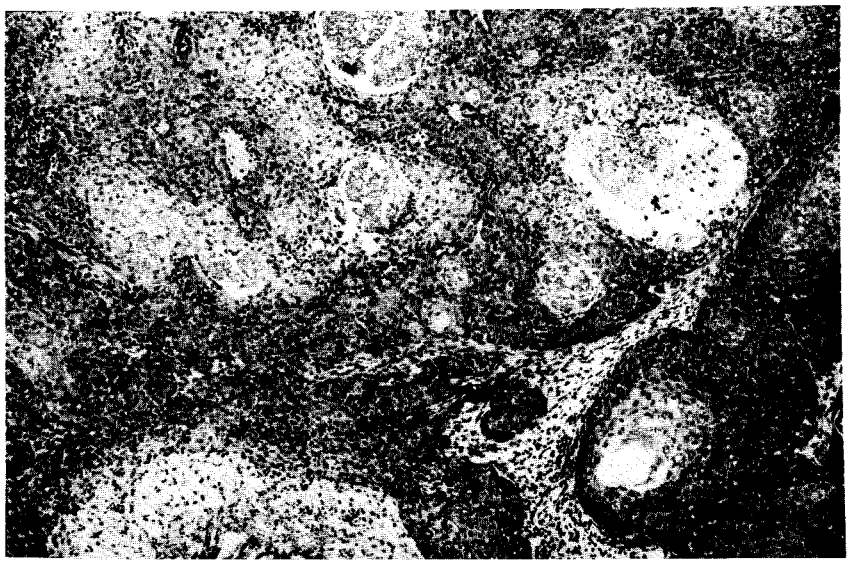

Figure 5. Higher magnification of adenoma in Figure 4.

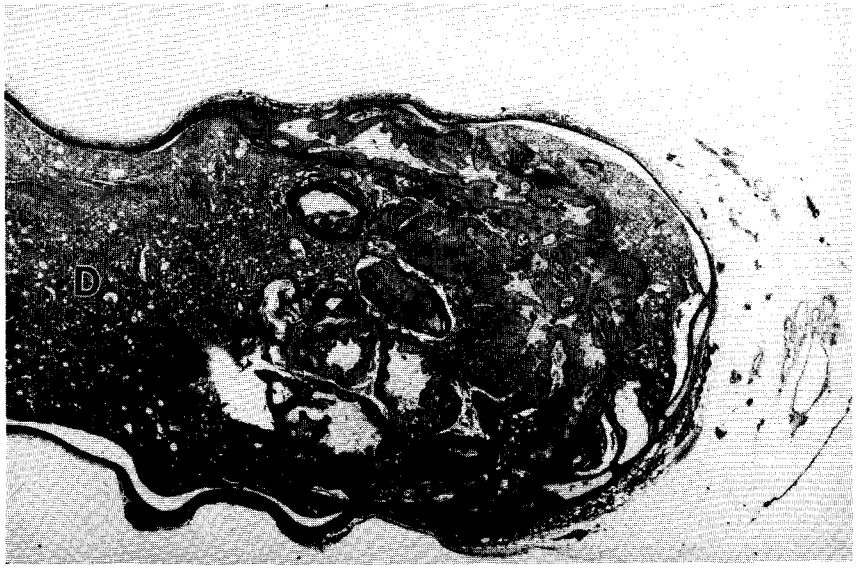

Figure 6. Clitoral gland papilloma in control female rat no. 122. Note the dilated excretory duct (D) filled with secretory debris.

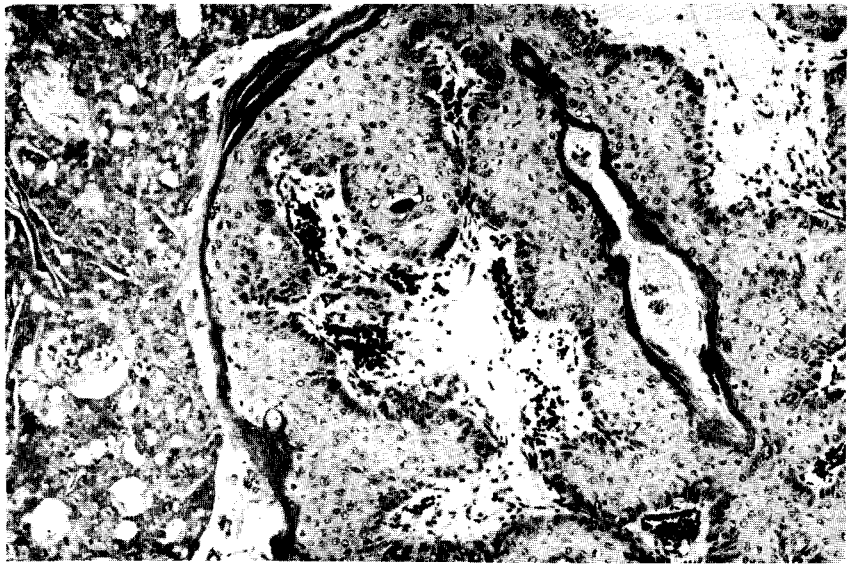

Figure 7. Higher magnification of papilloma in control female rat no. 122 showing the interconnecting cords of stratified squamous epithelium.

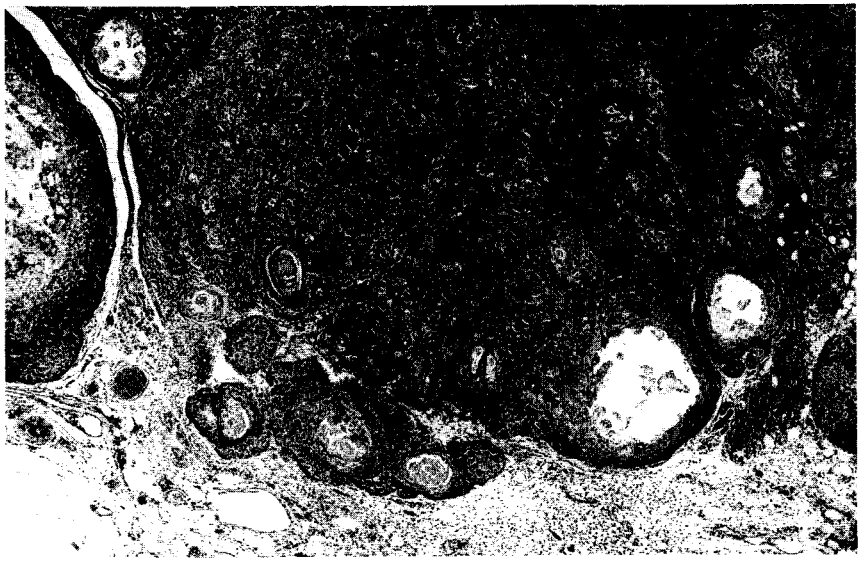

Figure 8. Preputial gland carcinoma in high dose male rat no. 95. Note the irregular border and small lobules of neoplastic glands invading adjacent tissue.

Figure 9. Higher magnification of preputial gland carcinoma in Figure 8. Note the loss of normal glandular structure.

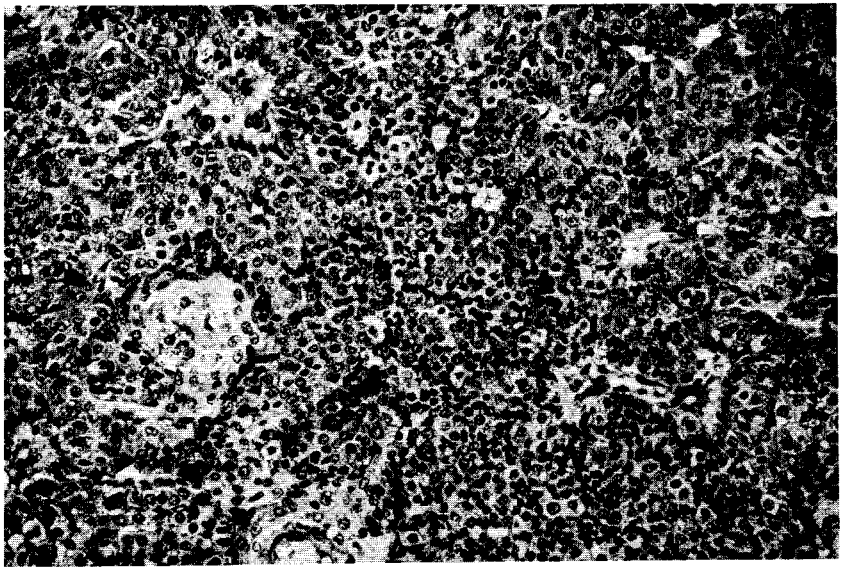

Figure 10. Cellular detail of preputial gland carcinoma in high dose male rat no. 95. Note the cellular pleomorphism.

Anterior Pituitary Gland: Adenomas in male rats occurred with a significant negative trend; the incidences in the dosed groups were significantly lower than that in the controls (Table 10). Carcinomas occurred in one control and one low dose male rat.

Mammary Gland: Fibroadenomas, adenocarcinomas, and fibroadenomas or adenocarcinomas (combined) in female rats occurred with significant negative trends; the incidence of fibroadenomas or adenocarcinomas (combined) in the high dose group was significantly lower than that in the controls (Table 11).

TABLE 10. ANTERIOR PITUITARY GLAND LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID (a)

	Control	2,000 ppm	4,000 ppm
Hyperplasia			
Overall Rates	1/49 (2%)	3/50 (6%)	2/50 (4%)
Adenoma (b)			
Overall Rates	(c) 11/49 (22%)	(d) 2/50 (4%)	2/50 (4%)
Terminal Rates	8/26 (31)	2/28 (7%)	1/27 (4%)
Week of First Observation	58	104	91
Incidental Tumor Tests	P=0.002N	P=0.007N	P=0.008N

(a) For a complete explanation of the entries in this table, see Table A3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods).
(b) Historical incidence of adenomas or carcinomas (combined) at study laboratory (mean ± SD): 95/390 (24% ± 9%); historical incidence in NTP studies: 400/1,540 (26% ± 10%)
(c) A carcinoma was seen in a 12th animal.
(d) A carcinoma was seen in a third animal.

TABLE 11. MAMMARY GLAND TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID (a)

	Control	2,000 ppm	4,000 ppm
Fibroadenoma			
Overall Rates	7/50 (14%)	6/50 (12%)	2/50 (4%)
Terminal Rates	2/22 (9%)	4/31 (13%)	0/29 (0%)
Week of First Observation	68	95	94
Incidental Tumor Tests	P=0.086N	P=0.540N	P=0.122N
Adenocarcinoma			
Overall Rates	3/50 (6%)	1/50 (2%)	0/50 (0%)
Terminal Rates	1/22 (5%)	1/31 (3%)	0/29 (0%)
Week of First Observation	92	104	
Incidental Tumor Tests	P=0.062N	P=0.330N	P=0.130N
Fibroadenoma or Adenocarcinoma (b)			
Overall Rates	10/50 (20%)	7/50 (14%)	2/50 (4%)
Terminal Rates	3/22 (14%)	5/31 (16%)	0/29 (0%)
Week of First Observation	68	95	94
Incidental Tumor Tests	P=0.017N	P=0.343N	P=0.026N

(a) For a complete explanation of the entries in this table, see Table B3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods).
(b) Historical incidence at study laboratory (mean ± SD): 114/397 (29% ± 10%); historical incidence in NTP studies: 552/1,643 (34% ± 12%)

THIRTEEN-WEEK STUDIES

No compound-related deaths occurred (Table 12). The final mean body weights of mice that received 8,000 or 16,000 ppm nalidixic acid were 10% or 13% lower than that of controls for males and 16% or 20% lower for females. Feed consumption by dosed groups was similar to that by controls. Liver weight to body weight ratios were significantly greater than those for controls for males at 2,000, 8,000, and 16,000 ppm and for females at 4,000, 8,000, and 16,000 ppm (Table 13). No compound-related histopathologic effects were observed.

Dose Selection Rationale: Because of lower body weight gain at higher doses, dietary concentrations of nalidixic acid selected for mice for the 2-year studies were 2,000 ppm and 4,000 ppm.

TABLE 12. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF NALIDIXIC ACID

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 6	Week 13
MALE							
0	10/10	25.9 ± 0.4	36.1 ± 0.9	+10.2 ± 0.6		4.0	3.9
1,000	10/10	25.2 ± 0.6	35.0 ± 0.6	+9.8 ± 0.4	97.0	3.8	3.7
2,000	10/10	25.0 ± 0.6	34.9 ± 0.7	+9.9 ± 0.5	96.7	3.7	3.3
4,000	10/10	24.1 ± 0.3	33.6 ± 0.4	+9.5 ± 0.5	93.1	3.7	3.3
8,000	10/10	23.8 ± 0.5	32.4 ± 0.5	+8.6 ± 0.5	89.8	3.9	3.3
16,000	10/10	22.5 ± 0.6	31.4 ± 0.7	+8.9 ± 0.4	87.0	3.6	3.1
FEMALE							
0	10/10	20.9 ± 0.5	29.4 ± 1.1	+8.5 ± 0.9		3.1	3.0
1,000	10/10	21.0 ± 0.3	28.2 ± 0.2	+7.2 ± 1.1	95.9	3.0	3.1
2,000	(e) 9/10	21.4 ± 0.4	28.7 ± 1.1	+7.4 ± 0.8	97.6	3.4	2.9
4,000	10/10	21.5 ± 0.5	27.1 ± 0.4	+5.6 ± 0.4	92.2	3.0	3.1
8,000	10/10	20.3 ± 0.3	24.8 ± 0.8	+4.5 ± 0.7	84.4	3.2	3.1
16,000	10/10	19.5 ± 0.4	23.6 ± 0.5	+4.1 ± 0.3	80.3	3.2	3.0

(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) Grams per animal per day; not corrected for scatter.
(e) Week of death: 4

TABLE 13. LIVER WEIGHTS FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF NALIDIXIC ACID (a)

Concentration (ppm)	Number Weighed	Final Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Final Body Weight (mg/g)
MALE				
0	10	36.1 ± 0.89	1,842 ± 114	50.9 ± 2.77
1,000	10	35.0 ± 0.64	2,020 ± 89	57.8 ± 2.21
2,000	10	34.9 ± 0.71	2,107 ± 100	*60.5 ± 3.10
4,000	10	*33.6 ± 0.41	1,892 ± 64	56.4 ± 1.75
8,000	10	**32.4 ± 0.47	2,097 ± 89	**64.7 ± 2.56
16,000	10	**31.4 ± 0.71	2,009 ± 93	**64.0 ± 2.40
FEMALE				
0	10	29.4 ± 1.13	1,324 ± 55	45.1 ± 1.05
1,000	10	28.2 ± 1.18	1,318 ± 37	47.3 ± 2.14
2,000	9	28.7 ± 1.06	1,423 ± 45	50.0 ± 1.85
4,000	10	27.1 ± 0.42	1,444 ± 65	**53.3 ± 1.98
8,000	10	**24.8 ± 0.84	1,486 ± 59	**59.8 ± 1.30
16,000	10	**23.6 ± 0.55	1,319 ± 32	**55.9 ± 1.33

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

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of high dose male mice were 1%-8% lower than those of controls throughout most of the study (Table 14 and Figure 11). Mean body weights of low dose and control male mice were similar. Mean body weights of high dose female mice were 5%-10% lower than those of controls from week 12 to week 27 and then were 11%-17% lower. Mean body weights of low dose female mice were 7%-11% lower than those of controls from week 39 to the end of the study. The average daily feed consumption by low dose and high dose male mice was 98% and 102% that by controls and by low dose and high dose female mice, 97% and 100% that by controls (Tables F3 and F4). The average amount of nalidixic acid consumed per day was approximately 225 or 490 mg/kg for low dose or high dose male mice and 220 or 460 mg/kg for low dose or high dose female mice. No compound-related clinical signs were observed. Fighting among male cagemates was observed in all groups.

TABLE 14. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

Weeks on Study	Control		2,000 ppm			4,000 ppm		
	Av. Wt. (grams)	Number of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	Number of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	Number of Survivors
MALE								
0	21.9	50	21.9	100	50	21.6	99	50
1	24.4	50	24.7	101	50	24.1	99	50
2	25.7	50	25.4	99	50	25.5	99	50
3	26.9	50	26.6	99	50	26.2	97	50
4	27.5	50	28.3	103	50	27.2	99	50
5	28.3	50	27.6	98	50	27.2	96	50
6	28.8	50	(a) 24.9	86	50	(a) 26.4	92	49
7	29.6	50	29.2	99	50	28.7	97	49
8	29.9	49	29.4	98	50	29.0	97	49
9	30.9	49	30.2	98	50	29.6	96	49
10	30.5	49	30.6	100	50	30.1	99	49
11	31.0	49	30.5	98	50	29.8	96	49
12	31.2	49	30.8	99	50	30.0	96	48
13	31.8	49	31.4	99	50	30.8	97	48
14	32.4	49	31.7	98	50	30.9	95	48
18	33.0	48	32.9	100	50	32.3	98	47
22	34.0	48	33.8	99	50	33.3	98	44
26	35.0	48	34.7	99	50	33.7	96	44
30	35.7	47	35.5	99	49	34.6	97	44
34	36.6	44	36.3	99	48	35.1	96	44
39	37.1	43	36.6	99	45	35.5	96	44
42	37.2	43	36.6	98	45	35.2	95	44
46	37.9	43	36.7	97	45	35.5	94	44
51	38.1	43	37.8	99	45	36.0	94	44
56	38.7	43	38.1	98	45	36.3	94	44
60	37.7	43	37.7	100	45	35.5	94	44
65	37.6	43	37.8	101	45	35.8	95	44
69	38.5	43	38.0	99	45	36.4	95	44
74	37.6	43	37.6	100	45	35.5	94	44
78	38.2	43	38.3	100	45	36.1	95	44
82	38.6	41	38.2	99	43	36.1	94	43
87	38.3	40	38.0	99	42	35.9	94	41
91	38.2	37	38.0	99	42	35.7	93	40
96	38.1	36	37.4	98	40	35.5	93	38
99	37.4	35	37.2	99	38	35.4	95	35
104	36.3	33	37.1	102	34	35.5	98	31
FEMALE								
0	18.1	50	18.3	101	50	18.4	102	50
1	18.8	50	18.9	101	50	18.9	101	50
2	19.8	50	18.6	94	50	18.8	95	50
3	20.3	50	19.3	95	50	19.6	97	50
4	21.4	50	21.6	101	50	20.7	97	50
5	21.9	50	21.5	98	50	21.2	97	50
6	22.9	50	22.4	98	50	22.3	97	50
7	23.1	50	23.1	100	50	22.7	98	50
8	23.5	50	23.2	99	50	22.7	97	50
9	23.6	50	23.4	99	50	22.6	96	50
10	24.0	50	23.9	100	50	23.5	98	50
11	24.7	50	24.5	99	50	24.0	97	50
12	25.4	50	24.8	98	50	24.1	95	50
15	26.3	50	25.4	97	50	24.9	95	50
19	28.0	50	26.3	94	50	26.1	93	50
23	29.3	50	28.0	96	50	27.0	92	50
27	31.3	50	29.7	95	50	28.2	90	50
31	32.4	50	30.4	94	50	28.7	89	50
36	33.1	49	30.8	93	50	29.1	88	49
39	33.1	49	30.9	93	50	29.1	88	49
43	34.2	49	31.4	92	50	29.8	87	49
48	35.1	49	31.7	90	50	30.2	86	49
53	36.5	49	32.4	89	50	30.9	85	49
57	36.8	49	32.7	89	50	31.2	85	49
62	37.2	49	33.5	90	49	31.6	85	49
66	36.6	49	33.5	92	49	31.4	86	49
71	37.1	49	33.1	89	49	31.6	85	47
75	38.7	48	35.5	92	49	33.4	86	46
79	39.1	48	36.1	92	49	34.0	87	46
84	39.6	48	36.9	93	49	34.2	86	45
88	40.9	47	37.3	91	48	34.9	85	44
93	41.0	46	37.9	92	47	34.3	84	40
96	41.0	46	38.3	93	47	34.3	84	39
101	42.3	41	39.2	93	45	35.1	83	36

(a) Some cages of low dose and high dose male mice experienced weight loss because of insufficient feed from 5/20/81 to 5/28/81 (weeks 5 and 6).

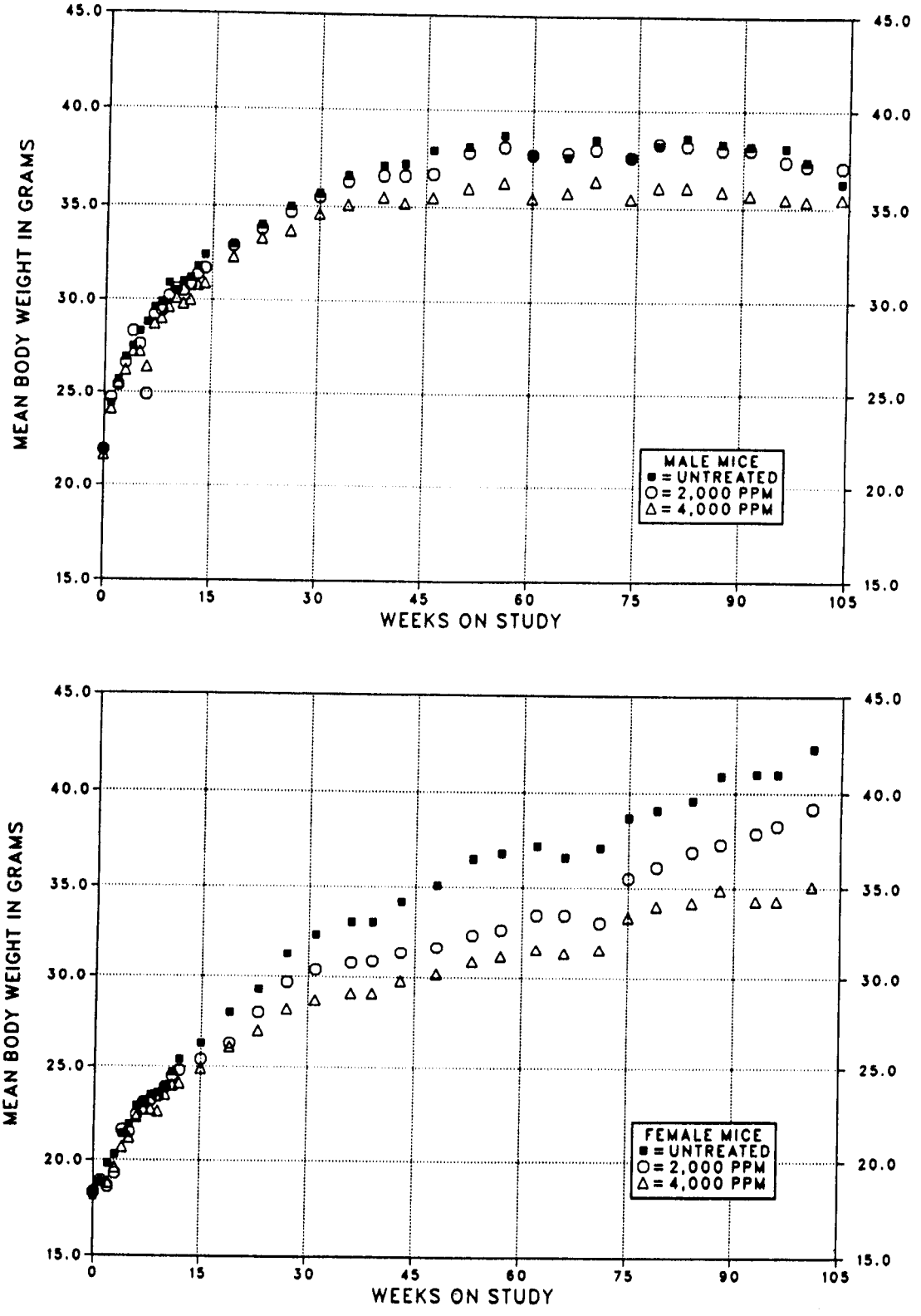

FIGURE 11. GROWTH CURVES FOR MICE FED DIETS CONTAINING NALIDIXIC ACID FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female mice fed diets containing nalidixic acid at the concentrations used in these studies and for controls are shown in Table 15 and in the Kaplan and Meier curves in Figure 12. No significant differences in survival were seen between any groups of either sex.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the subcutaneous tissue, lung, and ovary.

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 15. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

	Control	2,000 ppm	4,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Natural deaths	2	4	5
Moribund kills	15	12	14
Animals surviving until study termination	33	34	31
Survival P values (b)	0.799	0.861	0.870
FEMALE (a)			
Animals initially in study	50	50	50
Natural deaths	3	0	4
Moribund kills	7	7	14
Animals surviving until study termination	40	43	32
Survival P values (b)	0.067	0.563	0.108

(a) Termination period: week 104
(b) 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.

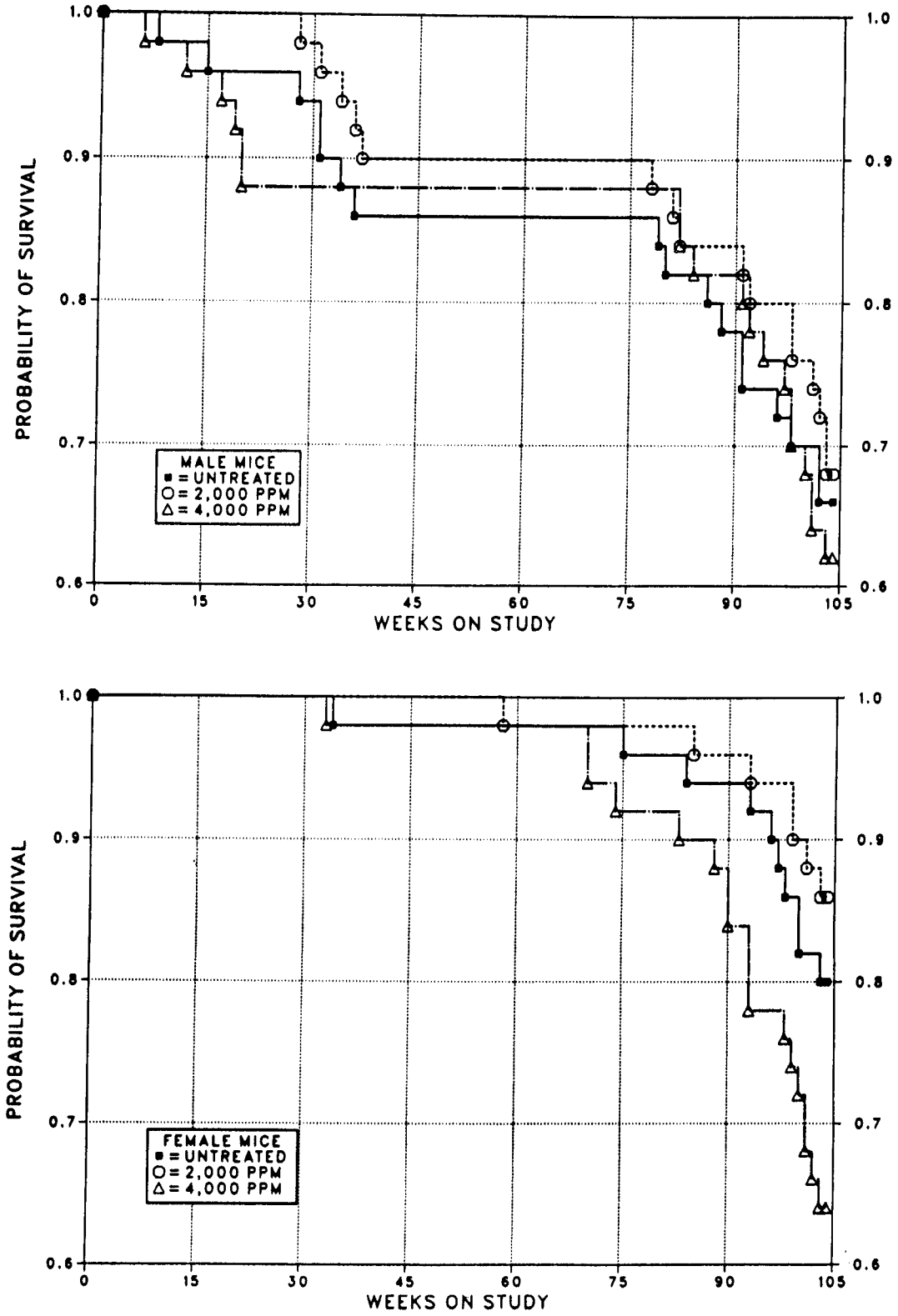

FIGURE 12. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING NALIDIXIC ACID FOR TWO YEARS

Subcutaneous Tissue: Fibrosarcomas and fibromas or fibrosarcomas (combined) in male mice occurred with significant positive trends; the incidences in the high dose group were significantly greater than those in the controls (Table 16). Photomicrographs comparing the fibromas and fibrosarcomas are presented in Figures 13 to 16.

Lung: Chronic bronchopneumonia was observed at increased incidences in dosed male mice (male: control, 0/49; low dose, 11/50, $P<0.01$; high dose, 6/50, $P<0.05$; female: 5/49; 5/50; 2/49).

Ovary: Neoplasms were observed in dosed but not in control female mice (Table 17). The historical incidence of ovarian neoplasms (cystadenomas, papillary cystadenomas, adenocarcinomas, adenomas, or papillary adenomas, combined) in $B6C3F_1$ mice in NTP studies is 14/1,577 (0.9%), and the highest observed incidence is 2/43. The historical incidence of luteomas, granulosa cell tumors, or granulosa cell carcinomas (combined) in NTP studies is 14/1,577 (0.9%), and the highest observed incidence is 3/47.

TABLE 16. SUBCUTANEOUS TISSUE TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID (a)

	Control	2,000 ppm (b)	4,000 ppm (b)
Fibroma			
Overall Rates	1/50 (2%)	3/50 (6%)	3/50 (6%)
Fibrosarcoma			
Overall Rates	4/50 (8%)	6/50 (12%)	11/50 (22%)
Terminal Rates	1/33 (3%)	2/34 (6%)	4/31 (13%)
Week of First Observation	88	81	84
Incidental Tumor Tests	P=0.048	P=0.339	P=0.080
Fibroma or Fibrosarcoma (c)			
Overall Rates	5/50 (10%)	9/50 (18%)	14/50 (28%)
Terminal Rates	2/33 (6%)	4/34 (12%)	7/31 (23%)
Week of First Observation	88	81	84
Incidental Tumor Tests	P=0.024	P=0.186	P=0.035

(a) For a complete explanation of the entries in this table, see Table C3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods).
(b) The estimated dose in milligrams per kilogram per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.
(c) Historical incidence at study laboratory (mean ± SD): 42/399 (11% ± 11%); historical incidence in NTP studies: 186/1,692 (11% ± 9%)

TABLE 17. NUMBERS OF MICE WITH OVARIAN NEOPLASMS IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID

	Control	2,000 ppm	4,000 ppm
Number examined	49	50	48
Cystadenoma, NOS	0	2	0
Luteoma	0	0	1
Granulosa cell tumor	0	1	1
Granulosa cell carcinoma	0	2	0
Tubular adenoma	0	1	0
Luteoma, granulosa cell tumor, or granulosa cell carcinoma (combined)	0	3	2

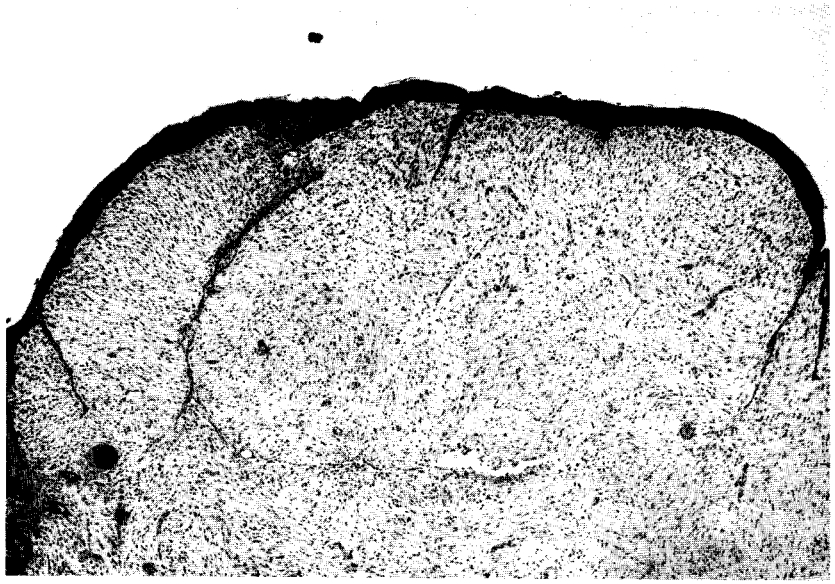

Figure 13. Subcutaneous tissue fibroma in control male mouse no. 145.

