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No. 357

TOXICOLOGY AND CARCINOGENESIS STUDIES OF HYDROCHLOROTHIAZIDE

(CAS NO. 58-93-5)

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 HYDROCHLOROTHIAZIDE

(CAS NO. 58-93-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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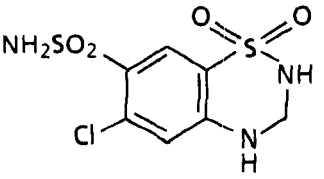

HYDROCHLOROTHIAZIDE

CAS No. 58-93-5

$C_7H_8ClN_3O_4S_2$ Molecular weight 297.7

Synonym: 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

Trade Names: Aquarius; Bremil; Chlorzide; Cidrex; Dichlorosal; Dichlotride; Diclotride; Direma; Disalunil; Esidrex; Esidrix; Fluvin; Hidroronol; Hydril; Hydro-Aquil; Hydro-Diuril; Hydrosaluric; Hydrothide; Hypothiazide; Ivaugan; Jen-Diril; Maschitt; Nefrix; Neo-Codema; Neoflumen; Oretic; Panurin; Ro-Hydrazide; Thiaretic; Thiuretic; Urodiazin; Vetidrex

ABSTRACT

Hydrochlorothiazide is a diuretic active at the distal convoluted tubule and collecting duct. Toxicology and carcinogenesis studies were conducted by feeding diets containing hydrochlorothiazide (USP grade, greater than 98% pure) to groups of F344/N rats and B6C3F$_1$ mice of each sex for 15 days, 13 weeks, 1 year, or 2 years. Additional studies were performed to evaluate teratologic effects in CD® rats and CD®-1 mice. Genetic toxicology studies were performed with Salmonella, Chinese hamster ovary (CHO) cells, mouse lymphoma cells, and Drosophila.

Fifteen-Day and Thirteen-Week Studies: All rats and mice lived to the end of the 15-day studies (dietary concentrations of 0 and 3,125-50,000 ppm). The final mean body weights of all dosed rat groups were 5%-11% lower than those of controls. The final mean body weights of the groups of male mice that received 6,250-50,000 ppm were 10%-14% lower than that of controls. The final mean body weights of dosed and control female mice were similar. Calculi were seen in the urinary bladder of 2/5 male and 2/5 female mice at 50,000 ppm and in 1/5 male and 1/5 female mice at 25,000 ppm.

All rats lived to the end of the first 13-week studies (dietary concentrations of 0 and 3,125-50,000 ppm). Final body weights of dosed rats were 7%-16% lower than those of controls. Mineralization in the kidney was observed in all dosed rats and because of this, additional 13-week studies in rats were conducted at lower dietary concentrations. All rats lived to the end of the second 13-week studies (dietary concentrations of 0 and 250