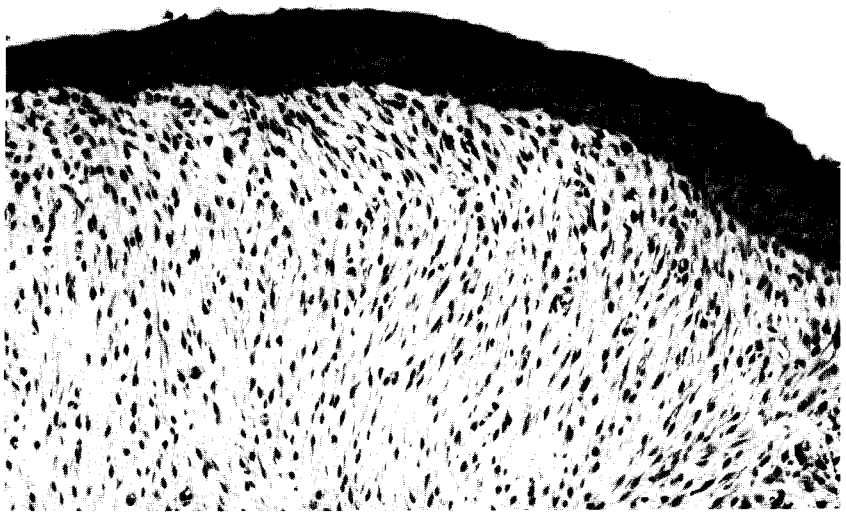

Figure 14. Higher magnification of fibroma shown in Figure 13. Note the uniform elongated nuclei of the neoplastic fibrocytes and relatively abundant intercellular collagen.

Figure 15. Subcutaneous tissue fibrosarcoma in high dose male mouse no. 72.

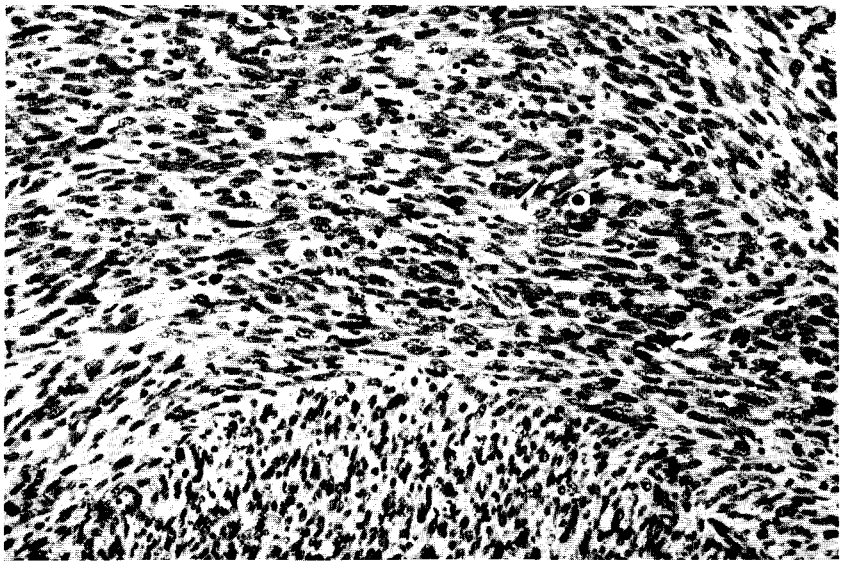

Figure 16. Higher magnification of fibrosarcoma shown in Figure 15. Note the greater degree of cellularity and anaplasia compared with the fibroma in Figures 13 and 14.

Nalidixic acid was not mutagenic in any of several in vitro short-term tests. No gene reversion was observed in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 after exposure to nalidixic acid in either the presence or absence of exogenous metabolic activation. Results of tests for induction of trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells were negative with or without metabolic activation. In Chinese hamster ovary cells, nalidixic acid did not induce sister chromatid exchanges or chromosomal aberrations in either the presence or absence of activation.

The experimental procedures and results are presented in Appendix I.

IV. DISCUSSION AND CONCLUSIONS

IV. DISCUSSION AND CONCLUSIONS

Nalidixic acid was evaluated for toxicity and carcinogenicity in 13-week and 2-year studies. The chemical was administered in the diet to F344/N rats and B6C3F$_1$ mice of each sex. Nalidixic acid was studied because of its widespread use as an antimicrobial agent for urinary tract infections.

In vitro genetic toxicology studies were performed with *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary cells. Nalidixic acid showed no evidence of genotoxicity in any of these short-term tests.

In the 13-week studies, the only compound-related death was one female rat in the 16,000-ppm group. Final body weights were lower in groups of male rats and mice given 4,000 ppm or more and in groups of female rats and mice given 8,000 ppm or more. Relative liver weights were increased at dietary concentrations as low as 2,000 ppm (male rats and male mice). In mice, feed consumption by dosed groups was similar to that by control groups, and no compound-related histopathologic lesions were seen. Male rats in the 16,000-ppm group consumed only about two-thirds as much feed as controls and weighed approximately half as much at the end of the study. In all rats in this group, a severe degeneration of the germinal epithelium of the seminiferous tubules of the testis was observed. There are several possible explanations for this finding: (1) Depressed feed intake and low body weight have been associated with testicular atrophy. Sprague Dawley rats whose feed consumption was restricted to 67% that of males fed ad libitum for 2 years had testes that were normal; 90% contained sperm (Berg, 1960). However, a group restricted to 54% of feed consumption by the freely feeding group had atrophic seminiferous tubules. (2) Nalidixic acid could possibly affect testicular function by complexing with metal ions. Zinc is necessary for normal testicular function in rats (Oishi and Hiraga, 1980), and there has been concern that chemicals causing zinc depletion may interfere with spermatogenesis (Thomas et al., 1982). Nalidixic acid has been shown to chelate zinc and other divalent cations (G.S. Goldstein, Sterling Drug Inc., personal communication to Bureau of Drugs, 1977). However, no measurements of zinc levels were performed in either the 13-week or 2-year studies in rats, the levels of free nalidixic acid reaching the testes were probably relatively low, and the nalidixic acid was probably protein bound. The hypothesis that nalidixic acid may cause decreased zinc levels in the testis has not been tested. (3) An interference with DNA replication may have caused the depletion of germ cells in the testis by interfering with divisions of spermatogonia, stem cells that produce spermatozoa. Nalidixic acid is thought to interfere with DNA replication by complexing with the α-subunit of DNA gyrase (Cozzarelli, 1980; McCoy et al., 1980), thus interfering with bacterial DNA replication/repair by disruption of the supercoiling process. However, bacterial gyrase is structurally and functionally different from human topoisomerase II (Compendium, 1988).

Nalidixic acid, like many other weak acids, is rapidly absorbed from the gastrointestinal tract of humans, rats, and mice (Rossoff, 1974; Gleckman et al., 1979); it then undergoes metabolism to hydroxynalidixic acid and/or the glucuronide or dicarboxylic acid derivatives, has a plasma half-life of about 2 hours (Mannisto, 1976; Martindale, 1977), and is excreted in the urine. These attributes would tend to limit the possibility of cumulative toxicity, and no clearly chemical-related toxic effects were observed in the 13-week studies.

The increase in relative liver weight for both rats (20%-90% increase) and mice (30% increase) may be biologically significant. No histopathologic lesions of the liver were associated with dietary exposure in either species, suggesting that the increase in relative weight for male rats was probably caused by proliferation of smooth endoplasmic reticulum and increased levels of hepatocyte microsomal enzymes, although these were not specifically examined. The lower final mean body weights affected the liver to body weight ratios, but absolute increases in liver weight were also observed at concentrations below 16,000 ppm in male rats.

Dietary concentrations for the 2-year studies in rats and mice were set at 2,000 and 4,000 ppm for both males and females. These levels were based primarily on body weight changes in the 13-week studies. In both species, dietary

concentrations of 8,000 ppm resulted in more than a 10% reduction in final body weight relative to control values after 13 weeks.

In the 2-year studies in rats, mean body weights of high dose male and female and low dose male groups were lower than those of control groups. Survival of rats in dosed groups was similar to that in control groups. Feed consumption was reduced by about 5% (at 2,000 ppm) to 10% (at 4,000 ppm) in dosed groups relative to controls.

Substantially increased incidences of neoplasms of the preputial gland and clitoral gland were observed in rats administered nalidixic acid for 2 years. The incidences of preputial gland adenomas and carcinomas in high dose male rats showed increases (adenomas, 21%; carcinomas, 26%) (see Table 6) far in excess of the control incidence (adenomas, 4%; carcinomas, 0%) or the historical control incidence at the study laboratory (adenomas, 6%; carcinomas, 1.5%) and in NTP studies (adenomas, 4%; carcinomas, 3%) (Table A4a). The incidences in historical control groups are based largely on the histopathologic evaluation of glands macroscopically observed to be enlarged, and it is possible that a small percentage of these neoplasms were missed in earlier studies. In the current studies, nearly all preputial and clitoral glands were examined microscopically. The effect of nalidixic acid on clitoral gland neoplasms was not as strong as the effect on the preputial gland. Clitoral gland neoplasms (collectively) were increased in the dosed groups (33% or 34%) (see Table 7) relative to the concurrent control group (11%) or the historical control incidence at the study laboratory (11%) and in NTP studies (7%) (Table B4a). These neoplasms were also first detected earlier in nalidixic acid groups (2,000 ppm, week 73; 4,000 ppm, week 58) than in the control group (week 100). In both the low dose and high dose groups, five neoplasms were observed before week 100. In most NTP studies, clitoral gland neoplasms in untreated controls are not found before week 100. There were only three studies in the data base in which lesions were observed before week 90 (at weeks 80, 86, and 88). The chemicals 5-nitro-*o*-anisidine (NCI, 1978a), 1,5-napthalenediamine (NCI, 1978b), glycidol (NTP, 1990a), and 5-nitroacenaphthene (NCI, 1978c) have also induced clitoral gland neoplasms in rats. In male rats, 2,4-diaminoanisole sulfate (NCI, 1978d) and 3,3'-dimethoxybenzidine dihydrochloride (NTP, 1990b) caused increases in preputial gland neoplasms. The clitoral gland of the female is homologous to the preputial gland of the male and that both had increased incidences of neoplasms. It is possible that there is a relationship between these tumor sites and proximity to the urinary tract. These increases in preputial and clitoral neoplasms constitute clear evidence of carcinogenicity for male and female rats.

Squamous cell carcinomas of the tongue, a relatively uncommon neoplasm (0.3% historical incidence of oral cavity neoplasms at the study laboratory; the highest incidence in untreated controls in any NTP study was 2/49) were observed in 2/49 high dose male rats in this study. From the limited information available from the data base, chemical induction of these neoplasms is usually associated with genotoxic compounds (as are preputial/clitoral gland neoplasms) (Ashby and Tennant, 1988) and is usually considered to be the result of direct contact of a chemical. Nalidixic acid's limited genotoxicity is probably restricted to indirect effects of secondary influences on DNA replication and repair and lacks the response spectrum commonly associated with chemicals that induce preputial and clitoral gland tumors. Although the response in the preputial and clitoral glands is unambiguous, the low incidence of oral cavity neoplasms cannot be related with certainty to administration of nalidixic acid. There were no preneoplastic lesions (hyperplasia or papillomas) in the oral cavity or preneoplastic or neoplastic lesions in the esophagus or forestomach (organs lined by stratified squamous epithelium) to support the association of squamous cell carcinoma with administration of nalidixic acid.

In contrast to the above findings, several neoplasms commonly observed in untreated control F344/N rats occurred at reduced incidences in those groups administered nalidixic acid. In male rats, anterior pituitary gland adenomas were decreased in dosed groups (4% each) compared with the concurrent control group (22%). High dose female rats had decreased incidences of leukemia (control, 40%; 4,000 ppm, 14%) and mammary gland neoplasms (control, 20%; 4,000

ppm, 4%). Female rats in the control group had a greater incidence of leukemia than the historical incidence at the study laboratory (28%) or on the overall mean control incidence in NTP studies (20%). Thus, the dosed groups did not show as large a decrease in the incidence of leukemia compared with historical controls as they did compared with concurrent controls. The cause of the reductions in pituitary gland and mammary gland neoplasms in the dosed groups is not known. One possible explanation, at least for the mammary neoplasms, is that reduced body weight may have been a factor. Rao et al. (1987) showed a relationship between body weight at 18 months and the incidence of benign mammary neoplasms. The mean body weight of the nalidixic acid control group was about 300 g at 18 months, compared with 250 g in the 4,000-ppm group (see Table 4). The estimated tumor incidence for the control group determined by using the regression line of Rao et al. (1987) for benign mammary neoplasms would be about 30% and that for the 4,000-ppm group would be approximately 6%.

Retinal degeneration and cataracts in rats have been reported to result from high levels of light (Rao, 1988). In these studies of nalidixic acid, significant increases in these lesions were observed in dosed groups of both male and female rats. There appeared to be a dose response in the incidence of cataracts in male rats, and the incidence and mean severity of these eye lesions were generally similar in both dosed groups of female rats. Cage placement may have contributed to these findings, since the low dose groups were on the top two tiers of the rack and the high dose groups were on the middle two tiers of the rack and the cages were not rotated. Because eye lesions were identified relatively early in the studies, a veterinary ophthalmologist examined the eyes of a number of rats. The examinations were inconclusive as to the etiology. Light intensity measurements in the study room were made during the course of these studies, and the intensity was found to decline from the top to bottom of each rack. Light intensity was higher near cage racks containing male rats, a finding that is in accord with the slightly higher incidence of cataracts in dosed males than in females, but light intensity within cages was not measured. Although the data support an association between cataracts and retinal degeneration and chemical exposure, there is some uncertainty because these eye lesions are frequently associated with high-intensity lighting and the cages were not rotated in these studies. Additional research is needed to confirm a relationship between nalidixic acid and eye lesions.

There was some speculation before the start of this study that an antimicrobial agent with the urinary tract as a target organ may affect development of kidney lesions in older rats. As judged by data generated during these studies, nalidixic acid did not reduce the incidence or severity of the spontaneous age-related nephropathy in rats or cause specific kidney lesions.

In the 2-year studies of nalidixic acid in mice, high dose animals of each sex and low dose females weighed 1%-17% less than their respective controls. Survival was not affected by compound administration. Feed consumption by groups administered nalidixic acid was similar to values by control groups. Certain early deaths of male mice were attributed to fighting among cagemates.

The incidence of subcutaneous fibromas or fibrosarcomas (combined) in the high dose male group (28%) was marginally greater than in the concurrent control group (10%) or historical control groups (11%). However, these tumors are variable in incidence, and the incidence in the high dose males is well within the range of historical controls. Although the marginal increase in subcutaneous neoplasms in these studies could possibly be chemically related, an increase in similar neoplasms was not reported by Kurokawa et al. (1986), who used 1,600 ppm nalidixic acid as the highest dietary concentration. It is probable that the shorter exposure duration (76 weeks), lower dose, or different mouse strain contributed to the difference in the results obtained in that study and in the current NTP studies.

Chronic bronchopneumonia in male mice was observed at incidences of 0% in the control group, 22% in the 2,000-ppm group, and 12% in the 4,000-ppm group. Serologic analysis indicated that mice in these studies were infected by Sendai virus. According to Hall et al. (1986), animals with Sendai are predisposed to secondary

bacterial and mycoplasmal infections. These infections are not usually severe enough to cause death but may interfere with intrinsic host defense mechanisms. Thus, it is possible that Sendai virus infections together with nalidixic acid affected the incidences of bronchopneumonia in these studies. The reason that bronchopneumonia did not occur in control males is unknown; however, nalidixic acid at the levels administered may have increased the susceptibility of the dosed mice to the infection.

Three mice in the 2,000-ppm group and two mice in the 4,000-ppm group had ovarian luteomas or granulosa cell tumors or granulosa cell carcinomas (combined). Although relatively uncommon in untreated control mice in NTP studies, these few neoplasms are not considered to be associated with nalidixic acid exposure.

For the studies in mice, it is unlikely that higher doses of nalidixic acid would have been tolerated. At the end of the 2-year study, female mice in the 4,000-ppm group weighed 17% less than the controls, a decrease similar to that observed after consumption of 8,000 ppm nalidixic acid in feed for 13 weeks. There was more than a 10% body weight reduction in male mice consuming 8,000 ppm nalidixic acid after 13 weeks of exposure.

Many of the side effects associated with nalidixic acid administration to some humans were not detected in these studies. Allergic reactions and central nervous system effects were not observed in rats or mice. Feed consumption was marginally decreased in rats but not in mice; the cause of the reductions is not known but could be due to feed aversion or to gastrointestinal disturbance.

The experimental and tabulated data for the NTP Technical Report on nalidixic acid were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix K, 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 feed studies, there was *clear evidence of carcinogenic activity** of nalidixic acid for F344/N rats, as indicated by increased incidences of preputial gland neoplasms in males and clitoral gland neoplasms in females. There was *equivocal evidence of carcinogenic activity* for male B6C3F$_1$ mice fed diets containing nalidixic acid, as indicated by marginally increased incidences of subcutaneous tissue neoplasms. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice fed diets containing 2,000 or 4,000 ppm nalidixic acid for 2 years.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 9.

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID

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

	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)
Papilloma, NOS	1 (2%)		
Squamous cell carcinoma			1 (2%)
Basal cell tumor			1 (2%)
Keratoacanthoma		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Carcinoma, NOS, metastatic	1 (2%)		
Fibroma	4 (8%)	3 (6%)	1 (2%)
Fibrosarcoma		2 (4%)	1 (2%)
Neurilemoma, malignant		1 (2%)	
RESPIRATORY SYSTEM			
#Nasal mucosa	(50)	(50)	(50)
Adenoma, NOS			1 (2%)
#Lung	(50)	(18)	(50)
Carcinoma, NOS, metastatic	1 (2%)		
Alveolar/bronchiolar adenoma		1 (6%)	
Pheochromocytoma, metastatic			1 (2%)
Osteosarcoma, metastatic			1 (2%)
Chordoma, metastatic			1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, histiocytic type	1 (2%)		
Leukemia, mononuclear cell	22 (44%)	11 (22%)	17 (34%)
#Spleen	(50)	(31)	(50)
Leukemia, mononuclear cell		2 (6%)	
#Mandibular lymph node	(49)	(14)	(50)
Carcinoma, NOS, metastatic	1 (2%)		
CIRCULATORY SYSTEM			
#Spleen	(50)	(31)	(50)
Hemangiosarcoma		1 (3%)	
*Soft tissue	(50)	(50)	(50)
Hemangioma			1 (2%)
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(50)
Squamous cell carcinoma			2 (4%)
#Salivary gland	(50)	(14)	(49)
Adenoma, NOS	1 (2%)		
#Liver	(50)	(44)	(50)
Neoplastic nodule	1 (2%)	1 (2%)	1 (2%)
Hepatocellular carcinoma	1 (2%)		
Histiocytic sarcoma			1 (2%)
#Jejunum	(50)	(15)	(50)
Neurilemoma, malignant	1 (2%)		

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

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney	(50)	(17)	(50)
Tubular cell adenoma	1 (2%)		
Tubular cell adenocarcinoma	1 (2%)		
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(50)	(50)
Carcinoma, NOS	1 (2%)	1 (2%)	
Adenoma, NOS	11 (22%)	2 (4%)	2 (4%)
#Adrenal	(50)	(50)	(49)
Cortical adenoma	2 (4%)		1 (2%)
#Adrenal cortex	(50)	(50)	(49)
Osteosarcoma, metastatic			1 (2%)
#Adrenal medulla	(50)	(50)	(49)
Pheochromocytoma	16 (32%)	19 (38%)	22 (45%)
Pheochromocytoma, malignant	3 (6%)	4 (8%)	5 (10%)
#Thyroid	(50)	(17)	(49)
Follicular cell carcinoma		2 (12%)	
C-cell adenoma	5 (10%)	2 (12%)	6 (12%)
C-cell carcinoma	1 (2%)		1 (2%)
#Parathyroid	(36)	(12)	(40)
Adenoma, NOS			1 (3%)
#Pancreatic islets	(50)	(49)	(50)
Islet cell adenoma	1 (2%)		
Islet cell carcinoma		1 (2%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Fibroadenoma	2 (4%)		1 (2%)
*Preputial gland	(50)	(50)	(50)
Carcinoma, NOS		10 (20%)	12 (24%)
Papilloma, NOS	1 (2%)		
Adenoma, NOS	2 (4%)	10 (20%)	10 (20%)
#Prostate	(50)	(14)	(50)
Adenocarcinoma, NOS			1 (2%)
#Testis	(50)	(50)	(50)
Interstitial cell tumor	47 (94%)	47 (94%)	50 (100%)
NERVOUS SYSTEM			
#Brain	(50)	(15)	(50)
Glioma, NOS	1 (2%)	1 (7%)	1 (2%)
Astrocytoma	1 (2%)		
*Spinal cord	(50)	(50)	(50)
Neurilemoma, malignant		1 (2%)	
SPECIAL SENSE ORGANS			
*Zymbal gland	(50)	(50)	(50)
Carcinoma, NOS		2 (4%)	2 (4%)
Carcinoma, NOS, metastatic	1 (2%)		
Papillary adenoma	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Vertebra	(50)	(50)	(50)
Osteosarcoma			1 (2%)

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

	Untreated Control	Low Dose	High Dose
BODY CAVITIES			
*Pleura	(50)	(50)	(50)
Mesothelioma, NOS	2 (4%)	1 (2%)	
*Pericardium	(50)	(50)	(50)
Mesothelioma, NOS	1 (2%)		
*Epicardium	(50)	(50)	(50)
Mesothelioma, NOS	1 (2%)		
*Tunica vaginalis	(50)	(50)	(50)
Mesothelioma, NOS	1 (2%)	2 (4%)	
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mesothelioma, NOS	2 (4%)	1 (2%)	
Connective tissue			
Lipoma			1
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	4	1	1
Moribund sacrifice	19	21	22
Terminal sacrifice	27	28	27
TUMOR SUMMARY			
Total animals with primary tumors**	50	50	50
Total primary tumors	136	129	144
Total animals with benign tumors	49	47	50
Total benign tumors	95	85	98
Total animals with malignant tumors	27	32	35
Total malignant tumors	33	39	45
Total animals with secondary tumors##	1		3
Total secondary tumors	4		4
Total animals with tumors uncertain-- benign or malignant	6	5	1
Total uncertain tumors	8	5	1

* Number of animals receiving complete necropsy examinations; 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 NALIDIXIC ACID: UNTREATED CONTROL

ANIMAL NUMBER	14401	14405	12401	11501	10106	10648	15004	13801	12106	14701	11401	11601	11801	10503	11301	12801	11101	11201	13001	10203
WEEKS ON STUDY	05606	05808	07808	08202	08505	08606	09001	09101	09202	09505	09905	09606	09707	09707	09808	09908	09808	09909	10000	10000	10101	10101	10301	10404	10404
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																			X						
Fibroma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																			X						
Trachea	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																			X						
Thymus	+	+	−	+	+	−	+	−	+	+	−	−	+	+	+	−	−	−	+	−	−	−	−	+	
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Hepatocellular carcinoma																								X	
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neurilemoma, malignant														X											
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																									
Tubular cell adenocarcinoma																							X		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS								X																	
Adenoma, NOS		X									X									X					
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																		X							
Pheochromocytoma							X						X	X		X		X			X	X	X	X	X
Pheochromocytoma, malignant																						X			
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C cell adenoma								X													X				
C cell carcinoma																									
Parathyroid	−	+	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+	−
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																		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	107	108	109	110	117	119	120	122	123	125	127	129	131	132	133	134	135	136	137	139	140	142	143	146	149	TOTAL. TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Papilloma, NOS														X												1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Carcinoma, NOS, metastatic																										1
Fibroma				X															X	X					X	4
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS, metastatic																										1
Thymus	–	–	+	–	+	+	+	+	–	+	–	+	–	+	–	–	+	–	–	+	+	+	+	–	+	27
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS															X											1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule				X																						1
Hepatocellular carcinoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neurilemoma, malignant																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma	X																									1
Tubular cell adenocarcinoma																										1
Urinary bladder	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS																										1
Adenoma, NOS				X				X						X					X	X	X	X	X			11
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma																								X		2
Pheochromocytoma		X		X	X		X	X														X				16
Pheochromocytoma, malignant											X	X														3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C cell adenoma								X							X			X								5
C-cell carcinoma																							X			1
Parathyroid	+	+	–	+	+	+	–	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	+	+	–	36
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																										1

* Animals necropsied

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

ANIMAL NUMBER	144	141	145	124	115	101	106	148	150	104	138	121	126	147	114	116	118	105	113	128	111	112	130	102	103
WEEKS ON STUDY	056	058	078	082	085	086	090	091	092	095	095	096	097	097	098	098	098	099	100	100	101	101	103	104	104
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+
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	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
Papilloma, NOS																					X				
Adenoma, NOS	X																			X					
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma, NOS												X													
Astrocytoma			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, metastatic																				X					
Papillary adenoma																							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
Mesothelioma, NOS																	X				X				
Pericardium	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																									
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
Mesothelioma, NOS														X											
Malignant lymphoma, histiocytic type																									
Leukemia, mononuclear cell				X	X	X	X		X	X	X	X	X	X	X	X	X	X	X		X	X		X	

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

ANIMAL NUMBER	107	108	109	110	117	119	120	122	123	125	127	129	131	132	133	134	135	136	137	139	140	142	143	146	149	TOTAL. TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
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	47
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial/clitoral 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
Papilloma, NOS																										1
Adenoma, NOS																										2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma, NOS																										1
Astrocytoma																										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, metastatic																										1
Papillary adenoma																										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
Mesothelioma, NOS																										2
Pericardium	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					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
Mesothelioma, NOS																		N								2
Malignant lymphoma, histiocytic type	X																									1
Leukemia, mononuclear cell	X						X					X													X	22

* Animals necropsied

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

ANIMAL NUMBER	0 3 2	0 1 6	0 2 7	0 0 4	0 4 5	0 0 2	0 0 6	0 1 9	0 2 4	0 4 4	0 1 7	0 3 0	0 1 3	0 4 0	0 4 6	0 3 5	0 4 2	0 4 8	0 4 3	0 2 6	0 0 9	0 1 2	0 0 1	0 0 3	0 0 5
WEEKS ON STUDY	0 3 4	0 3 7	0 4 8	0 7 5	0 7 5	0 7 7	0 7 7	0 7 7	0 8 3	0 8 5	0 8 6	0 8 6	0 8 9	0 8 9	0 9 6	0 9 7	0 9 8	0 9 8	0 9 9	1 0 0	1 0 3	1 0 3	1 0 4	1 0 4	1 0 4
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma					X					X		X													
Fibrosarcoma		X																							
Neurilemoma, malignant																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	~	–	–	–	–	+	–	–	+	–	–	–
Alveolar/bronchiolar adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	–	+
Hemangiosarcoma																									X
Leukemia, mononuclear cell															X										
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Thymus	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	+	–	–	–	–	–
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																			X						
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma					X			X										X	X			X			X
Pheochromocytoma, malignant																	X								
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	+
Follicular cell carcinoma																				X					
C cell adenoma												X													X
Parathyroid	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell carcinoma				X																					
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	N	N	+	+	+	N	+	+	N	N	N	N	N	N	N	N	N	N	N
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	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	X	X									X					X	X		
Adenoma, NOS																									X
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Glioma, NOS															X										
Spinal cord	+	N	N	+	N	N	N	N	N	N	N	+	N	+	N	N	N	N	N	N	N	N	N	N	N
Neurilemoma, malignant	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																						
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
Mesothelioma, NOS																				X					
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, 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
Mesothelioma, NOS												X													
Leukemia, mononuclear cell													X			X		X	X		X	X			

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

ANIMAL NUMBER	0007	0008	0110	0111	0114	0115	0118	0220	0221	0222	0223	0225	0228	0229	0231	0233	0234	0236	0237	0238	0239	0241	0247	0249	0250	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Keratoacanthoma																X										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																										3
Fibrosarcoma																									X	2
Neurilemoma, malignant													X													1
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	+	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	18
Alveolar/bronchiolar adenoma					X																					1
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12
Spleen	+	–	+	–	+	+	–	–	+	+	+	–	–	+	–	–	–	–	–	–	+	+	–	–	–	31
Hemangiosarcoma																										1
Leukemia, mononuclear cell														X												2
Lymph nodes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
Liver	+	+	+	–	+	+	+	–	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	44
Neoplastic nodule			X																							1
Bile duct	+	+	+	–	+	+	+	–	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	44
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	49
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
URINARY SYSTEM																										
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	17
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS																										1
Adenoma, NOS													X					X								2
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma		X	X		X	X			X	X		X	X				X		X		X		X		X	19
Pheochromocytoma, malignant								X											X						X	4
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	17
Follicular cell carcinoma																							X			2
C-cell adenoma																										2
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	49
Islet cell carcinoma																										1
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	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	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
Preputial/clitoral 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	X	X		X																					X	10
Adenoma, NOS	X		X					X			X					X	X			X		X	X			10
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15
Glioma, NOS																										1
Spinal cord	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, malignant																										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								X																		2
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
Mesothelioma, NOS																										1
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, 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
Mesothelioma, NOS																										1
Leukemia, mononuclear cell			X		X	X			X	X																11

* Animals necropsied

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

ANIMAL NUMBER	0060	0068	0056	0551	0894	0997	0843	0931	0811	0885	0558	0667	0977	1000	0662	0911	0552	0559	0799	0888	0711	0611	0700	0553	0544
WEEKS ON STUDY	0555	0664	0771	0881	0889	0900	0991	0991	0933	0993	0995	0995	0995	0996	0997	0997	0998	0999	0999	0999	1001	1002	1003	1004	1004
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Basal cell tumor	X																								
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																			X						
Fibrosarcoma		X																							
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma, metastatic																									
Osteosarcoma, metastatic															X										
Chordoma, metastatic																									X
Trachea	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																						X			
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Histiocytic sarcoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS							X																		X
Adrenal	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																									
Pheochromocytoma				X				X			X	X	X	X			X	X	X		X	X			
Pheochromocytoma, malignant																									
Osteosarcoma, metastatic															X										
Thyroid	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C cell adenoma							X								X		X								
C cell carcinoma																							X		
Parathyroid	+	+	–	+	–	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	–	+	+
Adenoma, NOS																									
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	X	X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Preputial/clitoral 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					X						X		X				X		X
Adenoma, NOS							X		X			X								X	X				
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma, NOS													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
Osteosarcoma															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		
Connective tissue, NOS																									
Lipoma																									
Soft tissue																									
Hemangioma																									

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

ANIMAL NUMBER	0055	0057	0063	0064	0065	0066	0069	0072	0073	0074	0075	0076	0078	0080	0082	0083	0086	0087	0089	0090	0092	0095	0096	0098	0099	TOTAL:
WEEKS ON STUDY	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma			X																							1
Basal cell tumor																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																										1
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma, metastatic														X												1
Osteosarcoma, metastatic																										1
Chordoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																										1
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	−	−	+	+	−	−	−	−	−	−	+	+	+	−	−	+	−	+	+	+	+	+	−	−	30
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											X															2
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																						X				1
Histiocytic sarcoma																						X				1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																										2
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cortical adenoma													X													1
Pheochromocytoma		X	X					X	X		X				X		X					X		X	X	X	22
Pheochromocytoma, malignant	X								X					X			X								X	5
Osteosarcoma, metastatic																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell adenoma					X			X												X						6
C cell carcinoma																										1
Parathyroid	−	+	−	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	40
Adenoma, NOS																			X							1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	*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	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS																		X								1
Preputial/clitoral 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							X		X			X	X				X					X				12
Adenoma, NOS									X										X	X		X		X		10
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma, NOS																										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																							X			2
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
Osteosarcoma																										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				X					X			X		17
Connective tissue, NOS																										
Lipoma															X											1
Soft tissue																										
Hemangioma																				X						1

* Animals necropsied

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

	Control	2,000 ppm	4,000 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	14.8%	6.9%	3.0%
Terminal Rates (c)	4/27 (15%)	0/28 (0%)	0/27 (0%)
Week of First Observation	104	75	99
Life Table Tests (d)	P=0.138N	P=0.508N	P=0.180N
Incidental Tumor Tests (d)	P=0.142N	P=0.450N	P=0.176N
Cochran-Armitage Trend Test (d)	P=0.133N		
Fisher Exact Test (d)		P=0.500N	P=0.181N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	4/50 (8%)	5/50 (10%)	2/50 (4%)
Adjusted Rates (b)	14.8%	12.1%	5.0%
Terminal Rates (c)	4/27 (15%)	1/28 (4%)	0/27 (0%)
Week of First Observation	104	37	64
Life Table Tests (d)	P=0.287N	P=0.494	P=0.337N
Incidental Tumor Tests (d)	P=0.284N	P=0.596N	P=0.333N
Cochran-Armitage Trend Test (d)	P=0.283N		
Fisher Exact Test (d)		P=0.500	P=0.339N
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	22/50 (44%)	13/50 (26%)	17/50 (34%)
Adjusted Rates (b)	48.6%	36.6%	44.7%
Terminal Rates (c)	5/27 (19%)	6/28 (21%)	8/27 (30%)
Week of First Observation	82	89	90
Life Table Tests (d)	P=0.222N	P=0.099N	P=0.263N
Incidental Tumor Tests (d)	P=0.141N	P=0.201N	P=0.182N
Cochran-Armitage Trend Test (d)	P=0.172N		
Fisher Exact Test (d)		P=0.047N	P=0.206N
Anterior Pituitary Gland: Adenoma			
Overall Rates (e)	11/49 (22%)	2/50 (4%)	2/50 (4%)
Adjusted Rates (b)	36.0%	7.1%	5.9%
Terminal Rates (c)	8/26 (31%)	2/28 (7%)	1/27 (4%)
Week of First Observation	58	104	91
Life Table Tests (d)	P=0.002N	P=0.007N	P=0.008N
Incidental Tumor Tests (d)	P=0.002N	P=0.007N	P=0.008N
Cochran-Armitage Trend Test (d)	P=0.002N		
Fisher Exact Test (d)		P=0.007N	P=0.007N
Anterior Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (e)	12/49 (24%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	37.5%	10.0%	5.9%
Terminal Rates (c)	8/26 (31%)	2/28 (7%)	1/27 (4%)
Week of First Observation	58	99	91
Life Table Tests (d)	P=0.001N	P=0.012N	P=0.005N
Incidental Tumor Tests (d)	P=0.001N	P=0.014N	P=0.005N
Cochran-Armitage Trend Test (d)	P=0.001N		
Fisher Exact Test (d)		P=0.010N	P=0.003N
Adrenal Gland Medulla: Pheochromocytoma			
Overall Rates (e)	16/50 (32%)	19/50 (38%)	22/49 (45%)
Adjusted Rates (b)	44.7%	56.5%	56.6%
Terminal Rates (c)	8/27 (30%)	14/28 (50%)	11/27 (41%)
Week of First Observation	90	75	81
Life Table Tests (d)	P=0.149	P=0.359	P=0.179
Incidental Tumor Tests (d)	P=0.133	P=0.204	P=0.142
Cochran-Armitage Trend Test (d)	P=0.112		
Fisher Exact Test (d)		P=0.338	P=0.133

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

	Control	2,000 ppm	4,000 ppm
Adrenal Medulla: Malignant Pheochromocytoma			
Overall Rates (e)	3/50 (6%)	4/50 (8%)	5/49 (10%)
Adjusted Rates (b)	10.5%	13.3%	18.5%
Terminal Rates (c)	2/27 (7%)	3/28 (11%)	5/27 (19%)
Week of First Observation	101	98	104
Life Table Tests (d)	P=0.286	P=0.510	P=0.355
Incidental Tumor Tests (d)	P=0.297	P=0.429	P=0.360
Cochran-Armitage Trend Test (d)	P=0.280		
Fisher Exact Test (d)		P=0.500	P=0.346
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (e)	18/50 (36%)	21/50 (42%)	24/49 (49%)
Adjusted Rates (b)	50.5%	60.8%	62.0%
Terminal Rates (c)	10/27 (37%)	15/28 (54%)	13/27 (48%)
Week of First Observation	90	75	81
Life Table Tests (d)	P=0.152	P=0.365	P=0.182
Incidental Tumor Tests (d)	P=0.136	P=0.193	P=0.147
Cochran-Armitage Trend Test (d)	P=0.114		
Fisher Exact Test (d)		P=0.341	P=0.135
Thyroid Gland: C-Cell Adenoma			
Overall Rates (e)	5/50 (10%)	(f) 2/17 (12%)	6/49 (12%)
Adjusted Rates (b)	16.1%		18.0%
Terminal Rates (c)	3/27 (11%)		3/27 (11%)
Week of First Observation	91		91
Life Table Test (d)			P=0.496
Incidental Tumor Test (d)			P=0.462
Fisher Exact Test (d)			P=0.486
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (e)	6/50 (12%)	(f) 2/17 (12%)	7/49 (14%)
Adjusted Rates (b)	19.6%		21.0%
Terminal Rates (c)	4/27 (15%)		3/27 (11%)
Week of First Observation	91		91
Life Table Test (d)			P=0.496
Incidental Tumor Test (d)			P=0.469
Fisher Exact Test (d)			P=0.484
Preputial Gland: Adenoma			
Overall Rates (a)	2/49 (4%)	10/49 (20%)	10/47 (21%)
Adjusted Rates (b)	5.2%	35.7%	29.6%
Terminal Rates (c)	0/27 (0%)	10/28 (36%)	5/26 (19%)
Week of First Observation	56	104	91
Life Table Tests (d)	P=0.016	P=0.017	P=0.018
Incidental Tumor Tests (d)	P=0.015	P=0.017	P=0.014
Cochran-Armitage Trend Test (d)	P=0.013		
Fisher Exact Test (d)		P=0.014	P=0.011
Preputial Gland: Adenoma or Papilloma			
Overall Rates (a)	3/49 (6%)	10/49 (20%)	10/47 (21%)
Adjusted Rates (b)	8.3%	35.7%	29.6%
Terminal Rates (c)	0/27 (0%)	10/28 (36%)	5/26 (19%)
Week of First Observation	56	104	91
Life Table Tests (d)	P=0.034	P=0.041	P=0.042
Incidental Tumor Tests (d)	P=0.033	P=0.035	P=0.034
Cochran-Armitage Trend Test (d)	P=0.028		
Fisher Exact Test (d)		P=0.035	P=0.029

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

	Control	2,000 ppm	4,000 ppm
Preputial Gland: Carcinoma			
Overall Rates (a)	0/49 (0%)	10/49 (20%)	12/47 (26%)
Adjusted Rates (b)	0.0%	28.1%	36.8%
Terminal Rates (c)	0/27 (0%)	5/28 (18%)	7/26 (27%)
Week of First Observation		77	90
Life Table Tests (d)	P<0.001	P=0.002	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P<0.001	P<0.001
Preputial Gland: Adenoma, Papilloma, or Carcinoma			
Overall Rates (a)	3/49 (6%)	19/49 (39%)	20/47 (43%)
Adjusted Rates (b)	8.3%	56.2%	53.6%
Terminal Rates (c)	0/27 (0%)	14/28 (50%)	10/26 (38%)
Week of First Observation	56	77	90
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P<0.001	P<0.001
Testis: Interstitial Cell Tumor			
Overall Rates (e)	47/50 (94%)	47/50 (94%)	50/50 (100%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	27/27 (100%)	28/28 (100%)	27/27 (100%)
Week of First Observation	56	75	55
Life Table Tests (d)	P=0.334	P=0.536	P=0.364
Incidental Tumor Tests (d)	P=0.038	P=0.150	P=0.113
Cochran-Armitage Trend Test (d)	P=0.101		
Fisher Exact Test (d)		P=0.661	P=0.121
All Sites: Mesothelioma			
Overall Rates (a)	5/50 (10%)	4/50 (8%)	0/50 (0%)
Adjusted Rates (b)	15.3%	12.4%	0.0%
Terminal Rates (c)	2/27 (7%)	2/28 (7%)	0/27 (0%)
Week of First Observation	97	86	
Life Table Tests (d)	P=0.034N	P=0.510N	P=0.038N
Incidental Tumor Tests (d)	P=0.032N	P=0.636	P=0.030N
Cochran-Armitage Trend Test (d)	P=0.029N		
Fisher Exact Test (d)		P=0.500N	P=0.029N
All Sites: Benign Tumors			
Overall Rates (a)	49/50 (98%)	47/50 (94%)	50/50 (100%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	27/27 (100%)	28/28 (100%)	27/27 (100%)
Week of First Observation	56	75	55
Life Table Tests (d)	P=0.454	P=0.466N	P=0.484
Incidental Tumor Tests (d)	P=0.276	P=0.612	P=0.486
Cochran-Armitage Trend Test (d)	P=0.378		
Fisher Exact Test (d)		P=0.309N	P=0.500
All Sites: Malignant Tumors			
Overall Rates (a)	27/50 (54%)	32/50 (64%)	35/50 (70%)
Adjusted Rates (b)	56.9%	72.1%	79.2%
Terminal Rates (c)	7/27 (26%)	16/28 (57%)	18/27 (67%)
Week of First Observation	78	34	64
Life Table Tests (d)	P=0.133	P=0.249	P=0.152
Incidental Tumor Tests (d)	P=0.056	P=0.127	P=0.060
Cochran-Armitage Trend Test (d)	P=0.061		
Fisher Exact Test (d)		P=0.208	P=0.074

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

	Control	2,000 ppm	4,000 ppm
All Sites: All Tumors			
Overall Rates (a)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	27/27 (100%)	28/28 (100%)	27/27 (100%)
Week of First Observation	56	34	55
Life Table Tests (d)	P=0.513	P=0.529	P=0.542
Incidental Tumor Tests (d)	(g)	(g)	(g)
Cochran-Armitage Trend Test (d)	(g)		
Fisher Exact Test (d)		P=1.000	P=1.000

(a) Number of tumor-bearing animals/number of animals examined grossly at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(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 than in controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined microscopically at the site
(f) Incomplete sampling of tissues
(g) No P value is reported because all animals had tumors.

TABLE A4a. HISTORICAL INCIDENCE OF PREPUTIAL GLAND TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	2/50	0/50	2/50
Phenylephrine hydrochloride	0/50	2/50	2/50
Erythromycin stearate	0/50	1/50	1/50
Tetracycline hydrochloride	5/50	0/50	5/50
Oxytetracycline hydrochloride	0/50	(b) 1/50	1/50
Nitrofurazone	8/50	1/50	9/50
Nalidixic acid	(c) 3/50	0/50	(c) 3/50
α-Methyldopa sesquihydrate	7/49	1/49	8/49
TOTAL	25/399 (6.3%)	6/399 (1.5%)	31/399 (7.8%)
SD (d)	6.50%	1.42%	6.34%
Range (e)			
High	8/50	2/50	9/50
Low	0/50	0/50	1/50
Overall Historical Incidence			
TOTAL	(c) 69/1,596 (4.3%)	(f) 49/1,596 (3.1%)	(c,f) 118/1,596 (7.4%)
SD (d)	5.03%	2.84%	5.21%
Range (e)			
High	8/50	5/50	9/50
Low	0/50	0/50	0/50

(a) Data as of May 12, 1988, for studies of at least 104 weeks
(b) Adenocarcinoma, NOS
(c) Includes one papilloma, NOS
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.
(f) Includes seven adenocarcinomas, NOS, and one squamous cell carcinoma

TABLE A4b. HISTORICAL INCIDENCE OF ORAL CAVITY SQUAMOUS CELL TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Number Examined	Number of Tumors	Site	Diagnosis
Historical Incidence at Physiological Research Laboratories			
399	(b) 1 (0.3%)	Palate, NOS	Squamous cell papilloma
Overall Historical Incidence			
	2	Palate, NOS	Squamous cell papilloma
	1	Tongue, NOS	Squamous cell papilloma
	2	Oral mucosa	Squamous cell carcinoma
	2	Palate, NOS	Squamous cell carcinoma
TOTAL 1,596	7 (0.4%)		

(a) Data as of May 12, 1988, for studies of at least 104 weeks; the greatest incidence observed in any untreated control group is 2/49.
(b) Observed in the erythromycin stearate study

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

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	4/49	1/49	5/49
Phenylephrine hydrochloride	11/48	0/48	11/48
Erythromycin stearate	14/49	1/49	15/49
Tetracycline hydrochloride	12/48	0/48	12/48
Oxytetracycline hydrochloride	20/50	(b) 1/50	21/50
Nitrofurazone	11/48	0/48	11/48
Nalidixic acid	11/49	1/49	12/49
α-Methyldopa sesquihydrate	8/49	0/49	8/49
TOTAL	91/390 (23.3%)	4/390 (1.0%)	95/390 (24.4%)
SD (c)	9.17%	1.09%	9.41%
Range (d)			
High	20/50	1/49	21/50
Low	4/49	0/49	5/49
Overall Historical Incidence			
TOTAL	(e) 377/1,540 (24.5%)	(f) 23/1,540 (1.5%)	(e,f) 400/1,540 (26.0%)
SD (c)	10.33%	2.05%	10.24%
Range (d)			
High	24/46	3/39	25/46
Low	4/50	0/50	4/50

(a) Data as of May 12, 1988, for studies of at least 104 weeks
(b) Adenocarcinoma, NOS
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes 12 chromophobe adenomas and 1 acidophil adenoma
(f) Includes five chromophobe carcinomas and one adenocarcinoma, NOS

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

	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)
Animal foreign body			1 (2%)
Epidermal inclusion cyst		1 (2%)	
Follicular cyst, NOS			1 (2%)
Ulcer, NOS			1 (2%)
Abscess, NOS			2 (4%)
Inflammation, active chronic		2 (4%)	3 (6%)
Inflammation, granulomatous			1 (2%)
Inflammation with fibrosis		1 (2%)	5 (10%)
Hyperplasia, epithelial		1 (2%)	6 (12%)
*Subcutaneous tissue	(50)	(50)	(50)
Abscess, NOS	1 (2%)		
Inflammation, active chronic			1 (2%)
Inflammation, acute/chronic			1 (2%)
Calcinosis, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#Nasal cavity	(50)	(50)	(50)
Infection, fungal	1 (2%)	2 (4%)	
Foreign material, NOS	2 (4%)		
#Nasal mucosa	(50)	(50)	(50)
Dilatation/ducts	2 (4%)		
Retention of content	2 (4%)		
Congestion, acute	1 (2%)	1 (2%)	
Hemorrhage		1 (2%)	
Inflammation, acute	1 (2%)	1 (2%)	3 (6%)
Inflammation, active chronic	11 (22%)	15 (30%)	14 (28%)
Inflammation, chronic	9 (18%)	12 (24%)	7 (14%)
Hyperplasia, epithelial	1 (2%)		3 (6%)
Angiectasis		1 (2%)	
#Nasal gland	(50)	(50)	(50)
Hyperplasia, NOS		1 (2%)	
*Larynx	(50)	(50)	(50)
Inflammation, chronic		1 (2%)	
#Trachea	(49)	(14)	(49)
Inflammation, chronic	3 (6%)	1 (7%)	1 (2%)
#Tracheal gland	(49)	(14)	(49)
Dilatation, NOS			1 (2%)
#Lung	(50)	(18)	(50)
Congestion, NOS	1 (2%)	1 (6%)	1 (2%)
Hemorrhage	1 (2%)		
Inflammation, interstitial	4 (8%)	2 (11%)	8 (16%)
Inflammation, acute/chronic	1 (2%)		
Inflammation, chronic focal	1 (2%)		1 (2%)
Granuloma, NOS			1 (2%)
Inflammation with fibrosis	1 (2%)		
Perivascular cuffing	1 (2%)		1 (2%)
Hemosiderosis	1 (2%)		2 (4%)
Hyperplasia, epithelial		1 (6%)	1 (2%)
Hyperplasia, mesothelial	1 (2%)		
Histiocytosis	2 (4%)		1 (2%)

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

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM			
*Nasolacrimal duct	(50)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)
#Bone marrow	(49)	(12)	(50)
Congestion, NOS			1 (2%)
Metaplasia, osseous	1 (2%)		
Hyperplasia, hematopoietic	11 (22%)	5 (42%)	19 (38%)
Hyperplasia, granulocytic	1 (2%)		
#Spleen	(50)	(31)	(50)
Accessory structure			1 (2%)
Congestion, NOS		1 (3%)	
Fibrosis, focal	2 (4%)	2 (6%)	1 (2%)
Necrosis, focal	1 (2%)		
Hemosiderosis	2 (4%)	2 (6%)	5 (10%)
Depletion, lymphoid			1 (2%)
Hyperplasia, granulocytic	1 (2%)		
Hematopoiesis	2 (4%)	1 (3%)	2 (4%)
#Splenic capsule	(50)	(31)	(50)
Hemosiderosis		1 (3%)	
#Lymph node	(49)	(14)	(50)
Cyst, NOS			1 (2%)
Hemorrhage	2 (4%)	1 (7%)	6 (12%)
Hemosiderosis		1 (7%)	1 (2%)
Hyperplasia, plasma cell	1 (2%)		
#Mandibular lymph node	(49)	(14)	(50)
Cyst, NOS	1 (2%)		1 (2%)
Hemorrhage			1 (2%)
Hyperplasia, NOS			4 (8%)
Hyperplasia, plasma cell	1 (2%)		1 (2%)
Hyperplasia, lymphoid	1 (2%)	1 (7%)	
#Mediastinal lymph node	(49)	(14)	(50)
Hemosiderosis	1 (2%)		2 (4%)
Hyperplasia, NOS	1 (2%)		1 (2%)
#Mesenteric lymph node	(49)	(14)	(50)
Cyst, NOS			2 (4%)
Hemorrhage	1 (2%)		
Hemosiderosis	1 (2%)		
Hyperplasia, reticulum cell		1 (7%)	
#Nasal mucosa	(50)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)
#Liver	(50)	(44)	(50)
Hematopoiesis	1 (2%)		
#Adrenal cortex	(50)	(50)	(49)
Hematopoiesis	1 (2%)		
#Thymus	(27)	(13)	(30)
Cyst, NOS		1 (8%)	
Hemorrhage	1 (4%)		
Hyperplasia, epithelial	6 (22%)	2 (15%)	15 (50%)
CIRCULATORY SYSTEM			
#Nasal mucosa	(50)	(50)	(50)
Thrombosis, NOS		1 (2%)	
#Heart/atrium	(50)	(50)	(50)
Mineralization	2 (4%)	4 (8%)	4 (8%)
Dilatation, NOS	1 (2%)	1 (2%)	8 (16%)
Thrombus, organized	1 (2%)	2 (4%)	4 (8%)
Inflammation, chronic	2 (4%)	2 (4%)	
Fibrosis			3 (6%)
Metaplasia, osseous		1 (2%)	1 (2%)
#Heart/ventricle	(50)	(50)	(50)
Thrombus, organized	1 (2%)		

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

	Untreated Control	Low Dose	High Dose
CIRCULATORY SYSTEM (Continued)			
#Myocardium	(50)	(50)	(50)
Mineralization		1 (2%)	5 (10%)
Inflammation, acute/chronic		3 (6%)	
Degeneration, NOS	39 (78%)	33 (66%)	45 (90%)
Pigmentation, NOS		1 (2%)	1 (2%)
Hemosiderosis			1 (2%)
#Endocardium	(50)	(50)	(50)
Mineralization			1 (2%)
Inflammation, acute/chronic	1 (2%)		
Fibrosis, focal	2 (4%)	2 (4%)	1 (2%)
Hyperplasia, NOS			2 (4%)
#Cardiac valve	(50)	(50)	(50)
Endocardiosis	1 (2%)	1 (2%)	2 (4%)
*Blood vessel	(50)	(50)	(50)
Inflammation, active chronic	1 (2%)		
*Coronary artery	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
Necrosis, fibrinoid	1 (2%)		
*Pulmonary artery	(50)	(50)	(50)
Mineralization			1 (2%)
Inflammation, chronic focal	1 (2%)		
Hypertrophy, NOS	1 (2%)		1 (2%)
*Hepatic vein	(50)	(50)	(50)
Hyperplasia, NOS			1 (2%)
#Liver	(50)	(44)	(50)
Thrombosis, NOS			2 (4%)
#Pancreas	(50)	(49)	(50)
Periarteritis	1 (2%)		
DIGESTIVE SYSTEM			
#Salivary gland	(50)	(14)	(49)
Inflammation, chronic focal		1 (7%)	1 (2%)
Inflammation with fibrosis			1 (2%)
Focal cellular change	1 (2%)		
Atrophy, focal	2 (4%)		1 (2%)
Hyperplasia, stromal	2 (4%)		
#Parotid gland	(50)	(14)	(49)
Inflammation, chronic focal			1 (2%)
Necrosis, diffuse			1 (2%)
Focal cellular change	1 (2%)		
Atrophy, focal	1 (2%)	1 (7%)	3 (6%)
#Sublingual gland	(50)	(14)	(49)
Hyperplasia, epithelial			1 (2%)
#Liver	(50)	(44)	(50)
Deformity, NOS	2 (4%)	1 (2%)	2 (4%)
Congestion, NOS		1 (2%)	1 (2%)
Hemorrhage			1 (2%)
Granuloma, NOS	5 (10%)		2 (4%)
Scar		2 (5%)	
Fibrosis, multifocal		1 (2%)	
Degeneration, cystic			1 (2%)
Degeneration, ballooning	2 (4%)	4 (9%)	3 (6%)
Necrosis, focal	1 (2%)	2 (5%)	
Metamorphosis, fatty	4 (8%)	1 (2%)	
Pigmentation, NOS		1 (2%)	
Mitotic alteration			1 (2%)
Basophilic cyto change	29 (58%)	25 (57%)	21 (42%)
Focal cellular change	13 (26%)	4 (9%)	15 (30%)
Eosinophilic cyto change	1 (2%)	2 (5%)	
Clear cell change	2 (4%)	3 (7%)	1 (2%)
Atrophy, focal		1 (2%)	

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

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM			
#Liver (Continued)	(50)	(44)	(50)
Hyperplasia, focal	3 (6%)	1 (2%)	
Angiectasis	2 (4%)	2 (5%)	4 (8%)
#Portal tract	(50)	(44)	(50)
Inflammation, chronic	1 (2%)		1 (2%)
#Liver/centrilobular	(50)	(44)	(50)
Inflammation, chronic		1 (2%)	
Degeneration, NOS		1 (2%)	1 (2%)
Cytoplasmic vacuolization	1 (2%)		
#Liver/periportal	(50)	(44)	(50)
Inflammation, acute/chronic			1 (2%)
Inflammation, chronic		3 (7%)	1 (2%)
#Bile duct	(50)	(44)	(50)
Hyperplasia, NOS	27 (54%)	32 (73%)	29 (58%)
Hyperplasia, focal	2 (4%)	1 (2%)	5 (10%)
#Pancreas	(50)	(49)	(50)
Inflammation, acute/chronic		1 (2%)	
Inflammation, chronic focal	4 (8%)	7 (14%)	9 (18%)
Inflammation, chronic diffuse	2 (4%)	1 (2%)	4 (8%)
#Pancreatic acinus	(50)	(49)	(50)
Cytoplasmic change, NOS	1 (2%)		
Cytoplasmic vacuolization	1 (2%)		
Basophilic cyto change		2 (4%)	
Atrophy, focal	16 (32%)	22 (45%)	22 (44%)
Atrophy, diffuse		1 (2%)	1 (2%)
Hyperplasia, focal			1 (2%)
#Esophagus	(49)	(14)	(50)
Infection, bacterial			1 (2%)
Hyperkeratosis			1 (2%)
#Glandular stomach	(50)	(14)	(50)
Dilatation, NOS	3 (6%)		
Cyst, NOS	14 (28%)	2 (14%)	6 (12%)
Sclerosis	2 (4%)	1 (7%)	
Necrosis, focal			1 (2%)
Amyloidosis			1 (2%)
Hyperplasia, epithelial	1 (2%)		
#Forestomach	(50)	(14)	(50)
Ulcer, NOS	1 (2%)		
Inflammation, acute		1 (7%)	
Inflammation, active chronic	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
Hyperkeratosis			1 (2%)
#Small intestine	(50)	(15)	(50)
Ectopia	1 (2%)		
#Jejunum	(50)	(15)	(50)
Cyst, NOS		1 (7%)	
Inflammation, necrotizing	1 (2%)		
Granulation tissue	1 (2%)		
Ulcer, perforated	1 (2%)		
#Large intestine	(50)	(14)	(50)
Cyst, NOS			1 (2%)
Sclerosis			1 (2%)
#Colon	(50)	(14)	(50)
Retention of content			1 (2%)

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

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney	(50)	(17)	(50)
Pyelonephritis, acute	1 (2%)		
Inflammation, chronic focal		1 (6%)	2 (4%)
Nephropathy	47 (94%)	13 (76%)	46 (92%)
#Kidney/cortex	(50)	(17)	(50)
Mineralization		1 (6%)	
Cyst, NOS	1 (2%)		1 (2%)
#Kidney/medulla	(50)	(17)	(50)
Mineralization	1 (2%)	1 (6%)	
Necrosis, NOS			1 (2%)
#Kidney/tubule	(50)	(17)	(50)
Abscess, chronic	1 (2%)		
Degeneration, NOS	2 (4%)		
Pigmentation, NOS	1 (2%)	1 (6%)	
#Kidney/pelvis	(50)	(17)	(50)
Mineralization	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
#Urinary bladder	(49)	(13)	(50)
Hemorrhage			1 (2%)
Inflammation, acute			1 (2%)
Inflammation, acute/chronic		1 (8%)	
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(50)	(50)
Hyperplasia, NOS	2 (4%)		
#Anterior pituitary	(49)	(50)	(50)
Cyst, NOS	3 (6%)	2 (4%)	5 (10%)
Focal cellular change	6 (12%)	2 (4%)	2 (4%)
Hyperplasia, focal	1 (2%)	3 (6%)	2 (4%)
Angiectasis	1 (2%)		
#Pituitary posterior	(49)	(50)	(50)
Cyst, NOS		1 (2%)	
#Adrenal/capsule	(50)	(50)	(49)
Ectopia	5 (10%)	8 (16%)	4 (8%)
Inflammation, chronic focal		1 (2%)	
#Adrenal cortex	(50)	(50)	(49)
Congestion, NOS			2 (4%)
Degeneration, lipoid	14 (28%)	2 (4%)	6 (12%)
Necrosis, focal		1 (2%)	
Metamorphosis, fatty		1 (2%)	
Focal cellular change	1 (2%)	3 (6%)	1 (2%)
Hyperplasia, focal	9 (18%)	8 (16%)	2 (4%)
Angiectasis	2 (4%)		2 (4%)
#Adrenal medulla	(50)	(50)	(49)
Mineralization		1 (2%)	1 (2%)
Nuclear enlargement	1 (2%)		
Hyperplasia, focal	15 (30%)	9 (18%)	13 (27%)
Angiectasis		1 (2%)	
#Thyroid	(50)	(17)	(49)
Cyst, NOS	1 (2%)		
Hyperplasia, C-cell	19 (38%)	2 (12%)	12 (24%)
#Thyroid follicle	(50)	(17)	(49)
Mineralization			1 (2%)
Dilatation, NOS	1 (2%)		
#Pancreatic islets	(50)	(49)	(50)
Hyperplasia, focal	3 (6%)		

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

	Untreated Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Dilatation, NOS	3 (6%)		1 (2%)
Cyst, NOS			1 (2%)
Pigmentation, NOS			1 (2%)
Hyperplasia, epithelial	10 (20%)	1 (2%)	5 (10%)
*Preputial gland	(50)	(50)	(50)
Retention of content	2 (4%)	4 (8%)	3 (6%)
Cyst, NOS			1 (2%)
Inflammation, acute			1 (2%)
Abscess, NOS		3 (6%)	3 (6%)
Inflammation, active chronic	1 (2%)	3 (6%)	2 (4%)
Inflammation, chronic	12 (24%)	9 (18%)	11 (22%)
Inflammation, granulomatous	27 (54%)	15 (30%)	11 (22%)
Hyperplasia, epithelial	1 (2%)	5 (10%)	1 (2%)
Hyperplasia, papillary		1 (2%)	
#Prostate	(50)	(14)	(50)
Mineralization			1 (2%)
Retention of content			2 (4%)
Mucocele	1 (2%)		
Hemorrhage			1 (2%)
Inflammation, suppurative	2 (4%)		
Inflammation, active chronic	10 (20%)	3 (21%)	2 (4%)
Inflammation, chronic	2 (4%)		
Hyperplasia, epithelial	1 (2%)	2 (14%)	
*Seminal vesicle	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		
Atrophy, NOS		1 (2%)	1 (2%)
Hyperplasia, epithelial	1 (2%)		
#Testis	(50)	(50)	(50)
Mineralization	11 (22%)	6 (12%)	18 (36%)
Infarct, NOS			1 (2%)
Atrophy, NOS	2 (4%)	6 (12%)	3 (6%)
Hyperplasia, interstitial cell	15 (30%)	13 (26%)	7 (14%)
*Epididymis	(50)	(50)	(50)
Dilatation, NOS			1 (2%)
Infarct, NOS			1 (2%)
*Ampulla of vas deferens	(50)	(50)	(50)
Retention of content			1 (2%)
*Scrotum	(50)	(50)	(50)
Hemorrhage	1 (2%)		
NERVOUS SYSTEM			
*Choroid plexus	(50)	(50)	(50)
Hyperplasia, papillary			1 (2%)
#Brain	(50)	(15)	(50)
Mineralization	1 (2%)		
Deformity, NOS	2 (4%)		
Hydrocephalus, internal	1 (2%)		
Hemorrhage	2 (4%)		
*Spinal cord	(50)	(50)	(50)
Demyelinization		1 (2%)	
Cytoplasmic vacuolization			1 (2%)

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

	Untreated Control	Low Dose	High Dose
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Hemorrhage	1 (2%)		
*Eye/cornea	(50)	(50)	(50)
Synechia, anterior		1 (2%)	1 (2%)
Vascularization	1 (2%)		1 (2%)
*Eye/iris	(50)	(50)	(50)
Inflammation, active chronic			1 (2%)
*Eye/retina	(50)	(50)	(50)
Degeneration, NOS	3 (6%)	2 (4%)	3 (6%)
*Eye/crystalline lens	(50)	(50)	(50)
Mineralization		1 (2%)	2 (4%)
Cataract	3 (6%)	2 (4%)	3 (6%)
*Nasolacrimal duct	(50)	(50)	(50)
Retention of content	1 (2%)		1 (2%)
Inflammation, active chronic	3 (6%)	3 (6%)	5 (10%)
Inflammation, chronic	20 (40%)	17 (34%)	21 (42%)
Foreign material, NOS	1 (2%)		
Hyperplasia, epithelial	1 (2%)		1 (2%)
*Harderian gland	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		1 (2%)
Fibrosis			1 (2%)
MUSCULOSKELETAL SYSTEM			
*Femur	(50)	(50)	(50)
Healed fracture			1 (2%)
Osteochondrodystrophy			1 (2%)
Metaplasia, cartilaginous			1 (2%)
Metaplasia, osseous	1 (2%)		
*Articular cartilage	(50)	(50)	(50)
Metaplasia, osseous			1 (2%)
BODY CAVITIES			
*Pleura	(50)	(50)	(50)
Inflammation, chronic focal	1 (2%)		
*Pericardium	(50)	(50)	(50)
Hyperplasia, mesothelial	1 (2%)		
*Epicardium	(50)	(50)	(50)
Inflammation, active chronic		1 (2%)	
Inflammation, chronic focal			1 (2%)
Hemosiderosis		1 (2%)	
*Mesentery	(50)	(50)	(50)
Accessory structure			3 (6%)
Necrosis, fat	1 (2%)		
Hemosiderosis		1 (2%)	1 (2%)
*Tunica vaginalis	(50)	(50)	(50)
Inflammation, active chronic	1 (2%)		
ALL OTHER SYSTEMS			
Adipose tissue			
Necrosis, NOS	5	2	2
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 B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID

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

	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)
Papilloma, NOS	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Squamous cell carcinoma, invasive	1 (2%)		
Paraganglioma, malignant		1 (2%)	
Fibroma	2 (4%)	1 (2%)	
Fibrosarcoma		2 (4%)	
Liposarcoma		1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(50)	(19)	(50)
Squamous cell carcinoma, metastatic	1 (2%)		
Alveolar/bronchiolar adenoma			1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	19 (38%)	8 (16%)	7 (14%)
#Spleen	(50)	(50)	(50)
Leukemia, mononuclear cell	1 (2%)	1 (2%)	
CIRCULATORY SYSTEM			
#Spleen	(50)	(50)	(50)
Hemangioma	1 (2%)		
#Myocardium	(50)	(11)	(50)
Neurilemoma	1 (2%)		
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Neoplastic nodule	1 (2%)		1 (2%)
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Pituitary intermedia	(50)	(37)	(50)
Adenoma, NOS			1 (2%)
#Anterior pituitary	(50)	(37)	(50)
Carcinoma, NOS	1 (2%)	1 (3%)	
Adenoma, NOS	19 (38%)	18 (49%)	17 (34%)
#Adrenal	(50)	(50)	(50)
Cortical adenoma	2 (4%)		2 (4%)
Cortical carcinoma	1 (2%)		
#Adrenal medulla	(50)	(50)	(50)
Pheochromocytoma	3 (6%)	1 (2%)	1 (2%)
Pheochromocytoma, malignant	1 (2%)		
#Thyroid	(50)	(12)	(50)
C-cell adenoma	2 (4%)	1 (8%)	3 (6%)
C-cell carcinoma	1 (2%)	1 (8%)	2 (4%)
#Pancreatic islets	(50)	(11)	(50)
Islet cell adenoma	1 (2%)		

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

	Untreated Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS	3 (6%)	1 (2%)	
Fibroadenoma	7 (14%)	6 (12%)	2 (4%)
*Clitoral gland	(50)	(50)	(50)
Carcinoma, NOS	1 (2%)	4 (8%)	4 (8%)
Papilloma, NOS	1 (2%)	1 (2%)	
Papillary carcinoma			1 (2%)
Adenoma, NOS	4 (8%)	10 (20%)	11 (22%)
#Uterus	(50)	(49)	(50)
Leiomyoma			1 (2%)
Endometrial stromal polyp	14 (28%)	15 (31%)	5 (10%)
Endometrial stromal sarcoma		1 (2%)	
#Uterus/endometrium	(50)	(49)	(50)
Adenocarcinoma, NOS			1 (2%)
Papillary cystadenocarcinoma, NOS		1 (2%)	
#Ovary	(50)	(12)	(50)
Granulosa cell tumor	2 (4%)		
NERVOUS SYSTEM			
#Brain	(50)	(12)	(50)
Astrocytoma		1 (8%)	
Oligodendroglioma	2 (4%)		1 (2%)
SPECIAL SENSE ORGANS			
*Zymbal gland	(50)	(50)	(50)
Squamous cell carcinoma	2 (4%)		
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	6	1	
Moribund sacrifice	22	18	21
Terminal sacrifice	22	31	29

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

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	47	43	41
Total primary tumors	93	76	61
Total animals with benign tumors	38	37	32
Total benign tumors	58	53	44
Total animals with malignant tumors	25	22	16
Total malignant tumors	32	23	16
Total animals with secondary tumors##	2		
Total secondary tumors	2		
Total animals with tumors uncertain--benign or malignant	3		1
Total uncertain tumors	3		1

* Number of animals receiving complete necropsy examinations; 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 NALIDIXIC ACID: UNTREATED CONTROL

ANIMAL NUMBER	1 3 6	1 0 7	1 2 3	1 1 7	1 4 7	1 1 4	1 1 8	1 4 6	1 0 4	1 4 3	1 4 8	1 3 5	1 4 0	1 2 9	1 3 1	1 3 8	1 2 8	1 0 5	1 2 5	1 4 2	1 2 4	1 3 9	1 0 9	1 1 0	1 3 3
WEEKS ON STUDY	0 3 7	0 5 7	0 6 0	0 6 8	0 7 0	0 7 5	0 7 5	0 8 2	0 8 5	0 8 9	0 9 0	0 9 1	0 9 1	0 9 2	0 9 2	0 9 2	0 9 3	0 9 4	0 9 9	0 9 9	1 0 0	1 0 0	1 0 1	1 0 2	1 0 2
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, invasive						X																			
Fibroma							X																		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic																			X						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Leukemia, mononuclear cell																									X
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	−	+	+	−	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neurilemoma																									
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																							X		
Adenoma, NOS															X			X	X						
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																						X			
Cortical carcinoma																									
Pheochromocytoma														X											
Pheochromocytoma, malignant																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma								X																	
C-cell carcinoma																X									
Parathyroid	−	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	+	+	+	−	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+
Adenocarcinoma, NOS															X					X					
Fibroadenoma				X	X					X		X													
Preputial/clitoral 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				
Papilloma, NOS																									
Adenoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Endometrial stromal polyp		X	X		X	X						X		X							X	X		X
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma														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
Squamous cell carcinoma						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 B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	1 0 2	1 0 8	1 4 5	1 0 1	1 0 3	1 0 6	1 1 1	1 1 2	1 1 3	1 1 5	1 1 6	1 1 9	1 2 0	1 2 1	1 2 2	1 2 6	1 2 7	1 3 0	1 3 2	1 3 4	1 3 7	1 4 1	1 4 4	1 4 9	1 5 0	TOTAL:
WEEKS ON STUDY	1 0 3	1 0 3	1 0 3	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Papilloma, NOS											X															1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma, invasive																										1
Fibroma									X																	2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Leukemia, mononuclear cell				X																						1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	−	+	+	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+	−	−	+	+	−	+	−	35
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neurilemoma																		X								1
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule											X															1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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				X	X	X	X			X	X	X			X	X		X	X	19
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma													X													2
Cortical carcinoma	X																									1
Pheochromocytoma					X									X												3
Pheochromocytoma, malignant																		X								1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma												X														2
C-cell carcinoma																										1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	−	−	+	+	−	37
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma							X																			1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenocarcinoma, NOS					X																					3
Fibroadenoma	X								X											X						7
Preputial/clitoral 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
Papilloma, NOS															X											1
Adenoma, NOS				X											X					X					X	4
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp		X			X						X			X										X		14
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor																		X	X							2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma																										2
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
Squamous cell carcinoma																										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
Leukemia, mononuclear cell	X	X	X		X	X				X							X									19

* Animals necropsied

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

ANIMAL NUMBER	00440	00505	00530	00629	00749	00814	00941	00906	00921	00936	00932	00902	00945	00924	00910	00917	00939	00920	00923	00931	00901	00903	00904	00907	00908	00909
WEEKS ON STUDY	04707	05303	06404	06605	06707	07703	07709	08000	08101	08202	08808	09404	09905	09906	09907	09909	10101	10303	10303	10404	10404	10404	10704	10804	10804	10904
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Paraganglioma, malignant					X																					
Fibroma																										
Fibrosarcoma															X											
Liposarcoma									X																	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	~	+	−	−	−	−	−	−	−	−	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	~	−	−	−	−	−	−	−	−	−	−
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia, mononuclear cell																										
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	~	+	−	−	−	−	−	+	+	+	
Carcinoma, NOS																										
Adenoma, NOS				X		X		X				X	X	X									X			X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																										
Thyroid	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
C-cell adenoma		X																								
C-cell carcinoma																										
Parathyroid	+	−	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	N	+	+	+	+	N	+	N	N	N	N	+	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS																										
Fibroadenoma													X					X								
Preputial/clitoral 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	N
Carcinoma, NOS						X				X							X									
Papilloma, NOS															X											
Adenoma, NOS											X						X			X	X					
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary cystadenocarcinoma, NOS																										
Endometrial stromal polyp	X		X				X		X				X						X	X						X
Endometrial stromal sarcoma												X														
Ovary	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
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	N
Leukemia, mononuclear cell								X			X					X	X									

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

ANIMAL NUMBER	0111	0112	0113	0115	0116	0118	0119	0221	0225	0226	0227	0228	0230	0231	0233	0234	0235	0237	0238	0242	0243	0244	0246	0247	0248	TOTAL:
WEEKS ON STUDY	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	1004	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	N	N	N	+	N		*50
Paraganglioma, malignant																										1
Fibroma																							X			1
Fibrosarcoma									X																	2
Liposarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	–	–	+	–	–	+	–	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–		19
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		11
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+		49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia, mononuclear cell														X												1
Lymph nodes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–		13
Thymus	+	–	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–		41
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		11
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		11
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Pancreas	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		11
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		11
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		12
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		11
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		11
URINARY SYSTEM																										
Kidney	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–		12
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		11
ENDOCRINE SYSTEM																										
Pituitary	–	–	+	+	+	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–		37
Carcinoma, NOS			X																							1
Adenoma, NOS			X		X					X					X	X		X		X	X	X		X		18
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Pheochromocytoma										X																1
Thyroid	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		12
C-cell adenoma																										1
C-cell carcinoma		X																								1
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		9
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	+	N	N	N	N	N	N	N	+	N	N	N	N	+	+	+	N	N	N	N	N	+	N	*50
Adenocarcinoma, NOS											X															1
Fibroadenoma			X													X	X	X								6
Preputial/clitoral 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												X														4
Papilloma, NOS																										1
Adenoma, NOS	X		X	X	X														X		X					10
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+		49
Papillary cystadenocarcinoma, NOS						X																				1
Endometrial stromal polyp					X		X		X		X	X			X					X						15
Endometrial stromal sarcoma																										1
Ovary	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		12
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		12
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
Leukemia, mononuclear cell	X				X											X						X				8

* Animals necropsied

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

ANIMAL NUMBER	0052	0090	0054	0057	0083	0070	0067	0073	0060	0082	0097	0074	0071	0076	0072	0088	0100	0062	0081	0065	0092	0051	0053	0055	0056
WEEKS ON STUDY	0588	0667	0777	0788	0799	0800	0833	0877	0911	0991	0991	0992	0994	0994	0995	0998	0999	1000	1011	1033	1033	1044	1044	1044	1044
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	●	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	−	+	−	+	−	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
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	X		X			X			X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																					X				
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma													X						X						
C-cell carcinoma									X																
Parathyroid	−	+	+	+	+	+	−	+	+	+	−	−	+	+	+	+	+	+	−	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma														X					X						
Preputial/clitoral 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		X																		X			
Papillary carcinoma								X																	
Adenoma, NOS	X		X															X	X	X	X		X		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Leiomyoma																									
Endometrial stromal polyp					X				X														X		
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma																		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				

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

ANIMAL NUMBER	0058	0059	0061	0063	0064	0066	0068	0069	0075	0077	0078	0079	0080	0084	0085	0086	0087	0089	0091	0093	0094	0095	0096	0098	0099	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma													X													1
Trachea	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	−	+	+	+	+	+	−	+	−	−	−	−	+	+	+	+	+	+	+	−	+	−	−	−	+	34
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule										X																1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS		X		X			X				X			X	X		X	X								18
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma																							X			2
Pheochromocytoma		X																								1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma																										3
C-cell carcinoma										X																2
Parathyroid	−	−	+	−	+	+	+	−	+	+	+	−	+	+	+	−	+	+	−	+	+	+	−	+	+	37
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma																										2
Preputial/clitoral 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									X																	4
Papillary carcinoma																										1
Adenoma, NOS																			X	X	X	X				11
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS	X																									1
Leiomyoma																					X					1
Endometrial stromal polyp																	X							X		5
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma																										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																										7

* Animals necropsied

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

	Control	2,000 ppm	4,000 ppm
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	6.7%	9.1%	0.0%
Terminal Rates (c)	1/22 (5%)	2/31 (6%)	0/29 (0%)
Week of First Observation	75	97	
Life Table Tests (d)	P=0.153N	P=0.600	P=0.203N
Incidental Tumor Tests (d)	P=0.196N	P=0.540	P=0.251N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.500	P=0.247N
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	20/50 (40%)	9/50 (18%)	7/50 (14%)
Adjusted Rates (b)	51.6%	24.7%	16.5%
Terminal Rates (c)	5/22 (23%)	5/31 (16%)	0/29 (0%)
Week of First Observation	75	80	79
Life Table Tests (d)	P=0.001N	P=0.008N	P=0.004N
Incidental Tumor Tests (d)	P=0.002N	P=0.040N	P=0.004N
Cochran-Armitage Trend Test (d)	P=0.002N		
Fisher Exact Test (d)		P=0.014N	P=0.003N
Anterior Pituitary Gland: Adenoma			
Overall Rates (e)	19/50 (38%)	18/37 (49%)	17/50 (34%)
Adjusted Rates (b)	67.1%	60.8%	45.6%
Terminal Rates (c)	13/22 (59%)	12/22 (55%)	10/29 (34%)
Week of First Observation	92	65	79
Life Table Tests (d)	P=0.136N	P=0.442N	P=0.165N
Incidental Tumor Tests (d)	P=0.269N	P=0.257	P=0.283N
Cochran-Armitage Trend Test (d)	P=0.379N		
Fisher Exact Test (d)		P=0.219	P=0.418N
Anterior Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (e)	20/50 (40%)	18/37 (49%)	17/50 (34%)
Adjusted Rates (b)	68.3%	60.8%	45.6%
Terminal Rates (c)	13/22 (59%)	12/22 (55%)	10/29 (34%)
Week of First Observation	92	65	79
Life Table Tests (d)	P=0.098N	P=0.356N	P=0.122N
Incidental Tumor Tests (d)	P=0.211N	P=0.296	P=0.224N
Cochran-Armitage Trend Test (d)	P=0.305N		
Fisher Exact Test (d)		P=0.279	P=0.340N
Adrenal Gland: Cortical Adenoma or Carcinoma			
Overall Rates (e)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted Rates (b)	11.4%	0.0%	6.6%
Terminal Rates (c)	1/22 (5%)	0/31 (0%)	1/29 (3%)
Week of First Observation	100		103
Life Table Tests (d)	P=0.317N	P=0.084N	P=0.404N
Incidental Tumor Tests (d)	P=0.420N	P=0.145N	P=0.529N
Cochran-Armitage Trend Test (d)	P=0.390N		
Fisher Exact Test (d)		P=0.121N	P=0.500N
Adrenal Medulla: Pheochromocytoma			
Overall Rates (e)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	11.5%	3.2%	3.4%
Terminal Rates (c)	2/22 (9%)	1/31 (3%)	1/29 (3%)
Week of First Observation	92	104	104
Life Table Tests (d)	P=0.143N	P=0.215N	P=0.231N
Incidental Tumor Tests (d)	P=0.155N	P=0.263N	P=0.244N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.309N	P=0.309N

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

	Control	2,000 ppm	4,000 ppm
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (e)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	16.0%	3.2%	3.4%
Terminal Rates (c)	3/22 (14%)	1/31 (3%)	1/29 (3%)
Week of First Observation	92	104	104
Life Table Tests (d)	P=0.060N	P=0.105N	P=0.117N
Incidental Tumor Tests (d)	P=0.067N	P=0.132N	P=0.125N
Cochran-Armitage Trend Test (d)	P=0.101N		
Fisher Exact Test (d)		P=0.181N	P=0.181N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (e)	2/50 (4%)	(f) 1/12 (8%)	3/50 (6%)
Adjusted Rates (b)	6.8%		8.9%
Terminal Rates (c)	1/22 (5%)		1/29 (3%)
Week of First Observation	82		94
Life Table Test (d)			P=0.576
Incidental Tumor Test (d)			P=0.487
Fisher Exact Test (d)			P=0.500
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (e)	3/50 (6%)	(f) 2/12 (17%)	5/50 (10%)
Adjusted Rates (b)	9.3%		14.3%
Terminal Rates (c)	1/22 (5%)		2/29 (7%)
Week of First Observation	82		91
Life Table Test (d)			P=0.446
Incidental Tumor Test (d)			P=0.349
Fisher Exact Test (d)			P=0.357
Mammary Gland: Fibroadenoma			
Overall Rates (a)	7/50 (14%)	6/50 (12%)	2/50 (4%)
Adjusted Rates (b)	20.6%	17.8%	5.7%
Terminal Rates (c)	2/22 (9%)	4/31 (13%)	0/29 (0%)
Week of First Observation	68	95	94
Life Table Tests (d)	P=0.041N	P=0.352N	P=0.062N
Incidental Tumor Tests (d)	P=0.086N	P=0.540N	P=0.122N
Cochran-Armitage Trend Test (d)	P=0.067N		
Fisher Exact Test (d)		P=0.500N	P=0.080N
Mammary Gland: Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (b)	10.0%	3.2%	0.0%
Terminal Rates (c)	1/22 (5%)	1/31 (3%)	0/29 (0%)
Week of First Observation	92	104	
Life Table Tests (d)	P=0.044N	P=0.238N	P=0.102N
Incidental Tumor Tests (d)	P=0.062N	P=0.330N	P=0.130N
Cochran-Armitage Trend Test (d)	P=0.060N		
Fisher Exact Test (d)		P=0.309N	P=0.121N
Mammary Gland: Fibroadenoma or Adenocarcinoma			
Overall Rates (a)	10/50 (20%)	7/50 (14%)	2/50 (4%)
Adjusted Rates (b)	28.9%	20.8%	5.7%
Terminal Rates (c)	3/22 (14%)	5/31 (16%)	0/29 (0%)
Week of First Observation	68	95	94
Life Table Tests (d)	P=0.006N	P=0.173N	P=0.012N
Incidental Tumor Tests (d)	P=0.017N	P=0.343N	P=0.026N
Cochran-Armitage Trend Test (d)	P=0.012N		
Fisher Exact Test (d)		P=0.298N	P=0.014N

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

	Control	2,000 ppm	4,000 ppm
Clitoral Gland: Adenoma			
Overall Rates (a)	4/46 (9%)	10/46 (22%)	11/47 (23%)
Adjusted Rates (b)	19.0%	29.8%	31.3%
Terminal Rates (c)	4/21 (19%)	8/31 (26%)	5/27 (19%)
Week of First Observation	104	88	58
Life Table Tests (d)	P=0.091	P=0.216	P=0.112
Incidental Tumor Tests (d)	P=0.059	P=0.153	P=0.067
Cochran-Armitage Trend Test (d)	P=0.044		
Fisher Exact Test (d)		P=0.072	P=0.049
Clitoral Gland: Adenoma or Papilloma			
Overall Rates (a)	4/46 (9%)	11/46 (24%)	11/47 (23%)
Adjusted Rates (b)	19.0%	31.7%	31.3%
Terminal Rates (c)	4/21 (19%)	8/31 (26%)	5/27 (19%)
Week of First Observation	104	88	58
Life Table Tests (d)	P=0.097	P=0.153	P=0.112
Incidental Tumor Tests (d)	P=0.060	P=0.086	P=0.067
Cochran-Armitage Trend Test (d)	P=0.047		
Fisher Exact Test (d)		P=0.044	P=0.049
Clitoral Gland: Carcinoma or Papillary Carcinoma			
Overall Rates (a)	1/46 (2%)	4/46 (9%)	5/47 (11%)
Adjusted Rates (b)	3.3%	10.3%	13.3%
Terminal Rates (c)	0/21 (0%)	1/31 (3%)	2/27 (7%)
Week of First Observation	100	73	67
Life Table Tests (d)	P=0.109	P=0.219	P=0.141
Incidental Tumor Tests (d)	P=0.074	P=0.105	P=0.105
Cochran-Armitage Trend Test (d)	P=0.084		
Fisher Exact Test (d)		P=0.181	P=0.107
Clitoral Gland: Adenoma, Papilloma, Carcinoma, or Papillary Carcinoma			
Overall Rates (a)	5/46 (11%)	15/46 (33%)	16/47 (34%)
Adjusted Rates (b)	21.8%	39.5%	41.5%
Terminal Rates (c)	4/21 (19%)	9/31 (29%)	7/27 (26%)
Week of First Observation	100	73	58
Life Table Tests (d)	P=0.028	P=0.062	P=0.030
Incidental Tumor Tests (d)	P=0.009	P=0.016	P=0.010
Cochran-Armitage Trend Test (d)	P=0.008		
Fisher Exact Test (d)		P=0.011	P=0.007
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	14/50 (28%)	15/50 (30%)	5/50 (10%)
Adjusted Rates (b)	38.6%	38.7%	14.4%
Terminal Rates (c)	4/22 (18%)	9/31 (29%)	3/29 (10%)
Week of First Observation	57	47	79
Life Table Tests (d)	P=0.010N	P=0.437N	P=0.013N
Incidental Tumor Tests (d)	P=0.031N	P=0.448	P=0.030N
Cochran-Armitage Trend Test (d)	P=0.021N		
Fisher Exact Test (d)		P=0.500	P=0.020N
All Sites: Benign Tumors			
Overall Rates (a)	38/50 (76%)	37/50 (74%)	32/50 (64%)
Adjusted Rates (b)	92.2%	78.4%	74.0%
Terminal Rates (c)	19/22 (86%)	21/31 (68%)	18/29 (62%)
Week of First Observation	57	47	58
Life Table Tests (d)	P=0.034N	P=0.109N	P=0.037N
Incidental Tumor Tests (d)	P=0.116N	P=0.471N	P=0.104N
Cochran-Armitage Trend Test (d)	P=0.112N		
Fisher Exact Test (d)		P=0.500N	P=0.138N

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

	Control	2,000 ppm	4,000 ppm
All Sites: Malignant Tumors			
Overall Rates (a)	25/50 (50%)	22/50 (44%)	16/50 (32%)
Adjusted Rates (b)	60.2%	51.4%	36.2%
Terminal Rates (c)	6/22 (27%)	11/31 (35%)	4/29 (14%)
Week of First Observation	75	67	67
Life Table Tests (d)	P=0.030N	P=0.167N	P=0.041N
Incidental Tumor Tests (d)	P=0.061N	P=0.525	P=0.079N
Cochran-Armitage Trend Test (d)	P=0.043N		
Fisher Exact Test (d)		P=0.345N	P=0.052N
All Sites: All Tumors			
Overall Rates (a)	47/50 (94%)	43/50 (86%)	41/50 (82%)
Adjusted Rates (b)	95.9%	86.0%	83.5%
Terminal Rates (c)	20/22 (91%)	24/31 (77%)	21/29 (72%)
Week of First Observation	57	47	58
Life Table Tests (d)	P=0.036N	P=0.047N	P=0.042N
Incidental Tumor Tests (d)	P=0.064N	P=0.306N	P=0.056N
Cochran-Armitage Trend Test (d)	P=0.049N		
Fisher Exact Test (d)		P=0.159N	P=0.061N

(a) Number of tumor-bearing animals/number of animals examined grossly at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(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 than in controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined microscopically at the site
(f) Incomplete sampling of tissues

TABLE B4a. HISTORICAL INCIDENCE OF CLITORAL GLAND TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	5/49	1/49	6/49
Phenylephrine hydrochloride	1/50	1/50	2/50
Erythromycin stearate	5/50	1/50	6/50
Tetracycline hydrochloride	1/49	0/49	1/49
Oxytetracycline hydrochloride	2/50	3/50	5/50
Nitrofurazone	3/49	5/49	8/49
Nalidixic acid	(b) 5/50	1/50	(b) 6/50
α-Methyldopa sesquihydrate	10/50	0/50	10/50
TOTAL	(b) 32/397 (8.0%)	12/397 (3.0%)	(b) 44/397 (11.1%)
SD (c)	5.95%	3.44%	5.89%
Range (d)			
High	10/50	5/49	10/50
Low	1/50	0/50	1/49
Overall Historical Incidence			
TOTAL	(b) 63/1,643 (3.8%)	(e) 53/1,643 (3.2%)	(b,e) 116/1,643 (7.1%)
SD (c)	4.44%	3.49%	4.91%
Range (d)			
High	10/50	6/49	10/50
Low	0/50	0/50	0/50

(a) Data as of May 12, 1988, for studies of at least 104 weeks
(b) Includes one papilloma, NOS
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes three squamous cell carcinomas and four adenocarcinomas, NOS

TABLE B4b. HISTORICAL INCIDENCE OF LEUKEMIA IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls
Historical Incidence at Physiological Research Laboratories	
Ephedrine sulfate	13/49
Phenylephrine hydrochloride	15/50
Erythromycin stearate	14/50
Tetracycline hydrochloride	11/49
Oxytetracycline hydrochloride	13/50
Nitrofurazone	15/49
Nalidixic acid	20/50
α-Methyldopa sesquihydrate	12/50
TOTAL	113/397 (28.5%)
SD (b)	5.43%
Range (c)	
High	20/50
Low	11/49
Overall Historical Incidence	
TOTAL	324/1,643 (19.7%)
SD (b)	8.10%
Range (c)	
High	20/50
Low	3/50

(a) Data as of May 12, 1988, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE B4c. HISTORICAL INCIDENCE OF MAMMARY GLAND TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Fibroadenoma	Adenocarcinoma	Fibroadenoma or Adenocarcinoma
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	10/49	1/49	11/49
Phenylephrine hydrochloride	11/50	1/50	11/50
Erythromycin stearate	(b) 13/50	2/50	(b) 14/50
Tetracycline hydrochloride	(c) 16/49	(d) 1/49	(c,d) 16/49
Oxytetracycline hydrochloride	(b) 22/50	1/50	(b) 22/50
Nitrofurazone	8/49	1/49	9/49
Nalidixic acid	7/50	3/50	10/50
α-Methyldopa sesquihydrate	19/50	2/50	21/50
TOTAL	106/397 (26.7%)	12/397 (3.0%)	114/397 (28.7%)
SD (e)	10.65%	1.50%	9.95%
Range (f)			
High	22/50	3/50	22/50
Low	7/50	1/50	9/49
Overall Historical Incidence			
TOTAL	(g) 520/1,643 (31.6%)	(h) 49/1,643 (3.0%)	(g,h) 552/1,643 (33.6%)
SD (e)	12.23%	2.07%	11.95%
Range (f)			
High	30/50	4/50	32/50
Low	5/50	0/50	6/50

(a) Data as of May 12, 1988, for studies of at least 104 weeks
(b) Includes one adenoma, NOS
(c) Includes three adenomas, NOS
(d) Papillary adenocarcinoma
(e) Standard deviation
(f) Range and SD are presented for groups of 35 or more animals.
(g) Includes 11 adenomas, NOS, 2 cystadenomas, NOS, and 1 papillary cystadenoma, NOS
(h) Includes two carcinomas, NOS, two papillary adenocarcinomas, and one papillary cystadenocarcinoma, NOS

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

	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, chronic focal	1 (2%)		1 (2%)
Hyperplasia, basal cell	1 (2%)		
Acanthosis	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Cyst, NOS	1 (2%)		
Hemorrhage, chronic	1 (2%)		
Abscess, NOS			1 (2%)
Inflammation, granulomatous focal			1 (2%)
Granulation tissue	1 (2%)		
Necrosis, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#Nasal cavity	(50)	(50)	(50)
Retention of content			1 (2%)
Empyema			1 (2%)
Infection, fungal	1 (2%)		
Foreign material, NOS	2 (4%)	1 (2%)	3 (6%)
#Nasal mucosa	(50)	(50)	(50)
Inflammation, acute	1 (2%)	3 (6%)	
Inflammation, active chronic	4 (8%)	1 (2%)	14 (28%)
Inflammation, chronic	11 (22%)	15 (30%)	16 (32%)
Foreign material, NOS			1 (2%)
Hyperplasia, epithelial			4 (8%)
#Nasal gland	(50)	(50)	(50)
Retention of content			1 (2%)
#Nasal turbinate	(50)	(50)	(50)
Metaplasia, osseous	1 (2%)		3 (6%)
#Maxillary sinus	(50)	(50)	(50)
Inflammation, acute			1 (2%)
Inflammation, active chronic			1 (2%)
Foreign material, NOS			1 (2%)
#Maxillary sinus gland	(50)	(50)	(50)
Inflammation, chronic			1 (2%)
#Trachea	(50)	(11)	(49)
Inflammation, chronic	4 (8%)		4 (8%)
#Lung	(50)	(19)	(50)
Emphysema, NOS	1 (2%)		2 (4%)
Congestion, acute		1 (5%)	2 (4%)
Hemorrhage	1 (2%)		
Bronchopneumonia, NOS			3 (6%)
Inflammation, acute/chronic	1 (2%)		
Pneumonia, interstitial chronic	6 (12%)	1 (5%)	5 (10%)
Inflammation, chronic focal		1 (5%)	1 (2%)
Inflammation, granulomatous	1 (2%)		1 (2%)
Reaction, foreign body			2 (4%)
Perivascular cuffing	1 (2%)	1 (5%)	1 (2%)
Foreign material, NOS			2 (4%)
Hemosiderosis	1 (2%)		
Hyperplasia, alveolar epithelium	1 (2%)	1 (5%)	
Histiocytosis	1 (2%)		1 (2%)

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

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(49)	(49)
Fibrosis			1 (2%)
Atrophy, NOS		2 (4%)	
Metaplasia, osseous	10 (20%)	4 (8%)	3 (6%)
Hyperplasia, hematopoietic	11 (22%)	15 (31%)	12 (24%)
Hyperplasia, reticulum cell	2 (4%)		1 (2%)
#Spleen	(50)	(50)	(50)
Congestion, NOS	1 (2%)		
Necrosis, focal		1 (2%)	
Hemosiderosis	32 (64%)	40 (80%)	41 (82%)
Hyperplasia, reticulum cell		1 (2%)	
Hyperplasia, lymphoid			1 (2%)
Hematopoiesis	6 (12%)	11 (22%)	11 (22%)
#Lymph node	(50)	(13)	(50)
Hemorrhage		1 (8%)	2 (4%)
Inflammation, granulomatous			1 (2%)
Hemosiderosis		1 (8%)	2 (4%)
Hyperplasia, plasma cell		1 (8%)	1 (2%)
Hyperplasia, reticulum cell		1 (8%)	
#Mandibular lymph node	(50)	(13)	(50)
Cyst, NOS			4 (8%)
Hemorrhage	1 (2%)		
Pigmentation, NOS			1 (2%)
Atrophy, focal			1 (2%)
Hyperplasia, NOS	2 (4%)		2 (4%)
Hyperplasia, plasma cell	2 (4%)		1 (2%)
#Mediastinal lymph node	(50)	(13)	(50)
Hemorrhage	3 (6%)		3 (6%)
Hemosiderosis	3 (6%)	1 (8%)	7 (14%)
Hyperplasia, NOS	1 (2%)		
Hyperplasia, plasma cell			1 (2%)
Hyperplasia, lymphoid			1 (2%)
#Pancreatic lymph node	(50)	(13)	(50)
Hemorrhage	1 (2%)		
#Mesenteric lymph node	(50)	(13)	(50)
Hemorrhage	2 (4%)		1 (2%)
Hyperplasia, reticulum cell			1 (2%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)
#Liver	(50)	(50)	(50)
Hematopoiesis			1 (2%)
#Large intestine	(50)	(11)	(50)
Hyperplasia, lymphoid	1 (2%)		
#Thymus	(35)	(41)	(34)
Hemorrhage		1 (2%)	
Hyperplasia, epithelial	11 (31%)	18 (44%)	22 (65%)
CIRCULATORY SYSTEM			
*Multiple organs	(50)	(50)	(50)
Periarteritis	1 (2%)		
#Heart	(50)	(11)	(50)
Hypertrophy, NOS	1 (2%)		
#Myocardium	(50)	(11)	(50)
Inflammation, acute/chronic			1 (2%)
Inflammation, chronic focal			1 (2%)
Degeneration, NOS	22 (44%)	3 (27%)	27 (54%)
#Endocardium	(50)	(11)	(50)
Inflammation, acute/chronic			1 (2%)
Infection, bacterial			1 (2%)
Hemosiderosis	1 (2%)		

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

	Untreated Control	Low Dose	High Dose
CIRCULATORY SYSTEM (Continued)			
#Cardiac valve	(50)	(11)	(50)
Inflammation, chronic			1 (2%)
Hyperplasia, NOS			1 (2%)
*Artery	(50)	(50)	(50)
Periarteritis			1 (2%)
*Pulmonary artery	(50)	(50)	(50)
Periarteritis			1 (2%)
Hypertrophy, focal			1 (2%)
#Liver	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
#Salivary gland	(50)	(11)	(50)
Inflammation, active chronic			1 (2%)
Inflammation, chronic focal		1 (9%)	
#Parotid gland	(50)	(11)	(50)
Inflammation, chronic focal	1 (2%)		1 (2%)
Atrophy, focal			1 (2%)
#Sublingual gland	(50)	(11)	(50)
Cyst, NOS			1 (2%)
#Submaxillary gland	(50)	(11)	(50)
Atrophy, focal	1 (2%)		
#Liver	(50)	(50)	(50)
Hernia, NOS	1 (2%)	3 (6%)	1 (2%)
Deformity, NOS	4 (8%)		3 (6%)
Inflammation, acute focal	1 (2%)		
Inflammation, acute/chronic	2 (4%)	1 (2%)	
Granuloma, NOS	9 (18%)	13 (26%)	15 (30%)
Necrosis, focal	1 (2%)	1 (2%)	4 (8%)
Infarct, NOS			1 (2%)
Metamorphosis, fatty	4 (8%)	2 (4%)	
Mitotic alteration	1 (2%)	1 (2%)	
Cytoplasmic vacuolization		1 (2%)	1 (2%)
Basophilic cyto change	38 (76%)	38 (76%)	35 (70%)
Ground glass cyto change	2 (4%)		1 (2%)
Focal cellular change	3 (6%)	5 (10%)	3 (6%)
Eosinophilic cyto change			1 (2%)
Clear cell change		1 (2%)	2 (4%)
Hepatocytomegaly	2 (4%)		
Angiectasis		6 (12%)	
#Hepatic capsule	(50)	(50)	(50)
Fibrosis, focal		1 (2%)	
#Portal tract	(50)	(50)	(50)
Inflammation, chronic	3 (6%)	3 (6%)	1 (2%)
Histiocytosis	1 (2%)		
#Liver/centrilobular	(50)	(50)	(50)
Necrosis, NOS		1 (2%)	
Metamorphosis, fatty	1 (2%)		
#Liver/periportal	(50)	(50)	(50)
Metamorphosis, fatty	1 (2%)	1 (2%)	
Cytoplasmic vacuolization	3 (6%)		
#Bile duct	(50)	(50)	(50)
Hyperplasia, NOS	7 (14%)	5 (10%)	1 (2%)
Hyperplasia, focal	3 (6%)	1 (2%)	1 (2%)

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

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Pancreas	(50)	(11)	(50)
Cyst, NOS			1 (2%)
Inflammation, active chronic	1 (2%)		
Inflammation, acute/chronic			1 (2%)
Inflammation, chronic focal	3 (6%)	1 (9%)	3 (6%)
Inflammation, chronic diffuse	1 (2%)		
Metaplasia, NOS			1 (2%)
#Pancreatic acinus	(50)	(11)	(50)
Degeneration, NOS			1 (2%)
Focal cellular change	2 (4%)		1 (2%)
Atrophy, focal	9 (18%)	2 (18%)	3 (6%)
Atrophy, diffuse	2 (4%)		
Hyperplasia, NOS			1 (2%)
Hyperplasia, focal	1 (2%)		
#Glandular stomach	(50)	(12)	(50)
Dilatation, NOS	5 (10%)	2 (17%)	6 (12%)
Cyst, NOS	20 (40%)		17 (34%)
Inflammation, acute/chronic	1 (2%)	1 (8%)	
#Forestomach	(50)	(12)	(50)
Edema, NOS	1 (2%)		
Ulcer, NOS	1 (2%)		
Inflammation, acute/chronic	2 (4%)	1 (8%)	
Granulation tissue	1 (2%)		
Erosion		1 (8%)	
Hyperplasia, epithelial	1 (2%)		
#Small intestine	(50)	(11)	(50)
Ectopia	1 (2%)		
*Rectum	(50)	(50)	(50)
Dilatation, NOS			1 (2%)
Inflammation, chronic		1 (2%)	
Erosion		1 (2%)	
URINARY SYSTEM			
#Kidney	(50)	(12)	(50)
Hydronephrosis	1 (2%)		
Hemorrhage		1 (8%)	
Abscess, NOS			1 (2%)
Inflammation, chronic focal			1 (2%)
Nephropathy	39 (78%)	7 (58%)	41 (82%)
#Kidney/medulla	(50)	(12)	(50)
Mineralization	2 (4%)	5 (42%)	3 (6%)
#Kidney/tubule	(50)	(12)	(50)
Pigmentation, NOS	2 (4%)	1 (8%)	1 (2%)
Hyperplasia, atypical		1 (8%)	
#Kidney/pelvis	(50)	(12)	(50)
Mineralization	6 (12%)		9 (18%)
#Urinary bladder	(49)	(11)	(50)
Inflammation, chronic		1 (9%)	
ENDOCRINE SYSTEM			
#Pituitary intermedia	(50)	(37)	(50)
Hyperplasia, focal		1 (3%)	1 (2%)
#Anterior pituitary	(50)	(37)	(50)
Cyst, NOS	22 (44%)	7 (19%)	14 (28%)
Multiple cysts		3 (8%)	
Hemosiderosis			1 (2%)
Focal cellular change	4 (8%)	1 (3%)	4 (8%)
Hyperplasia, focal	9 (18%)	7 (19%)	8 (16%)
Angiectasis	8 (16%)	4 (11%)	8 (16%)

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

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Adrenal	(50)	(50)	(50)
Angiectasis	5 (10%)	2 (4%)	5 (10%)
#Adrenal/capsule	(50)	(50)	(50)
Ectopia	3 (6%)	4 (8%)	4 (8%)
#Adrenal cortex	(50)	(50)	(50)
Degeneration, lipoid	14 (28%)	7 (14%)	6 (12%)
Metamorphosis, fatty	3 (6%)	1 (2%)	1 (2%)
Eosinophilic cyto change		2 (4%)	
Hypertrophy, focal	4 (8%)	4 (8%)	5 (10%)
Hyperplasia, focal	16 (32%)	11 (22%)	13 (26%)
#Adrenal medulla	(50)	(50)	(50)
Degeneration, NOS	1 (2%)		
Amyloid, NOS	1 (2%)		
Hyperplasia, focal	2 (4%)	5 (10%)	4 (8%)
#Thyroid	(50)	(12)	(50)
Embryonal duct cyst		1 (8%)	1 (2%)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, C-cell	20 (40%)	3 (25%)	19 (38%)
#Thyroid follicle	(50)	(12)	(50)
Dilatation, NOS	1 (2%)		
#Parathyroid	(37)	(9)	(37)
Hyperplasia, diffuse	1 (3%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Dilatation, NOS	21 (42%)	1 (2%)	6 (12%)
Dilatation/ducts	2 (4%)	1 (2%)	
Galactocele	1 (2%)	1 (2%)	2 (4%)
Inflammation, granulomatous	2 (4%)		
Hyperplasia, epithelial	12 (24%)		8 (16%)
Histiocytosis	1 (2%)		
*Mammary duct	(50)	(50)	(50)
Hyperplasia, epithelial		1 (2%)	
*Clitoral gland	(50)	(50)	(50)
Retention of content	1 (2%)	6 (12%)	5 (10%)
Cyst, NOS			1 (2%)
Inflammation, active chronic	1 (2%)	3 (6%)	5 (10%)
Inflammation, chronic	7 (14%)	9 (18%)	6 (12%)
Abscess, chronic		1 (2%)	
Inflammation, granulomatous	2 (4%)	1 (2%)	
Granulation tissue			1 (2%)
Hyperplasia, epithelial	3 (6%)	7 (14%)	5 (10%)
*Vagina	(50)	(50)	(50)
Polyp, inflammatory			1 (2%)
#Uterus	(50)	(49)	(50)
Dilatation, NOS	8 (16%)	3 (6%)	3 (6%)
Hemorrhage	2 (4%)	1 (2%)	
Inflammation, active chronic		2 (4%)	
Inflammation, acute/chronic	1 (2%)		1 (2%)
#Cervix uteri	(50)	(49)	(50)
Dilatation, NOS	1 (2%)		
Inflammation, acute	1 (2%)		
Fibrosis			2 (4%)
#Cervical mucous membrane	(50)	(49)	(50)
Metaplasia, NOS			1 (2%)
#Uterus/endometrium	(50)	(49)	(50)
Cyst, NOS	4 (8%)	4 (8%)	1 (2%)
Edema, NOS		1 (2%)	
Infarct, NOS			1 (2%)
Hyperplasia, epithelial	2 (4%)	2 (4%)	2 (4%)

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

	Untreated Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM (Continued)			
#Endometrial stroma	(50)	(49)	(50)
Necrosis, NOS		1 (2%)	
Hyperplasia, focal	1 (2%)		
NERVOUS SYSTEM			
*Choroid plexus	(50)	(50)	(50)
Hyperplasia, NOS			2 (4%)
#Brain	(50)	(12)	(50)
Deformity, NOS	3 (6%)		1 (2%)
Hemorrhage	3 (6%)	1 (8%)	2 (4%)
SPECIAL SENSE ORGANS			
*Eye/anterior chamber	(50)	(50)	(50)
Hemorrhage			2 (4%)
*Eye/sclera	(50)	(50)	(50)
Mineralization	7 (14%)	16 (32%)	20 (40%)
Metaplasia, osseous			1 (2%)
*Eye/ciliary body	(50)	(50)	(50)
Hemosiderosis			1 (2%)
*Eye/iris	(50)	(50)	(50)
Synechia, anterior			2 (4%)
Synechia, posterior		1 (2%)	2 (4%)
Hemosiderosis			1 (2%)
*Eye/retina	(50)	(50)	(50)
Detachment			1 (2%)
Fibrosis			1 (2%)
Degeneration, NOS	6 (12%)	42 (84%)	47 (94%)
*Eye/crystalline lens	(50)	(50)	(50)
Cataract	1 (2%)	22 (44%)	19 (38%)
*Nasolacrimal duct	(50)	(50)	(50)
Retention of content	2 (4%)		2 (4%)
Inflammation, active chronic	9 (18%)	1 (2%)	5 (10%)
Inflammation, chronic	11 (22%)	21 (42%)	16 (32%)
Foreign material, NOS		1 (2%)	5 (10%)
*Harderian gland	(50)	(50)	(50)
Inflammation with fibrosis			1 (2%)
Fibrosis	1 (2%)		1 (2%)
*Zymbal gland	(50)	(50)	(50)
Abscess, NOS	1 (2%)		
Infection, bacterial	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Bone	(50)	(50)	(50)
Chondrodystrophy			1 (2%)
*Epiphysis	(50)	(50)	(50)
Metaplasia, cartilaginous	1 (2%)		
*Vertebra	(50)	(50)	(50)
Degeneration, NOS			1 (2%)
*Femur	(50)	(50)	(50)
Deformity, NOS		1 (2%)	
Metaplasia, osseous	2 (4%)		1 (2%)
*Synovial tissue	(50)	(50)	(50)
Mineralization		1 (2%)	
*Intervertebral disc	(50)	(50)	(50)
Metaplasia, osseous			1 (2%)

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

	Untreated Control	Low Dose	High Dose
BODY CAVITIES			
*Mesentery	(50)	(50)	(50)
Accessory structure			2 (4%)
Inflammation, acute/chronic			1 (2%)
Inflammation, chronic		1 (2%)	
ALL OTHER SYSTEMS			
Adipose tissue			
Inflammation, chronic			1
Necrosis, NOS	7	10	7
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 NALIDIXIC ACID

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

	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)
Fibroma	1 (2%)	3 (6%)	3 (6%)
Fibrosarcoma	4 (8%)	6 (12%)	11 (22%)
RESPIRATORY SYSTEM			
#Lung	(49)	(50)	(50)
Hepatocellular carcinoma, metastatic	4 (8%)		
Alveolar/bronchiolar adenoma	4 (8%)	4 (8%)	4 (8%)
Alveolar/bronchiolar carcinoma	2 (4%)	2 (4%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, histiocytic type	1 (2%)	1 (2%)	1 (2%)
Malignant lymphoma, mixed type	3 (6%)		
#Spleen	(50)	(15)	(50)
Malignant lymphoma, mixed type	1 (2%)		
#Lymph node	(49)	(23)	(47)
Malignant lymphoma, mixed type	1 (2%)		
#Mesenteric lymph node	(49)	(23)	(47)
Malignant lymphoma, mixed type	1 (2%)		1 (2%)
CIRCULATORY SYSTEM			
#Spleen	(50)	(15)	(50)
Hemangioma	1 (2%)		
#Liver	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	2 (4%)	
#Urinary bladder	(50)	(10)	(49)
Hemangioma	1 (2%)		
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Hepatocellular adenoma	3 (6%)	6 (12%)	3 (6%)
Hepatocellular carcinoma	7 (14%)	7 (14%)	4 (8%)
#Cecum	(50)	(9)	(49)
Leiomyosarcoma	1 (2%)		
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Adrenal medulla	(50)	(50)	(49)
Pheochromocytoma	1 (2%)	1 (2%)	3 (6%)
#Thyroid	(49)	(8)	(49)
Follicular cell adenoma	1 (2%)		1 (2%)
Follicular cell carcinoma			1 (2%)

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

	Untreated Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM			
#Testis	(50)	(8)	(49)
Sertoli cell tumor	1 (2%)		
Interstitial cell tumor			2 (4%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(50)
Adenoma, NOS			2 (4%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Sarcoma, NOS	1 (2%)		
Fibrosarcoma, metastatic	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	2	4	5
Moribund sacrifice	15	12	14
Terminal sacrifice	33	34	31
TUMOR SUMMARY			
Total animals with primary tumors**	26	24	32
Total primary tumors	36	32	40
Total animals with benign tumors	11	12	15
Total benign tumors	13	14	18
Total animals with malignant tumors	18	16	20
Total malignant tumors	23	18	22
Total animals with secondary tumors##	5		
Total secondary tumors	5		

* Number of animals receiving complete necropsy examinations; 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 C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID: UNTREATED CONTROL

ANIMAL NUMBER	10 06	11 10	13 34	11 11	12 25	10 07	11 15	14 22	12 26	12 29	13 31	11 18	12 21	10 02	13 38	12 28	14 44	10 01	10 03	10 04	10 05	10 08	10 09	11 12	11 13
WEEKS ON STUDY	00 08	01 15	02 28	03 11	03 31	03 34	03 36	07 09	08 00	08 86	08 88	09 01	09 91	09 96	09 98	10 02	10 02	10 04	10 04	10 44	10 44	10 44	10 44	10 44	10 44
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Fibrosarcoma											X		X	X											
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																X									
Alveolar/bronchiolar adenoma												X													
Alveolar/bronchiolar carcinoma																									
Trachea	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																		X							
Malignant lymphoma, mixed type																					X				
Lymph nodes	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type															X										
Thymus	+	−	+	+	+	+	−	+	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																				X					
Hepatocellular carcinoma										X		X				X									
Hemangiosarcoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																					X				
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
ENDOCRINE SYSTEM																									
Pituitary	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																				X					
Thyroid	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																				X					
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	+
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sertoli cell tumor																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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
Sarcoma, NOS										X															
Fibrosarcoma, metastatic											X														
Malignant lymphoma, histiocytic type												X													
Malignant lymphoma, mixed type																						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	114	116	117	119	120	122	123	124	127	130	132	133	135	136	137	139	140	141	143	145	146	147	148	149	150	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																				X						1
Fibrosarcoma								X																		4
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic	X		X											X												4
Alveolar/bronchiolar adenoma		X		X			X																			4
Alveolar/bronchiolar carcinoma																					X		X			2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Malignant lymphoma, mixed type																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, mixed type							X																			2
Thymus	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	42
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma		X													X											3
Hepatocellular carcinoma	X		X				X							X												7
Hemangiosarcoma										X																1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma												X														1
ENDOCRINE SYSTEM																										
Pituitary	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma																										1
Parathyroid	–	+	+	–	+	–	+	+	+	–	+	+	+	–	+	+	+	–	–	+	–	+	+	+	–	32
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sertoli cell tumor																							X			1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
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
Sarcoma, NOS																										1
Fibrosarcoma, metastatic																										1
Malignant lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type									X					X												3

* Animals necropsied

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

ANIMAL NUMBER	0049	0331	0338	0329	0119	0110	0502	0113	0340	0098	0431	0111	0061	0339	0469	0012	0023	0044	0055	0077	0088	0124	0144
WEEKS ON STUDY	0288	0311	0346	0366	0377	0788	0811	0822	0911	0922	0998	0981	1011	1022	1033	1043	1044	1044	1044	1044	1044	1044	1044
INTEGUMENTARY SYSTEM																							
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	N	+	N	N	+	+	N	N	N	N	N	+	N	+	N	+
Fibroma														X											
Fibrosarcoma							X		X		X				X								X		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																	X					X			
Alveolar/bronchiolar carcinoma																	X	X							
Trachea	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nasal cavity	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	+	–	–	+	–	–	–	+	+	–
Lymph nodes	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	+	–	–	–	–	–	+	+	–
Thymus	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma											X		X				X		X	X					
Hepatocellular carcinoma								X		X			X												
Hemangiosarcoma									X																
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	+	+	+	+	+	+	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Esophagus	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Stomach	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Small intestine	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Large intestine	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma													X												
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	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
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, histiocytic type						X																			

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

ANIMAL NUMBER	015	016	017	018	020	021	022	023	024	025	026	027	028	030	032	035	036	037	040	041	042	044	045	047	048	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	N	N	+	N	+	N	N	N	+	+	+	N	N	N	+	+	N	N	+	N	N	N	N	N	N	*50
Fibroma					X					X																3
Fibrosarcoma									X																	6
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X												X										4
Alveolar/bronchiolar carcinoma																										2
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Nasal cavity	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Spleen	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	15
Lymph nodes	+	–	–	–	+	–	–	–	+	–	–	+	–	+	–	+	–	–	–	–	–	+	–	–	–	23
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma						X																				6
Hepatocellular carcinoma	X									X									X			X				7
Hemangiosarcoma																			X							2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	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
Pancreas	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
ENDOCRINE SYSTEM																										
Pituitary	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma																										1
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
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	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Prostate	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	12
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
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, histiocytic type																										1

* Animals necropsied

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

ANIMAL NUMBER	0059	0079	0058	0094	0071	0091	0083	0087	0078	0098	0099	0069	0070	0072	0086	0063	0074	0092	0097	0051	0052	0053	0054	0055	0056
WEEKS ON STUDY	006	012	017	019	020	020	082	082	084	091	092	094	097	098	098	100	101	101	103	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																				X					X
Fibrosarcoma									X	X				X	X	X	X		X			X			
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Trachea	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	−	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																									
Thymus	+	−	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																								X	
Hepatocellular carcinoma								X								X		X						
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																X									
Thyroid	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma															X										
Follicular cell carcinoma																								X	
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	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor											X														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																				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, histiocytic type													X												

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

ANIMAL NUMBER	0057	0060	0061	0062	0064	0065	0066	0067	0068	0073	0075	0076	0077	0080	0081	0082	0084	0085	0088	0089	0090	0093	0095	0096	0100	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																									X	3
Fibrosarcoma			X									X			X											11
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X							X				X							X						4
Alveolar/bronchiolar carcinoma							X	X				X		X												4
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Malignant lymphoma, mixed type						X																				1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	46
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma										X										X						3
Hepatocellular carcinoma																					X					4
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma					X															X						3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma																										1
Follicular cell carcinoma																										1
Parathyroid	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	–	+	+	–	–	37
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
Interstitial cell tumor																										2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
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																						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, histiocytic type																										1

* Animals necropsied

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

	Control	2,000 ppm	4,000 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	3.0%	8.4%	9.7%
Terminal Rates (c)	1/33 (3%)	2/34 (6%)	3/31 (10%)
Week of First Observation	104	102	104
Life Table Tests (d)	P=0.214	P=0.319	P=0.282
Incidental Tumor Tests (d)	P=0.251	P=0.350	P=0.282
Cochran-Armitage Trend Test (d)	P=0.238		
Fisher Exact Test (d)		P=0.309	P=0.309
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	4/50 (8%)	6/50 (12%)	11/50 (22%)
Adjusted Rates (b)	10.4%	14.9%	28.3%
Terminal Rates (c)	1/33 (3%)	2/34 (6%)	4/31 (13%)
Week of First Observation	88	81	84
Life Table Tests (d)	P=0.036	P=0.414	P=0.056
Incidental Tumor Tests (d)	P=0.048	P=0.339	P=0.080
Cochran-Armitage Trend Test (d)	P=0.031		
Fisher Exact Test (d)		P=0.370	P=0.045
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	5/50 (10%)	9/50 (18%)	14/50 (28%)
Adjusted Rates (b)	13.2%	22.4%	36.3%
Terminal Rates (c)	2/33 (6%)	4/34 (12%)	7/31 (23%)
Week of First Observation	88	81	84
Life Table Tests (d)	P=0.018	P=0.238	P=0.026
Incidental Tumor Tests (d)	P=0.024	P=0.186	P=0.035
Cochran-Armitage Trend Test (d)	P=0.015		
Fisher Exact Test (d)		P=0.194	P=0.020
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (e)	4/49 (8%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	11.4%	11.8%	12.9%
Terminal Rates (c)	3/33 (9%)	4/34 (12%)	4/31 (13%)
Week of First Observation	91	104	104
Life Table Tests (d)	P=0.544	P=0.624N	P=0.617
Incidental Tumor Tests (d)	P=0.531	P=0.631	P=0.601
Cochran-Armitage Trend Test (d)	P=0.562N		
Fisher Exact Test (d)		P=0.631N	P=0.631N
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (e)	2/49 (4%)	2/50 (4%)	4/50 (8%)
Adjusted Rates (b)	6.1%	5.9%	12.9%
Terminal Rates (c)	2/33 (6%)	2/34 (6%)	4/31 (13%)
Week of First Observation	104	104	104
Life Table Tests (d)	P=0.225	P=0.685N	P=0.307
Incidental Tumor Tests (d)	P=0.225	P=0.685N	P=0.307
Cochran-Armitage Trend Test (d)	P=0.259		
Fisher Exact Test (d)		P=0.684N	P=0.349
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (e)	6/49 (12%)	5/50 (10%)	8/50 (16%)
Adjusted Rates (b)	17.3%	14.7%	25.8%
Terminal Rates (c)	5/33 (15%)	5/34 (15%)	8/31 (26%)
Week of First Observation	91	104	104
Life Table Tests (d)	P=0.282	P=0.476N	P=0.342
Incidental Tumor Tests (d)	P=0.275	P=0.507N	P=0.329
Cochran-Armitage Trend Test (d)	P=0.340		
Fisher Exact Test (d)		P=0.486N	P=0.403

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

	Control	2,000 ppm	4,000 ppm
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	6/50 (12%)	(f) 0/50 (0%)	1/50 (2%)
Adjusted Rates (b)	17.5%	0.0%	3.2%
Terminal Rates (c)	5/33 (15%)	0/34 (0%)	1/31 (3%)
Week of First Observation	98		104
Life Table Tests (d)	P=0.018N	P=0.016N	P=0.067N
Incidental Tumor Tests (d)	P=0.014N	P=0.013N	P=0.051N
Cochran-Armitage Trend Test (d)	P=0.016N		
Fisher Exact Test (d)		P=0.014N	P=0.056N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	7/50 (14%)	(f) 1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	19.6%	2.2%	5.8%
Terminal Rates (c)	5/33 (15%)	0/34 (0%)	1/31 (3%)
Week of First Observation	91	78	97
Life Table Tests (d)	P=0.039N	P=0.031N	P=0.092N
Incidental Tumor Tests (d)	P=0.022N	P=0.009N	P=0.062N
Cochran-Armitage Trend Test (d)	P=0.036N		
Fisher Exact Test (d)		P=0.030N	P=0.080N
Circulatory System: Hemangioma or Hemangiosarcoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	9.1%	5.3%	0.0%
Terminal Rates (c)	3/33 (9%)	1/34 (3%)	0/31 (0%)
Week of First Observation	104	91	
Life Table Tests (d)	P=0.086N	P=0.482N	P=0.132N
Incidental Tumor Tests (d)	P=0.096N	P=0.526N	P=0.132N
Cochran-Armitage Trend Test (d)	P=0.082N		
Fisher Exact Test (d)		P=0.500N	P=0.122N
Liver: Hepatocellular Adenoma			
Overall Rates (e)	3/50 (6%)	6/50 (12%)	3/50 (6%)
Adjusted Rates (b)	9.1%	16.2%	9.7%
Terminal Rates (c)	3/33 (9%)	4/34 (12%)	3/31 (10%)
Week of First Observation	104	98	104
Life Table Tests (d)	P=0.544	P=0.267	P=0.635
Incidental Tumor Tests (d)	P=0.539N	P=0.299	P=0.635
Cochran-Armitage Trend Test (d)	P=0.573		
Fisher Exact Test (d)		P=0.243	P=0.661
Liver: Hepatocellular Carcinoma			
Overall Rates (e)	7/50 (14%)	7/50 (14%)	4/50 (8%)
Adjusted Rates (b)	18.8%	18.1%	10.8%
Terminal Rates (c)	4/33 (12%)	4/34 (12%)	1/31 (3%)
Week of First Observation	86	82	82
Life Table Tests (d)	P=0.241N	P=0.575N	P=0.286N
Incidental Tumor Tests (d)	P=0.182N	P=0.584	P=0.199N
Cochran-Armitage Trend Test (d)	P=0.221N		
Fisher Exact Test (d)		P=0.613	P=0.263N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (e)	10/50 (20%)	12/50 (24%)	7/50 (14%)
Adjusted Rates (b)	27.2%	30.8%	19.7%
Terminal Rates (c)	7/33 (21%)	8/34 (24%)	4/31 (13%)
Week of First Observation	86	82	82
Life Table Tests (d)	P=0.298N	P=0.451	P=0.336N
Incidental Tumor Tests (d)	P=0.228N	P=0.405	P=0.261N
Cochran-Armitage Trend Test (d)	P=0.263N		
Fisher Exact Test (d)		P=0.405	P=0.298N

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

	Control	2,000 ppm	4,000 ppm
Adrenal Medulla: Pheochromocytoma			
Overall Rates (e)	1/50 (2%)	1/50 (2%)	3/49 (6%)
Adjusted Rates (b)	3.0%	2.6%	9.1%
Terminal Rates (c)	1/33 (3%)	0/34 (0%)	2/31 (6%)
Week of First Observation	104	101	100
Life Table Tests (d)	P=0.190	P=0.748N	P=0.290
Incidental Tumor Tests (d)	P=0.262	P=0.713N	P=0.348
Cochran-Armitage Trend Test (d)	P=0.196		
Fisher Exact Test (d)		P=0.753	P=0.301
All Sites: Benign Tumors			
Overall Rates (a)	11/50 (22%)	12/50 (24%)	15/50 (30%)
Adjusted Rates (b)	32.1%	32.1%	43.5%
Terminal Rates (c)	10/33 (30%)	9/34 (26%)	12/31 (39%)
Week of First Observation	91	98	92
Life Table Tests (d)	P=0.170	P=0.542	P=0.202
Incidental Tumor Tests (d)	P=0.227	P=0.566	P=0.225
Cochran-Armitage Trend Test (d)	P=0.210		
Fisher Exact Test (d)		P=0.500	P=0.247
All Sites: Malignant Tumors			
Overall Rates (a)	18/50 (36%)	16/50 (32%)	20/50 (40%)
Adjusted Rates (b)	44.7%	37.3%	48.6%
Terminal Rates (c)	11/33 (33%)	8/34 (24%)	10/31 (32%)
Week of First Observation	86	78	82
Life Table Tests (d)	P=0.362	P=0.367N	P=0.392
Incidental Tumor Tests (d)	P=0.506	P=0.329N	P=0.588N
Cochran-Armitage Trend Test (d)	P=0.377		
Fisher Exact Test (d)		P=0.417N	P=0.418
All Sites: All Tumors			
Overall Rates (a)	26/50 (52%)	24/50 (48%)	32/50 (64%)
Adjusted Rates (b)	64.8%	55.4%	76.1%
Terminal Rates (c)	19/33 (58%)	15/34 (44%)	21/31 (68%)
Week of First Observation	86	78	82
Life Table Tests (d)	P=0.132	P=0.359N	P=0.145
Incidental Tumor Tests (d)	P=0.166	P=0.307N	P=0.203
Cochran-Armitage Trend Test (d)	P=0.135		
Fisher Exact Test (d)		P=0.421N	P=0.155

(a) Number of tumor-bearing animals/number of animals examined grossly at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(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 than in controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined microscopically at the site
(f) Fifteen spleens were examined microscopically.

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

Study	Incidence in Controls		
	Fibroma	Fibrosarcoma	Fibroma or Fibrosarcoma
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	0/50	0/50	0/50
Phenylephrine hydrochloride	2/50	(b) 1/50	(b) 3/50
Erythromycin stearate	3/50	(b) 13/50	(b) 15/50
Tetracycline hydrochloride	1/49	2/49	3/49
Oxytetracycline hydrochloride	2/50	(c) 11/50	(c) 13/50
α-Methyldopa sesquihydrate	0/50	0/50	0/50
Nitrofurazone	0/50	(b) 3/50	(b) 3/50
Nalidixic acid	1/50	4/50	5/50
TOTAL	9/399 (2.3%)	34/399 (8.5%)	42/399 (10.5%)
SD (d)	2.25%	10.01%	11.34%
Range (e)			
High	3/50	13/50	15/50
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	(f) 40/1,692 (2.4%)	(g) 153/1,692 (9.0%)	(f,g) 186/1,692 (11.0%)
SD (d)	2.98%	7.83%	9.09%
Range (e)			
High	6/50	15/50	19/50
Low	0/50	0/50	0/50

(a) Data as of May 12, 1988, for studies of at least 104 weeks
(b) Includes one sarcoma, NOS
(c) Includes three sarcomas, NOS
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.
(f) Includes one neurofibroma
(g) Includes 51 sarcomas, NOS, and 5 neurofibrosarcomas

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

	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	3 (6%)	2 (4%)	4 (8%)
Inflammation, active chronic			1 (2%)
Inflammation, chronic	2 (4%)	5 (10%)	2 (4%)
Hyperplasia, epithelial	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Mineralization			1 (2%)
Cyst, NOS	2 (4%)		1 (2%)
Hemorrhage			1 (2%)
Inflammation, active chronic	1 (2%)		1 (2%)
Inflammation, chronic	2 (4%)		
Abscess, chronic	1 (2%)		
Granuloma, foreign body			1 (2%)
Angiectasis			1 (2%)
RESPIRATORY SYSTEM			
#Nasal cavity	(49)	(8)	(50)
Hemorrhage	5 (10%)		6 (12%)
Lymphocytic inflammatory infiltrate	3 (6%)	3 (38%)	7 (14%)
#Maxillary sinus	(49)	(8)	(50)
Inflammation, active chronic			1 (2%)
#Trachea	(49)	(8)	(49)
Hemorrhage			1 (2%)
#Lung	(49)	(50)	(50)
Mineralization		1 (2%)	
Congestion, NOS	6 (12%)	4 (8%)	6 (12%)
Hemorrhage	9 (18%)	6 (12%)	4 (8%)
Lymphocytic inflammatory infiltrate	3 (6%)	26 (52%)	3 (6%)
Pneumonia, interstitial chronic	1 (2%)		
Bronchopneumonia, chronic		11 (22%)	6 (12%)
Hyperplasia, alveolar epithelium		3 (6%)	1 (2%)
Histiocytosis	1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukocytosis, NOS		1 (2%)	
#Bone marrow	(50)	(8)	(50)
Hyperplasia, granulocytic	40 (80%)	7 (88%)	30 (60%)
#Spleen	(50)	(15)	(50)
Congestion, NOS	1 (2%)		
Hemorrhage	1 (2%)		
Hematoma, NOS			1 (2%)
Necrosis, focal	1 (2%)		
Pigmentation, NOS	40 (80%)	8 (53%)	42 (84%)
Depletion, lymphoid			1 (2%)
Hyperplasia, reticulum cell		1 (7%)	
Hyperplasia, lymphoid	11 (22%)	4 (27%)	14 (28%)
Hematopoiesis	46 (92%)	12 (80%)	43 (86%)
#Lymph node	(49)	(23)	(47)
Histiocytosis			1 (2%)
Plasmacytosis	1 (2%)		
Hyperplasia, lymphoid	2 (4%)		1 (2%)

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

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Mandibular lymph node	(49)	(23)	(47)
Histiocytosis	1 (2%)		1 (2%)
Plasmacytosis	1 (2%)		
Hyperplasia, lymphoid	6 (12%)	2 (9%)	10 (21%)
#Mesenteric lymph node	(49)	(23)	(47)
Dilatation, NOS		1 (4%)	
Congestion, NOS	15 (31%)	6 (26%)	8 (17%)
Edema, NOS		2 (9%)	
Hemorrhage	1 (2%)	1 (4%)	
Inflammation, acute	1 (2%)		2 (4%)
Inflammation, chronic			1 (2%)
Inflammation, granulomatous			1 (2%)
Hyperplasia, lymphoid	3 (6%)	2 (9%)	5 (11%)
#Liver	(50)	(50)	(50)
Hematopoiesis	20 (40%)	8 (16%)	15 (30%)
#Peyer's patch	(50)	(9)	(50)
Hyperplasia, lymphoid	3 (6%)	1 (11%)	3 (6%)
#Kidney	(50)	(50)	(50)
Hematopoiesis			1 (2%)
#Thymus	(42)	(7)	(46)
Cyst, NOS	3 (7%)		2 (4%)
Multiple cysts	1 (2%)		1 (2%)
Hemorrhage			1 (2%)
Necrosis, NOS		1 (14%)	
Atrophy, NOS			1 (2%)
Hyperplasia, lymphoid			1 (2%)
CIRCULATORY SYSTEM			
*Subcutaneous tissue	(50)	(50)	(50)
Lymphangiectasis	1 (2%)		
#Heart	(50)	(9)	(50)
Lymphocytic inflammatory infiltrate	1 (2%)		
Inflammation, active chronic	1 (2%)		1 (2%)
Inflammation, chronic	4 (8%)		2 (4%)
Endocardiosis	1 (2%)		
*Artery	(50)	(50)	(50)
Periarteritis	1 (2%)		
DIGESTIVE SYSTEM			
*Tooth	(50)	(50)	(50)
Dysplasia, NOS	1 (2%)		1 (2%)
*Root of tooth	(50)	(50)	(50)
Inflammation, suppurative	2 (4%)		1 (2%)
Inflammation, acute	1 (2%)		
Inflammation, active chronic	1 (2%)		1 (2%)
*Pulp of tooth	(50)	(50)	(50)
Inflammation, suppurative	3 (6%)		
Inflammation, active chronic		1 (2%)	
#Salivary gland	(49)	(8)	(49)
Lymphocytic inflammatory infiltrate	3 (6%)	3 (38%)	1 (2%)
Atrophy, NOS	2 (4%)		

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

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Liver	(50)	(50)	(50)
Mineralization			1 (2%)
Cyst, NOS			2 (4%)
Congestion, NOS		2 (4%)	
Lymphocytic inflammatory infiltrate	1 (2%)	7 (14%)	1 (2%)
Inflammation, acute	1 (2%)		
Inflammation, active chronic	1 (2%)		
Inflammation, chronic	4 (8%)	1 (2%)	1 (2%)
Necrosis, NOS	4 (8%)	2 (4%)	3 (6%)
Metamorphosis, fatty	1 (2%)		1 (2%)
Cytoplasmic vacuolization	1 (2%)		
Focal cellular change	3 (6%)	2 (4%)	
Hepatocytomegaly	28 (56%)	24 (48%)	30 (60%)
#Liver/centrilobular	(50)	(50)	(50)
Congestion, NOS	1 (2%)		
Nuclear vacuolization	1 (2%)		
Cytoplasmic vacuolization	1 (2%)		
#Liver/periportal	(50)	(50)	(50)
Inflammation, acute	1 (2%)		
*Gallbladder	(50)	(50)	(50)
Cyst, NOS	1 (2%)		
#Bile duct	(50)	(50)	(50)
Hyperplasia, cystic		1 (2%)	
#Pancreas	(50)	(9)	(50)
Lymphocytic inflammatory infiltrate	1 (2%)		
#Pancreatic acinus	(50)	(9)	(50)
Cytoplasmic vacuolization			1 (2%)
Focal cellular change	5 (10%)		1 (2%)
Atrophy, NOS	3 (6%)		3 (6%)
#Esophagus	(49)	(11)	(49)
Hemorrhage	1 (2%)		
#Stomach	(50)	(8)	(50)
Lymphocytic inflammatory infiltrate	1 (2%)		
Inflammation, acute			1 (2%)
Inflammation, active chronic	1 (2%)		
#Gastric fundal gland	(50)	(8)	(50)
Dilatation, NOS	7 (14%)		9 (18%)
#Glandular stomach	(50)	(8)	(50)
Mineralization	1 (2%)		3 (6%)
Inflammation, acute			3 (6%)
Erosion	1 (2%)		
Hyperplasia, epithelial			3 (6%)
Metaplasia, glandular	1 (2%)		
#Forestomach	(50)	(8)	(50)
Mineralization	1 (2%)		
Multiple cysts			1 (2%)
Inflammation, active chronic	1 (2%)		
Ulcer, chronic	1 (2%)		
Hyperplasia, epithelial			1 (2%)
Hyperkeratosis	2 (4%)		
*Rectum	(50)	(50)	(50)
Ulcer, NOS		1 (2%)	
Inflammation, acute		1 (2%)	

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

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate	2 (4%)	38 (76%)	10 (20%)
Pyelonephritis, acute	3 (6%)		
Inflammation, acute	1 (2%)		
Inflammation, chronic	1 (2%)		
Nephropathy	3 (6%)		1 (2%)
Hyperplasia, tubular cell	39 (78%)	42 (84%)	40 (80%)
#Kidney/capsule	(50)	(50)	(50)
Mineralization			1 (2%)
Inflammation, chronic	1 (2%)		
#Kidney/cortex	(50)	(50)	(50)
Cyst, NOS		1 (2%)	1 (2%)
Infarct, healed	1 (2%)		
Atrophy, focal	1 (2%)		
Metaplasia, osseous	1 (2%)	3 (6%)	4 (8%)
#Kidney/tubule	(50)	(50)	(50)
Mineralization	30 (60%)	15 (30%)	28 (56%)
Dilatation, NOS	7 (14%)	16 (32%)	13 (26%)
Necrosis, NOS	13 (26%)	14 (28%)	12 (24%)
Cytoplasmic vacuolization	43 (86%)	32 (64%)	26 (52%)
Atrophy, NOS	2 (4%)	1 (2%)	
Hyperplasia, atypical			1 (2%)
#Kidney/pelvis	(50)	(50)	(50)
Hemorrhage		1 (2%)	
*Ureter	(50)	(50)	(50)
Inflammation, active chronic	1 (2%)		
#Urinary bladder	(50)	(10)	(49)
Calculus, gross observation only	2 (4%)		
Calculus, microscopic examination	2 (4%)	1 (10%)	1 (2%)
Lymphocytic inflammatory infiltrate	1 (2%)		1 (2%)
Inflammation, active chronic	2 (4%)		
Hyperplasia, epithelial	1 (2%)		
*Urethra	(50)	(50)	(50)
Calculus, microscopic examination	18 (36%)	2 (4%)	10 (20%)
ENDOCRINE SYSTEM			
#Pituitary intermedia	(48)	(9)	(50)
Multiple cysts		1 (11%)	
#Anterior pituitary	(48)	(9)	(50)
Cyst, NOS	2 (4%)	3 (33%)	3 (6%)
Multiple cysts	2 (4%)		
Hyperplasia, NOS	1 (2%)		2 (4%)
#Adrenal/capsule	(50)	(50)	(49)
Hyperplasia, NOS	48 (96%)	50 (100%)	44 (90%)
#Adrenal cortex	(50)	(50)	(49)
Cyst, NOS	2 (4%)		1 (2%)
Lymphocytic inflammatory infiltrate		1 (2%)	
Pigmentation, NOS	2 (4%)		3 (6%)
Hyperplasia, NOS	9 (18%)	12 (24%)	13 (27%)
Angiectasis	1 (2%)		
#Adrenal medulla	(50)	(50)	(49)
Hyperplasia, NOS	6 (12%)	4 (8%)	2 (4%)
#Thyroid	(49)	(8)	(49)
Cystic follicles	6 (12%)		5 (10%)
Abscess, NOS			1 (2%)
Atrophy, NOS			1 (2%)
Hyperplasia, follicular cell	1 (2%)		3 (6%)

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

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid follicle	(49)	(8)	(49)
Atrophy, NOS	1 (2%)		1 (2%)
#Parathyroid	(32)	(5)	(37)
Multiple cysts			1 (3%)
#Pancreatic islets	(50)	(9)	(50)
Hyperplasia, NOS	1 (2%)		
Hyperplasia, focal	2 (4%)	1 (11%)	2 (4%)
REPRODUCTIVE SYSTEM			
*Bulbourethral gland	(50)	(50)	(50)
Ectopia			1 (2%)
*Prepuce	(50)	(50)	(50)
Inflammation, acute			1 (2%)
Inflammation, active chronic	1 (2%)	1 (2%)	
Inflammation, chronic	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
*Preputial gland	(50)	(50)	(50)
Mineralization	1 (2%)		
Cystic ducts		2 (4%)	3 (6%)
Lymphocytic inflammatory infiltrate	1 (2%)		
Inflammation, suppurative	3 (6%)	2 (4%)	2 (4%)
Abscess, NOS		1 (2%)	
Inflammation, active chronic	4 (8%)	1 (2%)	4 (8%)
Inflammation, chronic	1 (2%)	4 (8%)	3 (6%)
#Prostate	(49)	(12)	(48)
Inflammation, suppurative	1 (2%)		
Inflammation, active chronic	2 (4%)		
Hyperplasia, NOS	2 (4%)		1 (2%)
*Seminal vesicle	(50)	(50)	(50)
Dilatation, NOS	3 (6%)	5 (10%)	4 (8%)
Inflammation, chronic		2 (4%)	1 (2%)
Hyperplasia, focal			1 (2%)
#Testis	(50)	(8)	(49)
Mineralization	1 (2%)		1 (2%)
Atrophy, NOS	5 (10%)		8 (16%)
#Testis/tubule	(50)	(8)	(49)
Mineralization	4 (8%)		5 (10%)
*Epididymis	(50)	(50)	(50)
Mineralization	1 (2%)		
Granuloma, spermatic			1 (2%)
*Scrotum	(50)	(50)	(50)
Steatitis		4 (8%)	4 (8%)
NERVOUS SYSTEM			
#Brain	(49)	(9)	(50)
Mineralization	25 (51%)	3 (33%)	16 (32%)
Hemorrhage			2 (4%)
*Spinal cord	(50)	(50)	(50)
Malacia			1 (2%)
SPECIAL SENSE ORGANS			
*Eye/lacrimal gland	(50)	(50)	(50)
Atrophy, NOS	1 (2%)		
*Nasolacrimal duct	(50)	(50)	(50)
Hemorrhage	7 (14%)	1 (2%)	5 (10%)
Lymphocytic inflammatory infiltrate		1 (2%)	
Inflammation, suppurative			1 (2%)
Inflammation, acute	1 (2%)		2 (4%)

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

	Untreated Control	Low Dose	High Dose
MUSCULOSKELETAL SYSTEM			
*Maxilla	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)		
Inflammation, active chronic			2 (4%)
*Coccyx	(50)	(50)	(50)
Abscess, NOS	1 (2%)		
*Knee joint	(50)	(50)	(50)
Ankylosis	1 (2%)		
*Tarsal joint	(50)	(50)	(50)
Ankylosis	16 (32%)	13 (26%)	7 (14%)
Inflammation, active chronic		1 (2%)	
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Hemorrhage	1 (2%)		
*Pericardium	(50)	(50)	(50)
Hemorrhage	1 (2%)		
*Mesentery	(50)	(50)	(50)
Hemorrhage		1 (2%)	
Steatitis	2 (4%)	3 (6%)	1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate	40 (80%)	4 (8%)	36 (72%)
Axilla			
Inflammation, active chronic		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 D

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF NALIDIXIC ACID

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

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	49	50	49
Animals examined histopathologically	49	50	49
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(49)	(50)	(49)
Sarcoma, NOS			1 (2%)
Fibrosarcoma	3 (6%)		
RESPIRATORY SYSTEM			
#Nasal cavity	(48)	(50)	(49)
Papilloma, NOS	1 (2%)		
#Lung	(49)	(50)	(49)
Adenocarcinoma, NOS, metastatic			1 (2%)
Hepatocellular carcinoma, metastatic	1 (2%)	1 (2%)	
Alveolar/bronchiolar adenoma		3 (6%)	2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)	2 (4%)	
Granulosa cell carcinoma, metastatic		1 (2%)	
Sarcoma, NOS, metastatic			1 (2%)
Hepatoblastoma, metastatic		1 (2%)	
Osteosarcoma, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(49)
Malignant lymphoma, lymphocytic type	1 (2%)	1 (2%)	
Malignant lymphoma, histiocytic type		1 (2%)	2 (4%)
Malignant lymphoma, mixed type	13 (27%)	10 (20%)	11 (22%)
*Skin	(49)	(50)	(49)
Mast cell tumor		1 (2%)	
#Spleen	(49)	(50)	(49)
Malignant lymphoma, mixed type	3 (6%)	3 (6%)	
#Mesenteric lymph node	(47)	(50)	(48)
Malignant lymphoma, mixed type			1 (2%)
CIRCULATORY SYSTEM			
*Subcutaneous tissue	(49)	(50)	(49)
Hemangioma			1 (2%)
Hemangiosarcoma	1 (2%)	1 (2%)	
#Spleen	(49)	(50)	(49)
Hemangiosarcoma			1 (2%)
#Uterus	(49)	(50)	(49)
Hemangiosarcoma			1 (2%)
DIGESTIVE SYSTEM			
#Liver	(49)	(50)	(49)
Hepatocellular adenoma	2 (4%)	4 (8%)	6 (12%)
Hepatocellular carcinoma	3 (6%)	2 (4%)	1 (2%)
Hepatoblastoma		1 (2%)	
Osteosarcoma, metastatic	1 (2%)		
#Duodenum	(49)	(50)	(49)
Adenomatous polyp, NOS		1 (2%)	
URINARY SYSTEM			
None			

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

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(50)	(49)
Adenoma, NOS	18 (37%)	18 (36%)	15 (31%)
#Adrenal medulla	(48)	(50)	(49)
Pheochromocytoma		2 (4%)	3 (6%)
#Thyroid	(49)	(50)	(49)
Adenoma, NOS		1 (2%)	1 (2%)
Follicular cell adenoma			1 (2%)
#Pancreatic islets	(48)	(50)	(49)
Islet cell carcinoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(49)	(50)	(49)
Adenocarcinoma, NOS	1 (2%)	2 (4%)	1 (2%)
#Uterus	(49)	(50)	(49)
Histiocytic sarcoma	1 (2%)		
Endometrial stromal polyp	1 (2%)	1 (2%)	
#Cervix uteri	(49)	(50)	(49)
Leiomyoma		1 (2%)	
#Ovary	(49)	(50)	(48)
Cystadenoma, NOS		2 (4%)	
Luteoma			1 (2%)
Granulosa cell tumor		1 (2%)	1 (2%)
Granulosa cell carcinoma		2 (4%)	
Tubular adenoma		1 (2%)	
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Nasolacrimal duct	(49)	(50)	(49)
Papilloma, NOS			1 (2%)
*Harderian gland	(49)	(50)	(49)
Adenoma, NOS		4 (8%)	1 (2%)
Sarcoma, NOS		1 (2%)	
*Ear canal	(49)	(50)	(49)
Squamous cell carcinoma		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*Cervical vertebra other	(49)	(50)	(49)
Osteoma		1 (2%)	
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
Knee			
Osteosarcoma	1		
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	3		4
Moribund sacrifice	7	7	14
Terminal sacrifice	40	43	32

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

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	36	39	35
Total primary tumors	51	68	52
Total animals with benign tumors	20	30	24
Total benign tumors	22	39	32
Total animals with malignant tumors	24	21	18
Total malignant tumors	29	27	19
Total animals with secondary tumors##	2	2	2
Total secondary tumors	3	3	2
Total animals with tumors uncertain--benign or malignant		2	1
Total uncertain tumors		2	1

* Number of animals receiving complete necropsy examinations; 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 NALIDIXIC ACID: UNTREATED CONTROL

ANIMAL NUMBER	123	107	104	141	109	116	139	135	138	125	101	102	103	105	106	108	110	111	112	113	114	115	117	118	119
WEEKS ON STUDY	034	075	084	093	096	097	098	100	100	103	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma							X			X										X					
Hemangiosarcoma			X																						
RESPIRATORY SYSTEM																									
Lungs and bronchi	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																			X						
Alveolar/bronchiolar carcinoma																									
Osteosarcoma, metastatic																									
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	A	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																						X			
HEMATOPOIETIC SYSTEM																									
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																									
Lymph nodes	A	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	A	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	A	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular carcinoma																			X						
Osteosarcoma, metastatic																									
Bile duct	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	A	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	A	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Small intestine	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS					X						X		X	X				X		X			X		
Adrenal	A	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	A	+	+	−	+	−	+	+	−	+	−	+	−	+	+	+	+	−	+	+	−	+	+	+	+
Pancreatic islets	A	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell carcinoma															X										
REPRODUCTIVE SYSTEM																									
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS										X															
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Endometrial stromal polyp																									
Ovary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	A	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																							
Malignant lymphoma, mixed type				X	X		X			X		X							X						
Knee, NOS	A																								
Osteosarcoma																									

+: 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	120	121	122	124	126	127	128	129	130	131	132	133	134	136	137	140	142	143	144	145	146	147	148	149	150	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Fibrosarcoma																										3
Hemangiosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar carcinoma												X														1
Osteosarcoma, metastatic					X																					1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Papilloma, NOS																										1
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, mixed type			X			X																	X			3
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma													X												X	2
Hepatocellular carcinoma			X																						X	3
Osteosarcoma, metastatic					X																					1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS	X			X	X				X				X	X			X	X	X				X		X	18
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid	+	+	+	−	−	−	+	+	+	−	−	+	+	−	+	−	−	−	+	−	−	+	+	+	+	31
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islet cell carcinoma																										1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Adenocarcinoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																									X	1
Endometrial stromal polyp																				X						1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
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	*49
Malignant lymphoma, lymphocytic type																										1
Malignant lymphoma, mixed type	X						X			X	X					X		X	X							13
Knee, NOS																										
Osteosarcoma					X																					1

* Animals necropsied

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

ANIMAL NUMBER	043	042	032	015	036	010	035	001	002	003	004	005	006	007	008	009	011	012	013	014	016	017	018	019	020
WEEKS ON STUDY	058	085	093	099	099	101	103	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma											X														
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic				X																					
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Granulosa cell carcinoma, metastatic						X																			
Hepatoblastoma, metastatic				X																					
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type					X																			X	
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma												X		X					X						
Hepatocellular carcinoma				X	X																				
Hepatoblastoma				X																					
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	N	+	N	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenomatous polyp, NOS														X											
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS								X						X	X		X					X		X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Parathyroid	+	+	−	−	−	+	−	+	+	+	−	+	+	+	+	−	−	+	−	−	−	+	−	+	−
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																							X		
Endometrial stromal polyp																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma, NOS				X																		X			
Granulosa cell tumor																								X	
Granulosa cell carcinoma						X																			
Tubular adenoma							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			X
Sarcoma, NOS																									X
Ear	+	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																								
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
Osteoma		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																									
Malignant lymphoma, histiocytic type			X																						
Malignant lymphoma, mixed type							X							X		X	X						X		X

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

ANIMAL NUMBER	0212	0222	0233	0244	0255	0266	0277	0288	0299	0300	0311	0333	0344	0377	0388	0399	0400	0411	0444	0455	0466	0477	0488	0499	0500	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mast cell tumor																			X							1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Hemangiosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar adenoma								X			X			X												3
Alveolar/bronchiolar carcinoma		X	X																							2
Granulosa cell carcinoma, metastatic																										1
Hepatoblastoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, mixed type																	X									3
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	48
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma			X																							4
Hepatocellular carcinoma																										2
Hepatoblastoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenomatous polyp, NOS																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS			X				X	X		X	X	X		X	X	X				X	X	X				18
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma	X														X											2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS											X															1
Parathyroid	+	–	–	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	+	–	–	+	+	+	30
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenocarcinoma, NOS			X				X																			2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma																										1
Endometrial stromal polyp													X													1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma, NOS																										2
Granulosa cell tumor																										1
Granulosa cell carcinoma																	X									2
Tubular adenoma																										1
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																										4
Sarcoma, NOS																										1
Ear	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
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																										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											1
Malignant lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type			X							X			X										X			10

* Animals necropsied

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

ANIMAL NUMBER	065	056	059	093	098	078	068	079	052	091	097	057	073	083	069	089	072	066	051	053	054	055	058	060	061
WEEKS ON STUDY	033	070	070	074	083	088	090	090	093	093	093	098	099	100	101	101	102	103	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS			X																						
Hemangioma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS, metastatic								X																	
Alveolar/bronchiolar adenoma																				X					
Sarcoma, NOS, *metastatic*			X																						
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								X	
Lymph nodes	A	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																									X
Thymus	A	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma												X													
Hepatocellular carcinoma														X											
Bile duct	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	A	+	+	+	+	+	+	+	N	+	–	+	+	N	+	+	+	+	+	+	+	+	+	+	+
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS				X					X	X		X	X											X	
Adrenal	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																					X				
Thyroid	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS														X											
Follicular cell adenoma																X									
Parathyroid	A	+	–	+	–	–	+	+	–	+	–	–	+	–	–	+	+	–	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS								X																	
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Ovary	A	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luteoma																			X						
Granulosa cell tumor																X									
NERVOUS SYSTEM																									
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Lacrimal gland	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Papilloma, NOS																									
Harderian gland	A	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	A	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, histiocytic type						X				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	0062	0063	0064	0067	0070	0071	0074	0075	0076	0077	0080	0081	0082	0084	0085	0086	0087	0088	0090	0092	0094	0095	0096	0099	0100	TOTAL TISSUES TUMORS
WEEKS ON STUDY	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Sarcoma, NOS																										1
Hemangioma																					X					1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, NOS, metastatic																										1
Alveolar/bronchiolar adenoma																			X							2
Sarcoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Malignant lymphoma, mixed type																										1
Thymus	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma														X	X	X		X						X		6
Hepatocellular carcinoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS					X	X	X						X	X		X		X			X		X			15
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma				X											X											3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS																										1
Follicular cell adenoma																										1
Parathyroid	–	+	+	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	–	–	+	+	+	34
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Adenocarcinoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma										X																1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Luteoma																										1
Granulosa cell tumor																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																										
Lacrimal 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	*49
Papilloma, NOS																					X					1
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	*49
Adenoma, NOS				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	*49
Malignant lymphoma, histiocytic type																										2
Malignant lymphoma, mixed type													X				X		X							11

* Animals necropsied

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

	Control	2,000 ppm	4,000 ppm
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	3/49 (6%)	0/50 (0%)	0/49 (0%)
Adjusted Rates (b)	6.9%	0.0%	0.0%
Terminal Rates (c)	1/40 (3%)	0/43 (0%)	0/32 (0%)
Week of First Observation	97		
Life Table Tests (d)	P=0.045N	P=0.115N	P=0.156N
Incidental Tumor Tests (d)	P=0.030N	P=0.153N	P=0.090N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.117N	P=0.121N
Subcutaneous Tissue: Sarcoma or Fibrosarcoma			
Overall Rates (a)	3/49 (6%)	0/50 (0%)	1/49 (2%)
Adjusted Rates (b)	6.9%	0.0%	2.0%
Terminal Rates (c)	1/40 (3%)	0/43 (0%)	0/32 (0%)
Week of First Observation	97		70
Life Table Tests (d)	P=0.202N	P=0.115N	P=0.355N
Incidental Tumor Tests (d)	P=0.100N	P=0.153N	P=0.178N
Cochran-Armitage Trend Test (d)	P=0.175N		
Fisher Exact Test (d)		P=0.117N	P=0.309N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (e)	0/49 (0%)	3/50 (6%)	2/49 (4%)
Adjusted Rates (b)	0.0%	7.0%	6.3%
Terminal Rates (c)	0/40 (0%)	3/43 (7%)	2/32 (6%)
Week of First Observation		104	104
Life Table Tests (d)	P=0.143	P=0.134	P=0.191
Incidental Tumor Tests (d)	P=0.143	P=0.134	P=0.191
Cochran-Armitage Trend Test (d)	P=0.201		
Fisher Exact Test (d)		P=0.125	P=0.247
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (e)	1/49 (2%)	5/50 (10%)	2/49 (4%)
Adjusted Rates (b)	2.5%	11.6%	6.3%
Terminal Rates (c)	1/40 (3%)	5/43 (12%)	2/32 (6%)
Week of First Observation	104	104	104
Life Table Tests (d)	P=0.312	P=0.120	P=0.422
Incidental Tumor Tests (d)	P=0.312	P=0.120	P=0.422
Cochran-Armitage Trend Test (d)	P=0.412		
Fisher Exact Test (d)		P=0.107	P=0.500
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	16/49 (33%)	13/50 (26%)	12/49 (24%)
Adjusted Rates (b)	36.1%	28.8%	30.8%
Terminal Rates (c)	12/40 (30%)	11/43 (26%)	6/32 (19%)
Week of First Observation	93	99	83
Life Table Tests (d)	P=0.419N	P=0.265N	P=0.474N
Incidental Tumor Tests (d)	P=0.209N	P=0.341N	P=0.229N
Cochran-Armitage Trend Test (d)	P=0.215N		
Fisher Exact Test (d)		P=0.307N	P=0.251N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	17/49 (35%)	15/50 (30%)	14/49 (29%)
Adjusted Rates (b)	37.4%	32.5%	34.0%
Terminal Rates (c)	12/40 (30%)	12/43 (28%)	6/32 (19%)
Week of First Observation	75	93	83
Life Table Tests (d)	P=0.519N	P=0.342N	P=0.561N
Incidental Tumor Tests (d)	P=0.209N	P=0.440N	P=0.216N
Cochran-Armitage Trend Test (d)	P=0.293N		
Fisher Exact Test (d)		P=0.388N	P=0.332N

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

	Control	2,000 ppm	4,000 ppm
Circulatory System: Hemangioma or Hemangiosarcoma			
Overall Rates (a)	1/49 (2%)	1/50 (2%)	3/49 (6%)
Adjusted Rates (b)	2.1%	2.3%	9.4%
Terminal Rates (c)	0/40 (0%)	1/43 (2%)	3/32 (9%)
Week of First Observation	84	104	104
Life Table Tests (d)	P=0.152	P=0.750N	P=0.244
Incidental Tumor Tests (d)	P=0.230	P=0.752N	P=0.348
Cochran-Armitage Trend Test (d)	P=0.201		
Fisher Exact Test (d)		P=0.748N	P=0.309
Liver: Hepatocellular Adenoma			
Overall Rates (e)	2/49 (4%)	4/50 (8%)	6/49 (12%)
Adjusted Rates (b)	5.0%	9.3%	17.8%
Terminal Rates (c)	2/40 (5%)	4/43 (9%)	5/32 (16%)
Week of First Observation	104	104	98
Life Table Tests (d)	P=0.050	P=0.371	P=0.078
Incidental Tumor Tests (d)	P=0.059	P=0.371	P=0.091
Cochran-Armitage Trend Test (d)	P=0.098		
Fisher Exact Test (d)		P=0.349	P=0.134
Liver: Hepatocellular Carcinoma			
Overall Rates (e)	3/49 (6%)	2/50 (4%)	1/49 (2%)
Adjusted Rates (b)	7.5%	4.3%	2.7%
Terminal Rates (c)	3/40 (7%)	0/43 (0%)	0/32 (0%)
Week of First Observation	104	99	100
Life Table Tests (d)	P=0.273N	P=0.464N	P=0.382N
Incidental Tumor Tests (d)	P=0.194N	P=0.541N	P=0.334N
Cochran-Armitage Trend Test (d)	P=0.221N		
Fisher Exact Test (d)		P=0.490N	P=0.309N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (e)	4/49 (8%)	6/50 (12%)	7/49 (14%)
Adjusted Rates (b)	10.0%	13.2%	20.0%
Terminal Rates (c)	4/40 (10%)	4/43 (9%)	5/32 (16%)
Week of First Observation	104	99	98
Life Table Tests (d)	P=0.127	P=0.419	P=0.159
Incidental Tumor Tests (d)	P=0.181	P=0.364	P=0.198
Cochran-Armitage Trend Test (d)	P=0.214		
Fisher Exact Test (d)		P=0.383	P=0.262
Anterior Pituitary Gland: Adenoma			
Overall Rates (e)	18/49 (37%)	18/50 (36%)	15/49 (31%)
Adjusted Rates (b)	43.8%	41.9%	39.2%
Terminal Rates (c)	17/40 (43%)	18/43 (42%)	10/32 (31%)
Week of First Observation	96	104	74
Life Table Tests (d)	P=0.513	P=0.476N	P=0.555
Incidental Tumor Tests (d)	P=0.461N	P=0.492N	P=0.467N
Cochran-Armitage Trend Test (d)	P=0.298N		
Fisher Exact Test (d)		P=0.553N	P=0.335N
Adrenal Medulla: Pheochromocytoma			
Overall Rates (e)	0/48 (0%)	2/50 (4%)	3/49 (6%)
Adjusted Rates (b)	0.0%	4.7%	9.4%
Terminal Rates (c)	0/40 (0%)	2/43 (5%)	3/32 (9%)
Week of First Observation		104	104
Life Table Tests (d)	P=0.050	P=0.254	P=0.085
Incidental Tumor Tests (d)	P=0.050	P=0.254	P=0.085
Cochran-Armitage Trend Test (d)	P=0.084		
Fisher Exact Test (d)		P=0.258	P=0.125

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

	Control	2,000 ppm	4,000 ppm
Ovary: Granulosa Cell Tumor or Carcinoma			
Overall Rates (e)	0/49 (0%)	3/50 (6%)	1/48 (2%)
Adjusted Rates (b)	0.0%	6.8%	2.8%
Terminal Rates (c)	0/40 (0%)	2/43 (5%)	0/32 (0%)
Week of First Observation		101	101
Life Table Tests (d)	P=0.324	P=0.138	P=0.474
Incidental Tumor Tests (d)	P=0.411	P=0.111	P=0.571
Cochran-Armitage Trend Test (d)	P=0.371		
Fisher Exact Test (d)		P=0.125	P=0.495
Ovary: Luteoma, Granulosa Cell Tumor, or Granulosa Cell Carcinoma			
Overall Rates (e)	0/49 (0%)	3/50 (6%)	2/48 (4%)
Adjusted Rates (b)	0.0%	6.8%	5.8%
Terminal Rates (c)	0/40 (0%)	2/43 (5%)	1/32 (3%)
Week of First Observation		101	101
Life Table Tests (d)	P=0.154	P=0.138	P=0.202
Incidental Tumor Tests (d)	P=0.213	P=0.111	P=0.252
Cochran-Armitage Trend Test (d)	P=0.195		
Fisher Exact Test (d)		P=0.125	P=0.242
Harderian Gland: Adenoma			
Overall Rates (a)	0/49 (0%)	4/50 (8%)	1/49 (2%)
Adjusted Rates (b)	0.0%	9.3%	3.1%
Terminal Rates (c)	0/40 (0%)	4/43 (9%)	1/32 (3%)
Week of First Observation		104	104
Life Table Tests (d)	P=0.312	P=0.073	P=0.455
Incidental Tumor Tests (d)	P=0.312	P=0.073	P=0.455
Cochran-Armitage Trend Test (d)	P=0.390		
Fisher Exact Test (d)		P=0.061	P=0.500
All Sites: Benign Tumors			
Overall Rates (a)	20/49 (41%)	30/50 (60%)	24/49 (49%)
Adjusted Rates (b)	48.6%	65.1%	60.8%
Terminal Rates (c)	19/40 (48%)	27/43 (63%)	17/32 (53%)
Week of First Observation	96	85	74
Life Table Tests (d)	P=0.048	P=0.073	P=0.071
Incidental Tumor Tests (d)	P=0.116	P=0.055	P=0.136
Cochran-Armitage Trend Test (d)	P=0.240		
Fisher Exact Test (d)		P=0.044	P=0.271
All Sites: Malignant Tumors			
Overall Rates (a)	24/49 (49%)	21/50 (42%)	18/49 (37%)
Adjusted Rates (b)	49.8%	42.8%	41.7%
Terminal Rates (c)	16/40 (40%)	15/43 (35%)	8/32 (25%)
Week of First Observation	75	58	70
Life Table Tests (d)	P=0.366N	P=0.275N	P=0.410N
Incidental Tumor Tests (d)	P=0.035N	P=0.391N	P=0.044N
Cochran-Armitage Trend Test (d)	P=0.131N		
Fisher Exact Test (d)		P=0.310N	P=0.154N
All Sites: All Tumors			
Overall Rates (a)	36/49 (73%)	39/50 (78%)	35/49 (71%)
Adjusted Rates (b)	74.9%	78.0%	75.8%
Terminal Rates (c)	28/40 (70%)	32/43 (74%)	21/32 (66%)
Week of First Observation	75	58	70
Life Table Tests (d)	P=0.187	P=0.537	P=0.224
Incidental Tumor Tests (d)	P=0.344N	P=0.346	P=0.400N
Cochran-Armitage Trend Test (d)	P=0.454N		
Fisher Exact Test (d)		P=0.385	P=0.500N

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

(a) Number of tumor-bearing animals/number of animals examined grossly at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(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 than in controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined microscopically at the site

TABLE D4. HISTORICAL INCIDENCE OF OVARIAN TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Cystadenoma	Granulosa Cell Neoplasms	Tubular Adenoma
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	0/41	0/41	0/41
Phenylephrine hydrochloride	0/49	0/49	0/49
Erythromycin stearate	0/48	(b) 2/48	0/48
Tetracycline hydrochloride	0/48	1/48	0/48
Oxytetracycline hydrochloride	2/44	0/44	0/44
α-Methyldopa sesquihydrate	0/47	0/47	0/47
Nitrofurazone	2/47	(c) 3/47	0/47
Nalidixic acid	0/49	0/49	0/49
TOTAL	4/373 (1.1%)	(d) 6/373 (1.6%)	0/373 (0.0%)
SD (e)	2.04%	2.46%	0.00%
Range (f)			
High	2/44	3/47	0/49
Low	0/49	0/49	0/49
Overall Historical Incidence			
TOTAL	(g) 14/1,577 (0.9%)	(h,i) 14/1,577 (0.9%)	(i) 2/1,577 (0.1%)
SD (e)	1.56%	1.63%	0.80%
Range (f)			
High	2/43	3/47	2/43
Low	0/50	0/49	0/50

(a) Data as of May 12, 1988, for studies of at least 104 weeks
(b) Includes one luteoma
(c) Includes two luteomas
(d) Includes three luteomas
(e) Standard deviation
(f) Range and SD are presented for groups of 35 or more animals.
(g) Includes one adenoma, NOS, one papillary adenoma, five cystadenomas, NOS, six papillary cystadenomas, NOS, and one adenocarcinoma, NOS
(h) Includes four luteomas and one granulosa cell carcinoma
(i) Two benign mixed tumors were also observed.

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

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	49	50	49
Animals examined histopathologically	49	50	49
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(49)	(50)	(49)
Inflammation, active chronic	† 1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#Nasal cavity	(48)	(50)	(49)
Hemorrhage	2 (4%)		5 (10%)
Lymphocytic inflammatory infiltrate	3 (6%)		3 (6%)
Inflammation, acute	1 (2%)		1 (2%)
Inflammation, active chronic			1 (2%)
Granuloma, foreign body			1 (2%)
#Lung	(49)	(50)	(49)
Mineralization		1 (2%)	
Congestion, NOS		3 (6%)	
Hemorrhage	1 (2%)	3 (6%)	6 (12%)
Lymphocytic inflammatory infiltrate			1 (2%)
Bronchopneumonia, chronic	5 (10%)	5 (10%)	2 (4%)
Histiocytosis		1 (2%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(49)
Leukocytosis, NOS			1 (2%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)
#Bone marrow	(49)	(50)	(49)
Fibrosis	19 (39%)	11 (22%)	19 (39%)
Hyperplasia, granulocytic	11 (22%)	7 (14%)	7 (14%)
Hyperplasia, lymphoid		1 (2%)	
#Spleen	(49)	(50)	(49)
Necrosis, NOS	1 (2%)		
Pigmentation, NOS	41 (84%)	33 (66%)	43 (88%)
Angiectasis			1 (2%)
Hyperplasia, lymphoid	21 (43%)	13 (26%)	22 (45%)
Hematopoiesis	43 (88%)	34 (68%)	45 (92%)
#Mandibular lymph node	(47)	(50)	(48)
Hyperplasia, lymphoid	10 (21%)	5 (10%)	14 (29%)
#Thoracic lymph node	(47)	(50)	(48)
Hyperplasia, lymphoid			1 (2%)
#Pancreatic lymph node	(47)	(50)	(48)
Congestion, NOS			1 (2%)
#Mesenteric lymph node	(47)	(50)	(48)
Congestion, NOS		5 (10%)	4 (8%)
Inflammation, active chronic			1 (2%)
Necrosis, NOS	1 (2%)		
Histiocytosis			1 (2%)
Hyperplasia, lymphoid	4 (9%)	4 (8%)	7 (15%)
#Renal lymph node	(47)	(50)	(48)
Hyperplasia, lymphoid	1 (2%)		
#Liver	(49)	(50)	(49)
Hematopoiesis	33 (67%)	22 (44%)	39 (80%)
#Peyer's patch	(49)	(50)	(49)
Hyperplasia, lymphoid	3 (6%)		3 (6%)

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

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Thymus	(47)	(48)	(47)
Cyst, NOS			1 (2%)
Multiple cysts	2 (4%)		1 (2%)
Congestion, NOS		3 (6%)	
Hemorrhage			1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	3 (6%)
CIRCULATORY SYSTEM			
#Heart	(49)	(50)	(49)
Mineralization	1 (2%)	2 (4%)	
Inflammation, active chronic			1 (2%)
Inflammation, chronic	3 (6%)	4 (8%)	5 (10%)
*Arteriole	(49)	(50)	(49)
Mineralization	1 (2%)		
*Artery	(49)	(50)	(49)
Mineralization		1 (2%)	
DIGESTIVE SYSTEM			
#Salivary gland	(47)	(50)	(49)
Lymphocytic inflammatory infiltrate			1 (2%)
Inflammation, chronic			2 (4%)
Atrophy, NOS		1 (2%)	3 (6%)
#Liver	(49)	(50)	(49)
Congestion, NOS	1 (2%)		1 (2%)
Inflammation, acute	1 (2%)		
Inflammation, active chronic	1 (2%)		1 (2%)
Peliosis hepatis	1 (2%)		
Necrosis, NOS	2 (4%)		6 (12%)
Cytoplasmic vacuolization		2 (4%)	
Focal cellular change	2 (4%)	2 (4%)	
Hepatocytomegaly	3 (6%)	1 (2%)	3 (6%)
Angiectasis		1 (2%)	
#Liver/centrilobular	(49)	(50)	(49)
Hepatocytomegaly			1 (2%)
#Liver/periportal	(49)	(50)	(49)
Inflammation, chronic		1 (2%)	
Metamorphosis, fatty	1 (2%)	1 (2%)	
*Gallbladder	(49)	(50)	(49)
Multiple cysts	1 (2%)		1 (2%)
#Pancreas	(48)	(50)	(49)
Cyst, NOS	1 (2%)		
Cystic ducts	1 (2%)		
Lymphocytic inflammatory infiltrate	1 (2%)		
#Pancreatic acinus	(48)	(50)	(49)
Focal cellular change			1 (2%)
Atrophy, NOS	3 (6%)	3 (6%)	1 (2%)
Hyperplasia, NOS		2 (4%)	1 (2%)
#Gastric fundal gland	(48)	(49)	(49)
Dilatation, NOS	21 (44%)	6 (12%)	10 (20%)
#Glandular stomach	(48)	(49)	(49)
Mineralization	3 (6%)	1 (2%)	
Inflammation, acute	1 (2%)	1 (2%)	
Inflammation, active chronic	2 (4%)		1 (2%)
Inflammation, chronic	1 (2%)		
Hyperplasia, epithelial		1 (2%)	

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

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Forestomach	(48)	(49)	(49)
Multiple cysts			1 (2%)
Inflammation, active chronic		1 (2%)	1 (2%)
Erosion		1 (2%)	
Hyperkeratosis			1 (2%)
Acanthosis		1 (2%)	
Dysplasia, epithelial			3 (6%)
#Small intestine	(49)	(50)	(49)
Granuloma, foreign body			1 (2%)
#Jejunum	(49)	(50)	(49)
Amyloidosis			1 (2%)
#Ileum	(49)	(50)	(49)
Inflammation, chronic	1 (2%)		
Necrosis, hemorrhagic	1 (2%)		
Amyloidosis	1 (2%)	2 (4%)	1 (2%)
URINARY SYSTEM			
#Kidney	(49)	(50)	(49)
Hydronephrosis			1 (2%)
Lymphocytic inflammatory infiltrate	1 (2%)		
Glomerulonephritis, acute			1 (2%)
Inflammation, chronic		1 (2%)	2 (4%)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, tubular cell	16 (33%)	28 (56%)	24 (49%)
#Kidney/capsule	(49)	(50)	(49)
Hemorrhage			1 (2%)
Inflammation, active chronic			1 (2%)
#Kidney/cortex	(49)	(50)	(49)
Cyst, NOS	1 (2%)		
Hemorrhage	1 (2%)		
Infarct, healed			1 (2%)
Metaplasia, osseous	3 (6%)	1 (2%)	1 (2%)
#Perirenal tissue	(49)	(50)	(49)
Inflammation, chronic	1 (2%)		
#Kidney/tubule	(49)	(50)	(49)
Mineralization	10 (20%)	5 (10%)	5 (10%)
Dilatation, NOS	5 (10%)	1 (2%)	5 (10%)
Cyst, NOS		1 (2%)	
Degeneration, hyaline			1 (2%)
Degeneration, hydropic			1 (2%)
Nephrosis, NOS			1 (2%)
Necrosis, NOS	3 (6%)	8 (16%)	3 (6%)
Pigmentation, NOS	2 (4%)	1 (2%)	2 (4%)
Cytoplasmic vacuolization	44 (90%)	33 (66%)	41 (84%)
Atrophy, NOS			1 (2%)
#Urinary bladder	(49)	(49)	(49)
Metaplasia, squamous		1 (2%)	
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(50)	(49)
Cyst, NOS	1 (2%)	2 (4%)	1 (2%)
Multiple cysts			1 (2%)
Hyperplasia, NOS	14 (29%)	16 (32%)	11 (22%)
Angiectasis	1 (2%)		
#Adrenal/capsule	(48)	(50)	(49)
Cyst, NOS			1 (2%)
Hyperplasia, NOS	48 (100%)	50 (100%)	49 (100%)

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

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Adrenal cortex	(48)	(50)	(49)
Accessory structure	2 (4%)		
Congestion, NOS			1 (2%)
Metamorphosis, fatty	1 (2%)		1 (2%)
Pigmentation, NOS	43 (90%)	22 (44%)	41 (84%)
Cytomegaly		1 (2%)	
Hyperplasia, NOS	2 (4%)	8 (16%)	2 (4%)
#Adrenal medulla	(48)	(50)	(49)
Hyperplasia, NOS	1 (2%)		
Hyperplasia, focal	1 (2%)		1 (2%)
#Thyroid	(49)	(50)	(49)
Embryonal duct cyst			1 (2%)
Cystic follicles	12 (24%)	5 (10%)	5 (10%)
Lymphocytic inflammatory infiltrate	1 (2%)		
Inflammation, acute	1 (2%)		
Inflammation, chronic			2 (4%)
Hyperplasia, follicular cell	5 (10%)	4 (8%)	7 (14%)
#Thyroid follicle	(49)	(50)	(49)
Atrophy, NOS			1 (2%)
#Parathyroid	(31)	(30)	(34)
Hyperplasia, NOS		1 (3%)	
#Pancreatic islets	(48)	(50)	(49)
Hyperplasia, NOS	1 (2%)		
Hyperplasia, focal	3 (6%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(49)	(50)	(49)
Multiple cysts	7 (14%)	8 (16%)	13 (27%)
Inflammation, chronic	1 (2%)		
Hyperplasia, cystic		1 (2%)	
#Uterus	(49)	(50)	(49)
Hydrometra	3 (6%)		
Hemorrhage	1 (2%)		1 (2%)
Hematoma, NOS		1 (2%)	
Inflammation, acute			1 (2%)
Angiectasis		4 (8%)	
#Uterus/endometrium	(49)	(50)	(49)
Inflammation, acute	1 (2%)		2 (4%)
Hyperplasia, cystic	49 (100%)	50 (100%)	48 (98%)
Angiectasis	1 (2%)		
#Ovary	(49)	(50)	(48)
Mineralization	1 (2%)		1 (2%)
Follicular cyst, NOS	5 (10%)		2 (4%)
Parovarian cyst	4 (8%)	8 (16%)	6 (13%)
Hematoma, NOS		1 (2%)	
Hemorrhagic cyst			1 (2%)
Abscess, NOS			1 (2%)
#Mesovarium	(49)	(50)	(48)
Steatitis	1 (2%)		
#Ovary/follicle	(49)	(50)	(48)
Multiple cysts	1 (2%)		
NERVOUS SYSTEM			
#Brain	(49)	(50)	(49)
Compression, NOS		1 (2%)	4 (8%)
Mineralization	38 (78%)	33 (66%)	21 (43%)
Hydrocephalus, internal		1 (2%)	
Inflammation, active chronic		1 (2%)	

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

	Untreated Control	Low Dose	High Dose
SPECIAL SENSE ORGANS			
*Eye	(49)	(50)	(49)
Retinopathy		1 (2%)	
Phthisis bulbi		1 (2%)	
*Eye/crystalline lens	(49)	(50)	(49)
Cataract		1 (2%)	
*Nasolacrimal duct	(49)	(50)	(49)
Foreign body, NOS		1 (2%)	
Cyst, NOS	1 (2%)		
Hemorrhage	9 (18%)	3 (6%)	15 (31%)
Lymphocytic inflammatory infiltrate			1 (2%)
Inflammation, acute	1 (2%)	1 (2%)	
Hyperplasia, epithelial			2 (4%)
*Harderian gland	(49)	(50)	(49)
Inflammation, chronic		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*Skull	(49)	(50)	(49)
Fibrosis		2 (4%)	
BODY CAVITIES			
*Mesentery	(49)	(50)	(49)
Steatitis	2 (4%)		
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(50)	(49)
Lymphocytic inflammatory infiltrate	45 (92%)	49 (98%)	43 (88%)
SPECIAL MORPHOLOGY SUMMARY			
Autolysis/no necropsy	1		1

* 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 once only.

APPENDIX E

SENTINEL ANIMAL PROGRAM

APPENDIX E. SENTINEL ANIMAL PROGRAM

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

Results

Results are presented in Table E1.

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

Interval (months)	Number of Animals	Positive Serologic Reaction for
RATS		
6	2/10	RCV
12	9/10	Sendai
	6/10	RCV
18	5/9	Sendai
24	5/10	RCV/SDA
	7/10	Sendai
	9/10	*M. pul.* (b)
MICE		
6	--	None positive
12	1/9	Sendai
18	5/5	Sendai
24	1/10	Sendai
	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 these results were considered to be false positive.

APPENDIX F

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

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

Week	Control		Low Dose			High Dose		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
2	17	203	16	194	165	15	180	333
8	18	311	17	300	113	17	281	242
12	16	349	16	332	96	15	309	194
16	17	371	17	359	95	16	330	194
20	16	399	16	382	84	16	352	182
24	16	409	15	386	78	14	356	157
28	16	423	15	397	76	14	365	153
32	18	438	17	411	83	16	377	170
37	16	449	15	420	71	15	381	157
40	16	454	15	426	70	14	384	146
44	17	459	17	431	79	16	389	165
49	16	453	15	420	71	14	380	147
54	17	466	16	430	74	15	392	153
58	16	461	14	423	66	14	383	146
63	16	456	15	417	72	14	376	149
67	14	454	13	415	63	12	373	129
72	16	453	15	415	72	14	373	150
76	16	449	15	410	73	14	369	152
80	15	451	14	410	68	13	366	142
85	14	456	12	410	59	12	362	133
89	13	453	12	409	59	12	362	133
94	12	439	13	403	65	11	349	126
97	13	432	12	392	61	12	344	140
101	15	439	13	390	67	12	338	142
Mean	15.7	422	14.8	391	78	14.0	353	164
SD (c)	1.5		1.6		22	1.6		44
CV(d)	9.6		10.8		28.2	11.4		26.8

(a) Average grams of feed removed from feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of nalidixic acid consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

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

	Control		Low Dose			High Dose		
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
2	12	145	11	139	158	11	133	331
8	12	192	11	185	119	11	172	256
12	10	205	11	202	109	10	183	219
16	11	214	10	211	95	10	191	209
20	10	224	11	220	100	10	201	199
24	10	229	9	223	81	9	201	179
28	10	236	10	228	88	9	206	175
32	12	245	11	237	93	10	212	189
37	11	254	10	243	82	10	218	183
40	11	256	10	247	81	10	220	182
44	12	260	11	254	87	11	225	196
49	11	259	11	249	88	10	219	183
54	12	276	11	261	84	11	231	190
58	12	286	11	269	82	10	235	170
63	12	293	11	276	80	10	236	169
67	10	293	10	280	71	10	237	169
72	12	300	12	291	82	11	249	177
76	13	307	12	299	80	11	255	173
80	13	313	11	305	72	9	258	140
85	12	322	11	314	70	10	266	150
89	10	324	11	320	69	10	272	147
94	11	326	11	317	69	10	269	149
97	11	322	10	314	64	10	271	148
101	11	321	11	317	69	11	271	162
Mean	11.3	267	10.8	258	86	10.2	226	185
SD (c)	1.0		0.7		20	0.6		40
CV (d)	8.8		6.5		23.3	5.9		21.6

(a) Average grams of feed removed from feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of nalidixic acid consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

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

	Control		Low Dose			High Dose		
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
3	3.5	26.9	3.6	26.6	271	3.5	26.2	534
8	3.5	29.9	3.3	29.4	224	2.4	29.0	331
14	6.0	32.4	4.1	31.7	259	4.7	30.9	608
18	4.2	33.0	4.1	32.9	249	4.2	32.3	520
22	4.2	34.0	4.1	33.8	243	4.5	33.3	541
26	4.0	35.0	4.0	34.7	231	4.2	33.7	499
30	4.0	35.7	4.1	35.5	231	4.4	34.6	509
34	4.2	36.6	4.3	36.3	237	4.6	35.1	524
39	4.2	37.1	4.5	36.6	246	4.5	35.5	507
42	4.2	37.2	4.3	36.6	235	4.3	35.2	489
46	4.3	37.9	4.3	36.7	234	4.6	35.5	518
51	4.3	38.1	4.2	37.8	222	4.4	36.0	489
56	4.0	38.7	4.2	38.1	220	4.5	36.3	496
60	4.1	37.7	4.2	37.7	223	4.5	35.5	507
65	4.1	37.6	4.0	37.8	212	4.4	35.8	492
69	4.2	38.5	4.2	38.0	221	4.4	36.4	484
74	4.0	37.6	3.9	37.6	207	4.1	35.5	462
78	4.0	38.2	3.9	38.3	204	4.6	36.1	510
82	3.8	38.6	3.8	38.2	199	3.9	36.1	432
87	3.9	38.3	3.8	38.0	200	3.9	35.9	435
91	3.9	38.2	3.6	38.0	189	3.9	35.7	437
96	4.0	38.1	3.8	37.4	203	4.1	35.5	462
99	3.4	37.4	3.9	37.2	210	4.3	35.4	486
Mean	4.1	36.2	4.0	35.9	225	4.2	34.4	490
SD (c)	0.5		0.3		21	0.5		52
CV (d)	12.2		7.5		9.3	11.9		10.6

(a) Average grams of feed removed from feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of nalidixic acid consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

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

	Control		Low Dose			High Dose		
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
5	3.1	21.9	3.0	21.5	279	3.0	21.2	566
11	3.3	24.7	3.2	24.5	261	3.3	24.0	550
15	3.3	26.3	3.2	25.4	252	3.1	24.9	498
19	3.3	28.0	3.2	26.3	243	3.3	26.1	506
23	3.3	29.3	3.2	28.0	229	3.1	27.0	459
27	3.1	31.3	3.1	29.7	209	3.1	28.2	440
31	3.4	32.4	3.2	30.4	211	3.4	28.7	474
36	3.3	33.1	3.3	30.8	214	3.3	29.1	454
39	3.2	33.1	3.2	30.9	207	3.2	29.1	440
43	3.5	34.2	3.3	31.4	210	3.3	29.8	443
48	3.7	35.1	3.4	31.7	215	3.4	30.2	450
53	3.5	36.5	3.5	32.4	216	3.5	30.9	453
57	3.7	36.8	3.5	32.7	214	3.6	31.2	462
62	3.6	37.2	3.6	33.5	215	3.5	31.6	443
66	3.6	36.6	3.7	33.5	221	3.5	31.4	446
71	3.6	37.1	3.7	33.1	224	3.5	31.6	443
75	3.9	38.7	3.8	35.5	214	3.8	33.4	455
79	3.6	39.1	3.5	36.1	194	3.6	34.0	424
84	3.6	39.6	3.4	36.9	184	3.5	34.2	409
88	3.6	40.9	3.2	37.3	172	3.5	34.9	401
93	3.9	41.0	3.9	37.9	206	3.8	34.3	443
96	4.0	41.0	3.8	38.3	198	4.1	34.3	478
101	4.1	42.3	3.9	39.2	199	4.0	35.1	456
Mean	3.5	34.6	3.4	32.0	217	3.5	30.2	461
SD (c)	0.3		0.3		24	0.3		39
CV (d)	8.6		8.8		11.1	8.6		8.5

(a) Average grams of feed removed from feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of nalidixic acid consumed per day per kilogram of body weight
(c) Standard deviation
(d) 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)

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) NCI, 1976; NIH, 1978
(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
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)			
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)			
Linoleic	2.40	2.37-2.44	2
Linolenic	0.284	0.259-0.308	2
Vitamins			
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			
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 lots of diet analyzed for nutrients reported in this table were manufactured in 1983 or 1984.
(b) One lot (July 22, 1981) was 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)	<10.0	<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) Detection limit was reduced from 10 ppb to 5 ppb after July 1981.
(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 value of 150 MPN for the lot produced on August 26, 1982.
(h) Mean, standard deviation, and range include the high value given in (g).
(i) All values were corrected for percent recovery.
(j) Mean, standard deviation, and range exclude one value of 100.3 ppb obtained for the lot produced on April 27, 1981.
(k) Mean, standard deviation, and range include the high value given in (j).
(l) Mean, standard deviation, and range exclude one value of 99.0 ppb obtained for the lot produced on April 27, 1981.
(m) Mean, standard deviation, and range include the high value given in (l).
(n) BHC = hexachlorocyclohexane or benzene hexachloride
(o) One observation was above the detection limit. The value and date it was obtained are given under the range.
(p) Two observations were above the detection limit. The values and dates they were obtained are given under the range.
(q) Ten lots contained more than 0.05 ppm.
(r) Analysis for endosulfan I, endosulfan II, and endosulfan sulfate began on December 23, 1981.

APPENDIX H

CHEMICAL CHARACTERIZATION, ANALYSIS, AND DOSE PREPARATION OF NALIDIXIC ACID FOR THE TOXICOLOGY STUDIES

APPENDIX H. CHEMICAL CHARACTERIZATION

Procurement and Characterization of Nalidixic Acid

Nalidixic acid was obtained in one lot (lot no. H120779) from Hilton-Davis Chemical Company (Cincinnati, OH). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on the analyses performed in support of the nalidixic acid studies are on file at the National Institute of Environmental Health Sciences.

The study chemical was identified as nalidixic acid by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Infrared and nuclear magnetic resonance spectra (Figures H1 and H2) were consistent with those expected for the structure and with the literature spectra (Sadtler Standard Spectra). The ultraviolet/visible spectrum was consistent with that expected for the structure of nalidixic acid and with the spectrum of a USP standard. The absorptivity of the study material at 258 nm was within National Formulary specifications when compared with a USP standard (USP, 1975).

The purity of nalidixic acid was determined by elemental analysis, Karl Fischer water analysis, potentiometric titration in pyridine solution of the carboxylic acid group with 0.1 N tetrabutylammonium hydroxide (in methanol:2-propanol, 1:9), thin-layer chromatography, and high-performance liquid chromatography. Thin-layer chromatography was performed on silica gel plates with *n*-butanol saturated with ammonium hydroxide (system 1) and with absolute ethanol:concentrated ammonium hydroxide (90:10) (system 2).

Visualization was with visible and 254-nm ultraviolet light and with a commercially prepared ninhydrin spray reagent (0.5% in butanol). High-performance liquid chromatography was performed with a Waters μBondapak C_{18} column with a Whatman CO:PELL ODS guard column, a solvent system of aqueous 1% acetic acid:acetonitrile containing 1% acetic acid (70:30, isocratic), and a detection wavelength of 254 nm.

Analysis of the cumulative data indicated that the nalidixic acid study material was approximately 99% pure. Results of the elemental analysis for carbon, hydrogen, and nitrogen agreed with the theoretical values. Water content was less than 0.01%. Titration of the carboxylic acid group indicated a purity of 102% (National Formulary specifications are 98%-102%). Thin-layer chromatography by system 1 indicated one trace and two slight trace impurities; system 2 indicated one trace and one slight trace impurity; similar results were obtained using a USP standard of nalidixic acid. High-performance liquid chromatography indicated one impurity with a peak area of 0.25% relative to the major peak area. Major peak comparisons with a USP standard by high-performance liquid chromatography indicated a purity of 98.8%.

Stability studies performed by high-performance liquid chromatography, using the same system as before but with a 50:50 solvent ratio, indicated that nalidixic acid was stable as a bulk chemical when stored protected from light for 2 weeks at temperatures up to 60° C. During the 2-year studies, the bulk chemical was stored at room temperature. The study material was identified at the study laboratory by infrared spectroscopy. Confirmation of the stability of the bulk chemical during the course of the studies was obtained by nonaqueous titration of the carboxylic acid group and high-performance liquid chromatography. No notable degradation was observed during the studies.

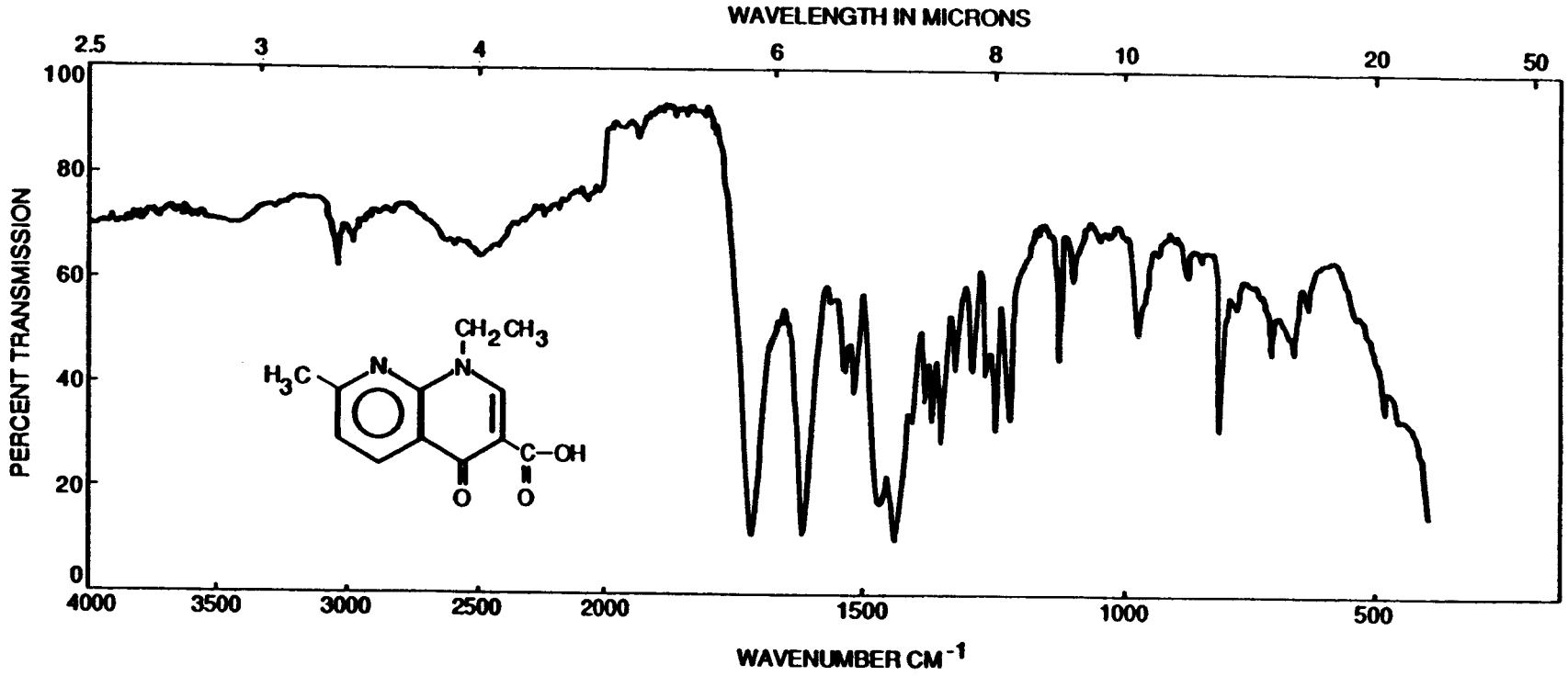

FIGURE H1. INFRARED ABSORPTION SPECTRUM OF NALIDIXIC ACID (LOT NO. H120779)

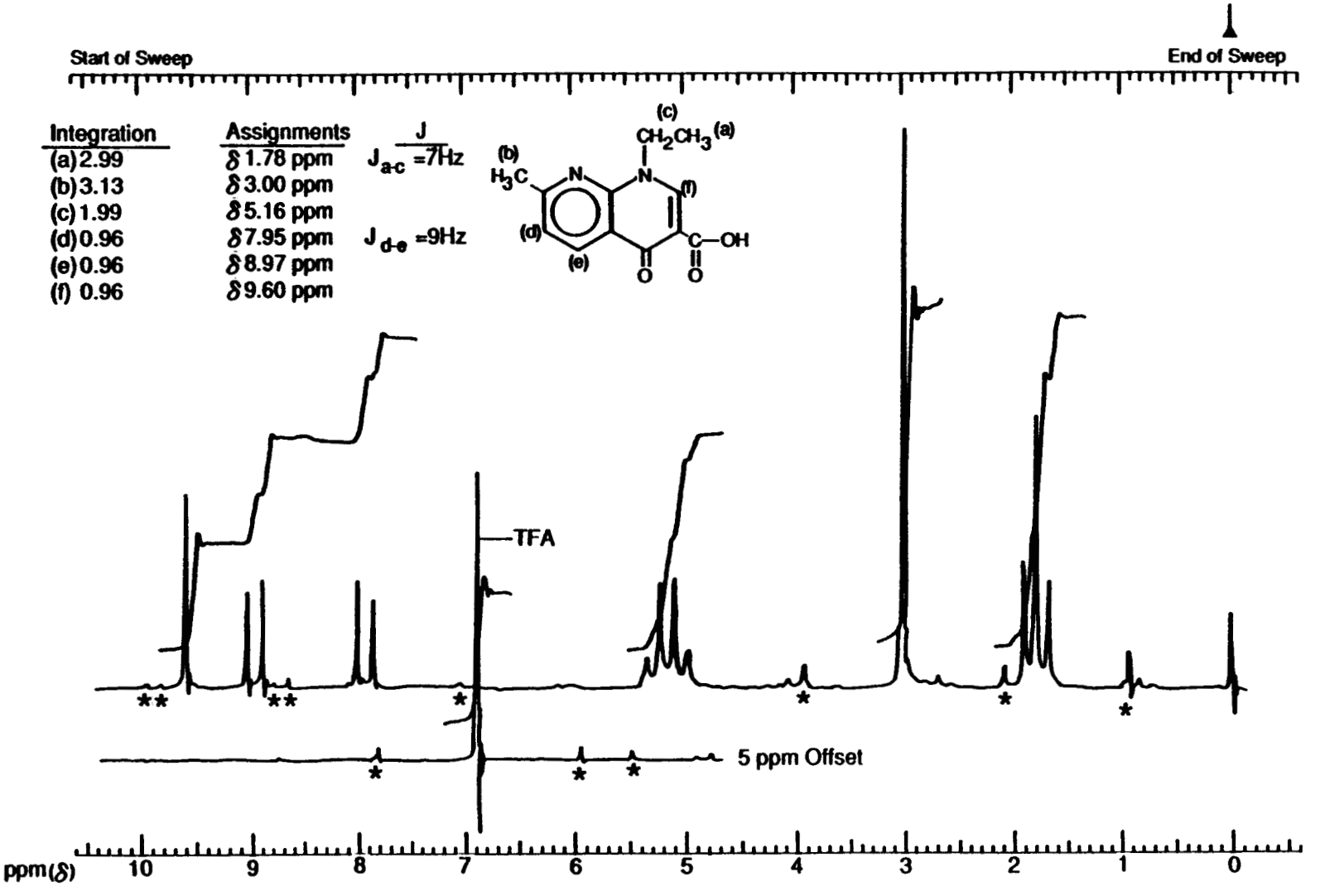

FIGURE H2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF NALIDIXIC ACID (LOT NO. H120779)

Preparation and Characterization of Formulated Diets

Formulated diets were prepared by adding a dry premix of feed and nalidixic acid to the appropriate amount of feed and blending for 15 minutes (Table H1). The nalidixic acid content of feed mixtures was determined by ultraviolet (at 257 nm) spectroscopy of acetonitrile extracts. The homogeneity of formulated diets containing 8,000 ppm nalidixic acid was demonstrated to be within 1% of the target concentration by the analytical chemistry laboratory and within 3% by the study laboratory in samples taken from three locations in the blender. The chemical in feed at a concentration of 8,000 ppm was shown to be stable for 2 weeks in the dark at temperatures up to 25° C. A loss of approximately 5% was demonstrated after 2 weeks' storage at 45° C. In the 13-week studies, the formulated diets were stored protected from light at room temperature and used within 11 days of formulation. In the 2-year studies, the formulated diets were stored protected from light at room temperature for no longer than 2 weeks.

Periodic analysis of formulated diets of nalidixic acid was conducted at the study laboratory and at the analytical chemistry laboratory. Feed samples were extracted with acetonitrile; the extracts were diluted to a concentration of approximately 3-5 µg/ml, and the absorbance was read at 257 nm. Formulated diets were analyzed once at the beginning of the 13-week studies. The results ranged from 90% to 104% of the target concentrations (Table H2). During the 2-year studies, the formulated diets were analyzed at approximately 8-week intervals. Based on the number of times that concentrations were within specifications, it is estimated that the mixtures were formulated within ± 10% of the target concentrations approximately 96% (27/28) of the time throughout the studies (Table H3). Results of referee analysis periodically performed by the analytical chemistry laboratory indicated generally good agreement with the results from the study laboratory (Table H4).

TABLE H1. PREPARATION AND STORAGE OF FORMULATED DIETS IN THE FEED STUDIES OF NALIDIXIC ACID

Thirteen-Week Studies	Two-Year Studies
Dietary Preparation	
Weighed portions of nalidixic acid were transferred to a beaker and mixed by spatula with weighed portions of feed to form a premix. Premix was layered with feed in an 8-qt Patterson-Kelly Twin-Shell® V-blender for 5 min with and 10 min without the intensifier bar.	Same as 13-wk studies
Maximum Storage Time	
11 d	2 wk
Storage Conditions	
Room temperature, protected from light and moisture	25° C in opaque plastic pails

TABLE H2. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF NALIDIXIC ACID

Date Mixed	Concentration of Nalidixic Acid in Feed (ppm) Target	Concentration of Nalidixic Acid in Feed (ppm) Determined	Determined as a Percent of Target
06/26/80	(a) 1,000	971	97.1
	(b) 1,000	973	97.3
	(c) 1,000	974	97.4
	1,000	978	97.8
	2,000	1,970	98.5
	4,000	4,000	100.0
	9,000	8,120	90.2
	(a) 16,000	16,300	101.9
	(b) 16,000	16,600	103.7
	(c) 16,000	16,200	101.2
	16,000	16,500	103.1

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

TABLE H3. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

Date Mixed	Concentration of Nalidixic Acid in Feed for Target Concentration (ppm) (a) 2,000 ppm	4,000 ppm
04/08/81	2,020	4,050
04/29/81	1,990	4,000
07/01/81	(b) 1,980	3,960
08/26/81	2,000	3,950
09/23/81	1,980	4,030
12/23/81	1,950	3,950
02/10/82	1,950	3,850
04/14/82	1,980	3,970
06/02/82	1,920	3,930
07/07/82	1,940	4,040
10/07/82	2,110	4,130
10/20/82	1,960	3,770
12/29/82	1,930	3,960
03/23/83	(c) 1,780	4,100
03/24/83	(d) 1,920	--
Mean (ppm)	1,964	3,978
Standard deviation	70.8	93.8
Coefficient of variation (percent)	3.6	2.4
Range (ppm)	1,780-2,110	3,770-4,130
Number of samples	14	14

(a) Results of duplicate analysis
(b) Results of single analysis
(c) Out of specifications; not used in the studies.
(d) Remix; not included in the mean.

TABLE H4. RESULTS OF REFEREE ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory (a)	Referee Laboratory (b)
04/08/81	2,000	2,020	1,950
09/23/81	4,000	4,030	3,800
04/14/82	2,000	1,980	1,960
10/07/82	4,000	4,130	3,890

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

APPENDIX I

GENETIC TOXICOLOGY

OF NALIDIXIC ACID

APPENDIX I. GENETIC TOXICOLOGY

METHODS

Salmonella Protocol: Testing was performed as reported by Ames et al. (1975) with modifications listed below and described in greater detail by Zeiger et al. (1988). Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). The study chemical was incubated with the *Salmonella typhimurium* tester strains (TA97, TA98, TA100, and TA1535) 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 four strains. All trials were repeated. Repeated trials with S9 were performed 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.

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, TX). The highest dose of the study compound was determined by solubility or toxicity and did not exceed 5 mg/ml. Mouse L5178Y/TK lymphoma 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 6×10^6 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, 3×10^6 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, TX). 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 ± 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 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.

RESULTS

Nalidixic acid did not induce gene mutation in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 when tested in a preincubation protocol with doses up to 33 µg/plate in the presence or absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Zeiger et al., 1988; Table I1). Results of tests for induction of Tft resistance in mouse L5178Y/TK lymphoma cells were negative with or without Aroclor 1254-induced male F344 rat liver S9; nalidixic acid concentrations ranged from 30 to 100 µg/ml (Table I2). In cytogenetic tests with CHO cells, nalidixic acid, at concentrations up to 160 µg/ml, did not induce SCEs (Table I3) or chromosomal aberrations (Table I4) with or without liver S9 from Aroclor 1254-induced male Sprague Dawley rats. Although a slight increase in SCEs occurred in the first trial without S9, it was not repeated in a second trial, and the assay results overall were considered to be negative.

TABLE I1. MUTAGENICITY OF NALIDIXIC ACID IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b)							
		−S9		+S9 (hamster)			+S9 (rat)		
		Trial 1	Trial 2	10%	30%	30%	10%	30%	
TA100	0	80 ± 5.5	106 ± 6.1	64 ± 4.4	93 ± 4.7	115 ± 7.1	81 ± 8.5	101 ± 6.4	
	0.03	83 ± 7.3	93 ± 3.7	--	--	--	--	--	
	0.1	89 ± 2.3	97 ± 2.5	74 ± 3.2	91 ± 8.5	--	93 ± 3.2	109 ± 16.6	
	0.3	83 ± 7.2	114 ± 3.6	85 ± 5.0	97 ± 2.1	--	75 ± 5.5	101 ± 6.2	
	1	89 ± 6.0	100 ± 7.6	82 ± 3.8	90 ± 5.2	133 ± 4.2	89 ± 4.3	114 ± 4.2	
	3.3	(c) 101 ± 3.3	108 ± 0.9	90 ± 3.8	103 ± 4.5	122 ± 5.2	112 ± 4.1	125 ± 11.8	
	10	--	--	(c) 40 ± 7.3	133 ± 8.7	135 ± 7.8	(c) 92 ± 8.6	(c) 27 ± 8.4	
	16.5	--	--	--	--	150 ± 7.2	--	--	
	33	--	--	--	--	Toxic	--	--	
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative	Negative	
Positive control (d)		1,242 ± 58.6	1,277 ± 21.1	920 ± 24.3	1,949 ±145.7	685 ± 42.5	1,456 ± 65.5	1,181 ± 1.2	
		−S9		+S9 (hamster)			+S9 (rat)		
		Trial 1	Trial 2	10%	30%		10%	30%	
TA1535	0	23 ± 2.2	24 ± 0.9	16 ± 1.2	11 ± 0.7		14 ± 1.8	13 ± 0.3	
	0.03	12 ± 3.4	24 ± 3.5	--	--		--	--	
	0.1	22 ± 3.2	26 ± 2.0	7 ± 0.7	12 ± 1.9		8 ± 1.5	10 ± 1.5	
	0.3	19 ± 3.4	20 ± 1.5	10 ± 1.9	10 ± 1.8		10 ± 2.8	13 ± 2.2	
	1	23 ± 1.9	23 ± 3.5	11 ± 0.7	14 ± 1.7		12 ± 2.8	14 ± 1.8	
	3.3	(c) 15 ± 2.0	21 ± 2.6	10 ± 1.2	11 ± 0.3		11 ± 3.2	14 ± 0.9	
	10	--	--	(c) 3 ± 2.4	12 ± 2.5		(c) 0 ± 0.0	(c) 2 ± 0.7	
Trial summary		Negative	Negative	Negative	Negative		Negative	Negative	
Positive control (d)		947 ± 2.6	1,027 ± 20.5	93 ± 5.0	259 ± 17.7		157 ± 3.2	176 ± 9.2	
TA97	0	97 ± 6.2	91 ± 4.8	110 ± 4.4	158 ± 9.5		149 ± 2.1	225 ± 12.2	
	0.03	87 ± 2.5	87 ± 0.7	--	--		--	--	
	0.1	95 ± 7.5	77 ± 10.0	101 ± 5.5	162 ± 15.6		149 ± 5.6	221 ± 19.4	
	0.3	86 ± 4.2	107 ± 12.0	115 ± 5.8	166 ± 4.3		124 ± 3.8	192 ± 8.6	
	1	81 ± 3.2	100 ± 3.5	116 ± 10.5	157 ± 2.6		129 ± 9.5	190 ± 9.0	
	3.3	98 ± 5.9	94 ± 1.5	94 ± 2.3	148 ± 6.2		121 ± 2.8	183 ± 12.1	
	10	--	--	(c) 49 ± 7.0	140 ± 14.5		(c) 5 ± 2.4	(c) 31 ± 25.9	
Trial summary		Negative	Negative	Negative	Negative		Negative	Negative	
Positive control (d)		570 ± 12	2,442 ± 63.5	957 ± 41.6	1,041 ± 48.8		1,157 ± 7.0	697 ± 7.4	
TA98	0	20 ± 2.4	16 ± 0.9	27 ± 2.5	31 ± 5.0		31 ± 5.8	34 ± 1.5	
	0.03	19 ± 0.6	18 ± 1.2	--	--		--	--	
	0.1	24 ± 2.6	19 ± 1.2	26 ± 2.1	32 ± 6.0		30 ± 1.8	27 ± 4.7	
	0.3	20 ± 2.3	18 ± 1.7	32 ± 2.8	27 ± 3.2		31 ± 4.3	33 ± 2.5	
	1	22 ± 3.8	16 ± 2.0	28 ± 3.3	31 ± 1.2		34 ± 7.2	35 ± 4.7	
	3.3	19 ± 1.0	16 ± 1.5	31 ± 3.5	27 ± 2.9		37 ± 3.8	26 ± 1.5	
	10			(c) 16 ± 2.6	32 ± 2.0		(c) 0 ± 0.0	(c) 6 ± 1.5	
Trial summary		Negative	Negative	Negative	Negative		Negative	Negative	
Positive control (d)		1,945 ± 81.4	1,875 ± 38.1	1,753 ± 53.2	1,481 ± 31.7		1,952 ± 54.7	819 ± 7.9	

(a) Study performed at EG&G Mason Research Institute. The detailed protocol is presented by Zeiger et al. (1988). 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) Slight toxicity
(d) 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 TA97.

TABLE I2. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE BY NALIDIXIC ACID IN MOUSE L5178Y LYMPHOMA CELLS (a,b)

Compound	Concentration (µg/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
-S9					
Trial 1					
Dimethyl sulfoxide (d)		90.0 ± 3.6	100.0 ± 14.4	102.0 ± 10.7	37.8 ± 4.3
Nalidixic acid	30	64.3 ± 6.7	88.0 ± 5.0	60.7 ± 7.5	31.3 ± 1.5
	40	63.7 ± 10.4	101.7 ± 13.4	72.7 ± 17.3	37.0 ± 3.2
	50	83.7 ± 3.2	64.7 ± 4.1	93.3 ± 2.4	37.3 ± 0.3
	(e) 60	79.0 ± 3.0	74.0 ± 10.0	93.0 ± 11.0	39.5 ± 3.5
	80	82.0 ± 3.1	77.0 ± 2.6	69.3 ± 3.2	28.3 ± 2.0
	100	77.7 ± 10.4	99.0 ± 15.6	77.0 ± 11.4	33.3 ± 3.4
Methyl methanesulfonate (f)	5	53.5 ± 5.5	53.0 ± 5.0	599.5 ± 44.5	(g) 372.5 ± 10.5
Trial 2					
Dimethyl sulfoxide		97.0 ± 12.7	100.0 ± 6.7	80.3 ± 18.5	29.3 ± 7.7
Nalidixic acid	30	99.0 ± 6.2	86.7 ± 3.5	102.7 ± 5.8	34.3 ± 1.2
	40	94.7 ± 4.3	83.0 ± 22.3	120.7 ± 10.7	42.7 ± 4.3
	50	102.3 ± 8.1	94.0 ± 4.2	95.7 ± 1.3	31.7 ± 3.0
	60	96.0 ± 2.5	85.0 ± 4.9	108.3 ± 7.5	37.7 ± 1.9
	80	92.3 ± 7.2	91.7 ± 11.3	106.3 ± 9.0	38.7 ± 0.3
	(f) 100	87.0 ± 3.0	80.0 ± 2.0	96.0 ± 11.0	37.0 ± 3.0
Methyl methanesulfonate	5	80.7 ± 5.4	60.7 ± 1.8	471.0 ± 14.7	(g) 195.7 ± 7.2
+S9 (h)					
Trial 1					
Dimethyl sulfoxide (d)		101.5 ± 3.5	99.8 ± 2.1	59.8 ± 8.1	19.8 ± 3.4
Nalidixic acid	30	104.7 ± 4.5	88.0 ± 4.6	59.3 ± 11.6	19.0 ± 4.6
	40	96.3 ± 7.4	82.3 ± 4.9	53.0 ± 3.5	19.0 ± 3.0
	50	95.7 ± 10.5	79.7 ± 5.8	48.0 ± 12.2	16.3 ± 3.4
	(i) 60	113	69	68	20
	80	107.7 ± 5.8	87.3 ± 5.2	88.7 ± 4.7	27.7 ± 1.3
	(f) 100	103.0 ± 3.0	80.0 ± 3.0	75.0 ± 10.0	24.0 ± 4.0
Methylcholanthrene	2.5	49.0 ± 8.7	17.0 ± 5.0	575.3 ± 27.2	(g) 409.0 ± 53.0
Trial 2					
Dimethyl sulfoxide (f)		104.5 ± 0.5	100.0 ± 8.0	75.5 ± 8.5	24.0 ± 3.0
Nalidixic acid	30	100.0 ± 2.5	83.0 ± 7.5	85.7 ± 10.8	28.7 ± 4.1
	40	97.0 ± 9.1	84.3 ± 2.4	78.7 ± 15.6	27.3 ± 5.8
	50	94.7 ± 6.8	88.3 ± 3.2	102.0 ± 25.9	35.3 ± 6.7
	(f) 60	109.5 ± 4.5	102.5 ± 3.5	89.0 ± 4.0	27.0 ± 0.0
	80	104.0 ± 6.7	83.0 ± 6.4	72.7 ± 5.5	23.3 ± 0.3
	(f) 100	105.5 ± 5.5	90.5 ± 3.5	73.0 ± 8.0	23.0 ± 1.0
Methylcholanthrene	2.5	59.0 ± 4.7	13.3 ± 0.3	771.3 ± 22.3	(g) 440.3 ± 30.2

TABLE I2. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE BY NALIDIXIC ACID 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 noted; the average for the tests is presented in the table. Cells (6×10^5/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, 3×10^6 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 from replicate trials of approximately 1×10^6 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 1×10^6 cells treated); MF = mutant fraction.
(d) Data presented are for four tests.
(e) Data presented are for two tests; the dose in one test was lethal.
(f) Data presented are for two tests.
(g) 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.
(h) 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 (dimethyl sulfoxide).
(i) Data presented are for one test.

TABLE I3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY NALIDIXIC ACID (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
−S9 (c)								
Trial 1--Summary: Weakly positive								
Dimethyl sulfoxide		50	1,044	410	0.39	8.2	27.5	
Nalidixic acid	5	50	1,039	431	0.41	8.6	27.5	104.9
	16	50	1,039	488	0.47	9.8	27.5	119.5
	50	50	1,041	426	0.41	8.5	27.5	103.7
	160	50	1,042	502	0.48	10.0	27.5	122.0
Mitomycin C	0	50	1,045	539	0.52	10.8	27.5	131.7
	0.001	10	208	164	0.79	16.4	27.5	200.0
Trial 2--Summary: Negative								
Dimethyl sulfoxide		50	1,048	450	0.43	9.0	26.5	
Nalidixic acid	50	50	1,048	476	0.45	9.5	26.5	105.6
	75	50	1,049	512	0.49	10.2	26.5	113.3
	100	50	1,049	501	0.48	10.0	26.5	111.1
	150	50	1,047	509	0.49	10.2	26.5	113.3
Mitomycin C	0	50	1,052	644	0.61	12.9	26.5	143.3
	0.01	10	210	580	2.76	58.0	26.5	644.4
+S9 (d)								
Trial 1--Summary: Negative								
Dimethyl sulfoxide		50	1,050	467	0.44	9.3	26.0	
Nalidixic acid	5	50	1,051	458	0.44	9.2	26.0	98.9
	16	50	1,053	423	0.40	8.5	26.0	91.4
	50	50	1,051	437	0.42	8.7	26.0	93.5
	160	50	1,050	505	0.48	10.1	26.0	108.6
Cyclophosphamide	0.3	50	1,044	607	0.58	12.1	26.0	130.1
	2	10	210	381	1.81	38.1	26.0	409.7
Trial 2--Summary: Negative								
Dimethyl sulfoxide		50	1,048	418	0.4	8.4	26.0	
Nalidixic acid	50	50	1,046	425	0.41	8.5	26.0	101.2
	75	50	1,049	434	0.41	8.7	26.0	103.6
	100	50	1,049	404	0.39	8.1	26.0	96.4
	150	50	1,046	396	0.38	7.9	26.0	94.0
Cyclophosphamide	0.3	50	1,050	605	0.58	12.1	26.0	144.0
	2	10	209	414	1.98	41.4	26.0	492.9

TABLE I3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY NALIDIXIC ACID (Continued)

(a) Study performed at Environmental Health Research and Testing, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) 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 I4. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY NALIDIXIC ACID (a)

−S9 (b)					+S9 (c)				
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
Harvest time: 12 hours					Harvest time: 12.5 hours				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	100	0	0.00	0.0		100	1	0.01	1.0
Nalidixic acid					Nalidixic acid				
5	100	0	0.00	0.0	5	100	1	0.01	1.0
16	100	2	0.02	2.0	16	100	0	0.00	0.0
50	100	2	0.02	2.0	50	100	0	0.00	0.0
160	100	1	0.01	1.0	160	100	1	0.01	1.0
Summary: Negative					Summary: Negative				
Mitomycin C					Cyclophosphamide				
0.25	100	23	0.23	17.0	15	100	42	0.42	29.0
1	50	35	0.70	40.0	50	50	52	1.04	54.0

(a) Study performed at Environmental Health Research and Testing, Inc.; Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) as indicated in (b) and (c). 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) 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.

APPENDIX J

RETINAL DEGENERATION AND CATARACTS AND CAGE POSITION FOR RATS IN THE TWO-YEAR FEED STUDIES OF NALIDIXIC ACID

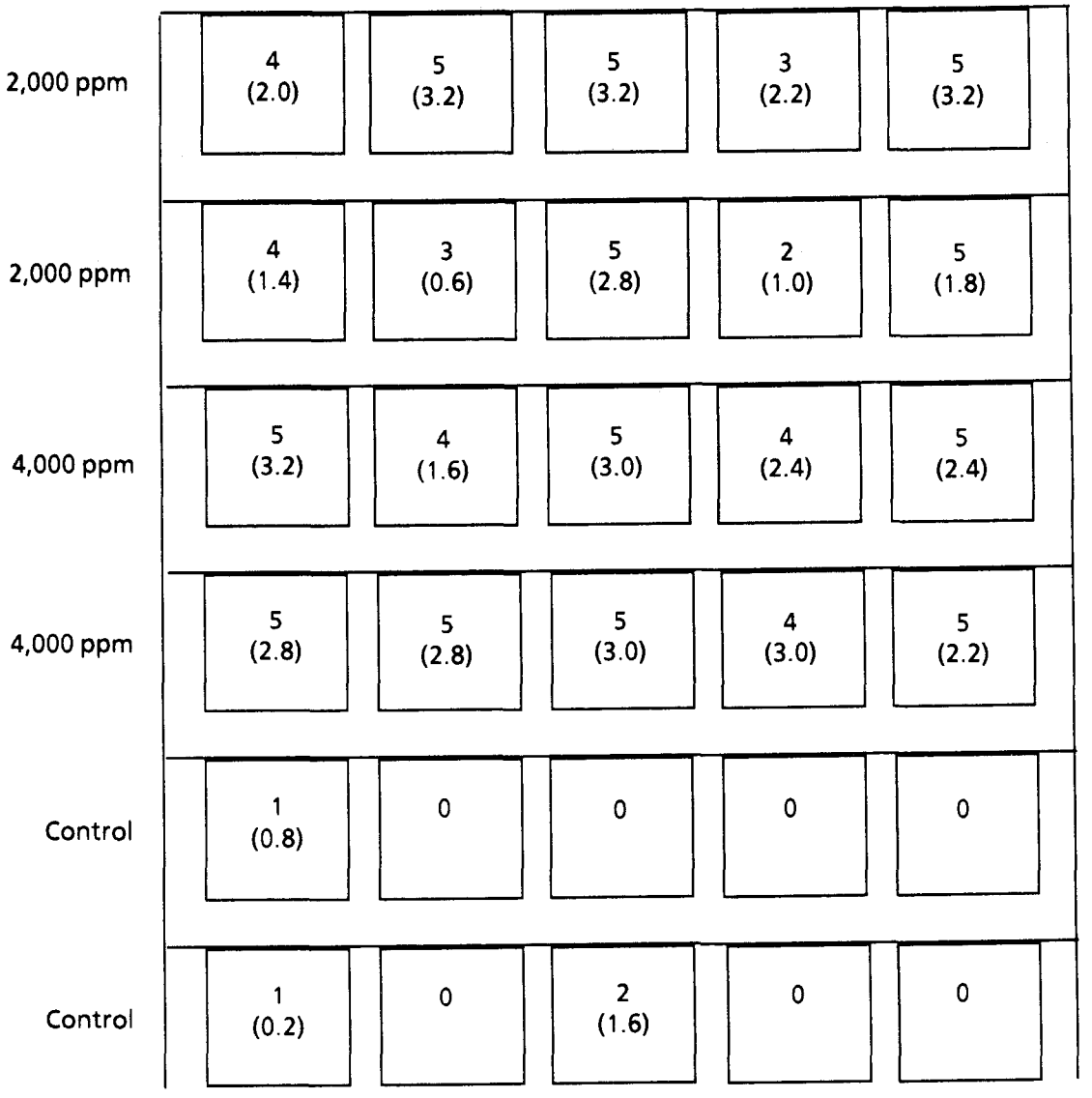

FIGURE J1. INCIDENCE OF RETINAL DEGENERATION BY CAGE POSITION IN MALE RATS FED NALIDIXIC ACID FOR TWO YEARS
(Number in parentheses is the severity score.)

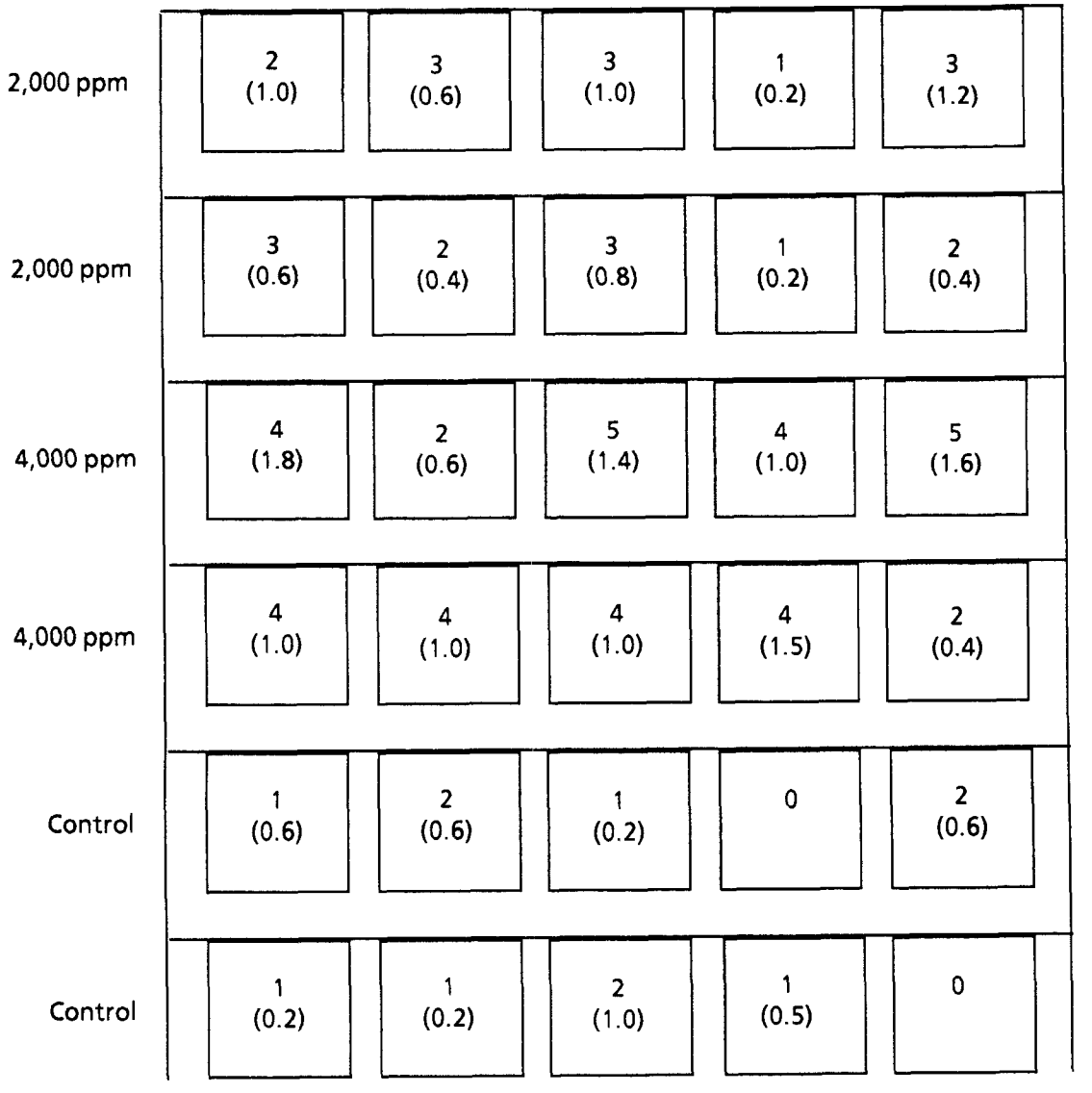

FIGURE J2. INCIDENCE OF CATARACTS BY CAGE POSITION IN MALE RATS FED NALIDIXIC ACID FOR TWO YEARS
(Number in parentheses is the severity score.)

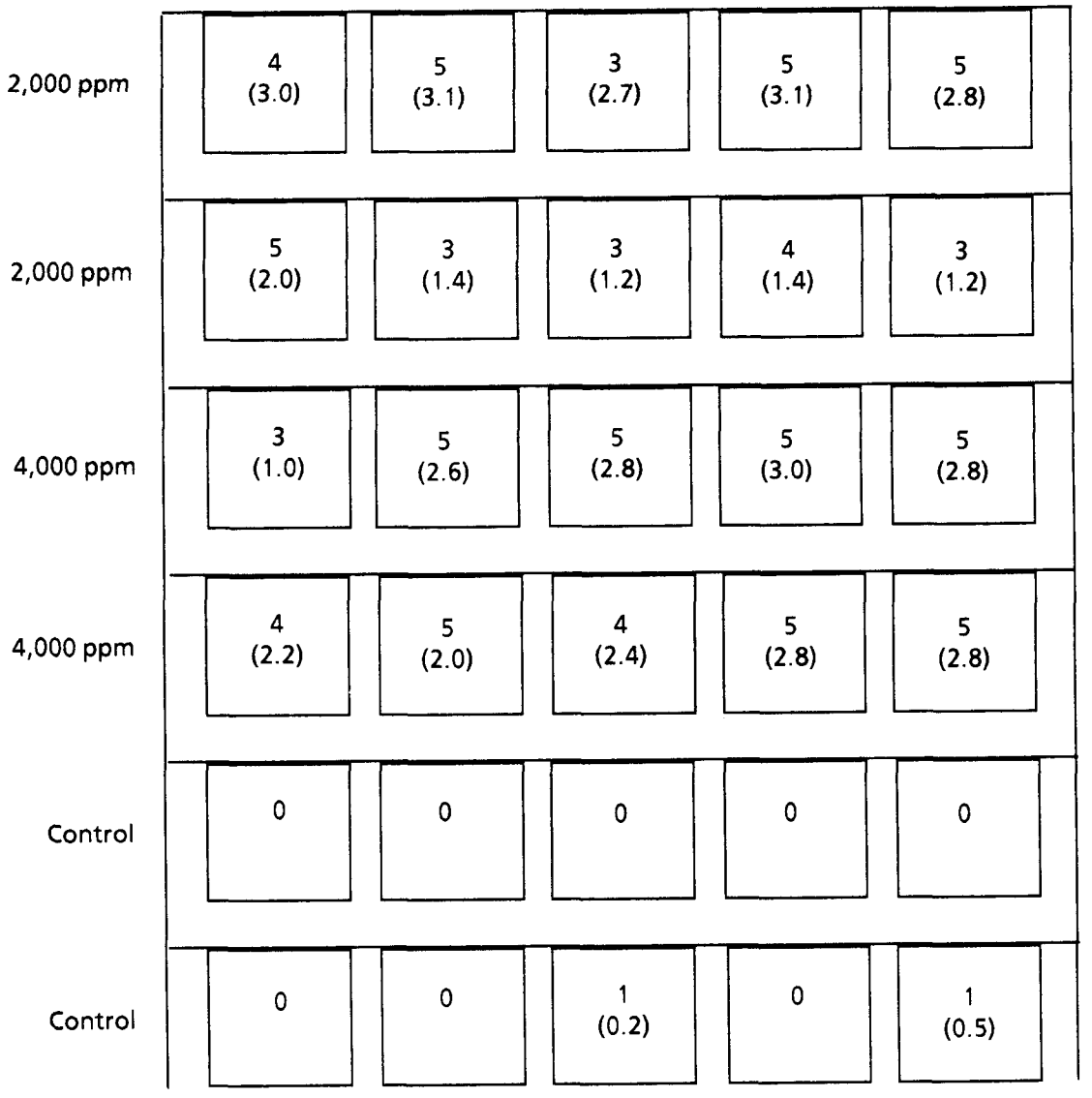

FIGURE J3. INCIDENCE OF RETINAL DEGENERATION BY CAGE POSITION IN FEMALE RATS FED NALIDIXIC ACID FOR TWO YEARS
(Number in parentheses is the severity score.)

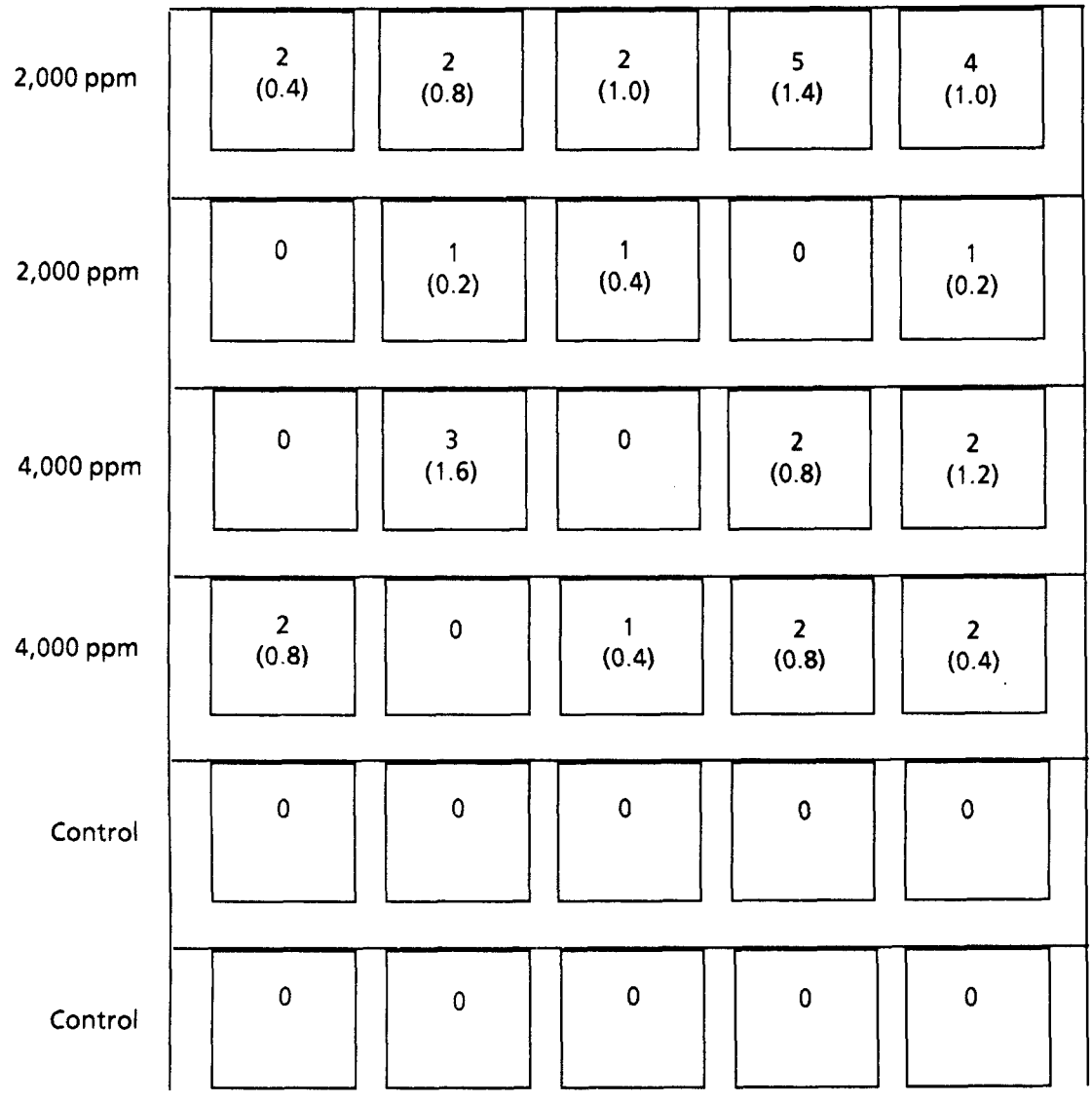

FIGURE J4. INCIDENCE OF CATARACTS BY CAGE POSITION IN FEMALE RATS FED NALIDIXIC ACID FOR TWO YEARS
(Number in parentheses is the severity score.)

APPENDIX K

AUDIT SUMMARY

APPENDIX K. AUDIT SUMMARY

The pathology specimens, experimental data, study documents, and draft of NTP Technical Report No. 368 for the 2-year studies of nalidixic acid in rats and mice were audited for the National Institute of Environmental Health Sciences (NIEHS) at the National Toxicology Program (NTP) Archives by Integrated Laboratory Systems. 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, animal husbandry, environmental conditions, dosing, external masses, mortality, animal identification, and serology for 2-year study animals.
(3) Body weight, feed consumption, and clinical observation data; all data were scanned before individual data for a random 10% sample of animals in each study group were reviewed in detail.
(4) All chemistry records.
(5) All postmortem records for individual animals concerning date of death, disposition code, condition code, tissue accountability, correlation of masses or clinical signs recorded at or near the last inlife observation with gross observations and microscopic diagnoses, and correlation between gross observations and microscopic diagnoses.
(6) All wet tissue bags for inventory and wet tissues from a random 20% sample of 2-year study animals in each group, plus other relevant cases, to evaluate the integrity of identity for individual animals and to examine for untrimmed potential lesions.
(7) Blocks and slides of tissues from a random 20% sample of 2-year study animals in each group, plus animals with less than complete or correct identification, to examine for proper match and inventory.
(8) Necropsy record forms for data entry errors and all microscopic diagnoses for a random 20% sample of animals, plus all redlined diagnoses on the preliminary pathology tables, to verify incorporation of changes into the final pathology tables.
(9) The extent of correlation between the data, factual information, and procedures for the 2-year studies as presented in the draft Technical Report and the study records available at the NTP Archives.

Procedures and events for the exposure phase of the studies were documented adequately by the archival records, with the exception that some or all of the records for balance calibration, animal quarantine, disposition of surplus rats and mice, rack changes, feed inventory, dose preparation and use, and ultraviolet spectra from dose mixture analyses were not available at the Archives. Review of data for the entire exposure phase indicated that the protocol procedures for animal care were followed adequately during the course of the studies. Although dose mixture preparation and use records were not available, records for formulation schedule, dose analysis, and feed consumption indicated that animals were dosed. Recalculation of approximately 10% of the group mean body weight and feed consumption values showed all to be correct. All of the external masses observed inlife in rats and mice correlated with necropsy observations. The disposition code and date of death recorded at necropsy for each unscheduled-death animal (136 rats and 87 mice) had matching entries in the inlife records.

Individual animal identifiers (punched ears and clipped toes) were present and correct in the residual tissue bags for 56/64 rats and 37/61 mice examined. Review of the entire data trail for the 8 rats and 24 mice with less than complete and correct identifiers indicated that the integrity of individual animal identity had been maintained. No untrimmed potential lesions were found in the wet tissues of 61 mice examined, whereas 14 lesions were found in 14/64 rats examined; none involved target organs. Intestinal segments were not completely opened for 4/64 rats and 19/61 mice; however, no potential lesions were evident by external examination. All gross observations made at necropsy were correlated with microscopic diagnoses, except for three that involved nontarget organs of three rats.

Tissue blocks and slides matched each other properly. All post-Pathology Working Group changes in diagnoses had been incorporated into the final pathology tables.

Full details about these and other audit findings are presented in audit reports that are on file at NIEHS.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PRINTED AS OF SEPTEMBER 1989

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-p-dioxin (Dermal)
206	Dibromochloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane (Inhalation)
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butylbenzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-*p*-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
229	Guar Gum
230	Agar
231	Stannous Chloride
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	D-Mannitol
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos
250	Benzyl Acetate
251	Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	Propylene Oxide
269	Telone II®
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four strains of rats)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl)phosphonium Sulfate and Tetrakis(hydroxymethyl)phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
353	2,4-Dichlorophenol
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
361	Hexachloroethane

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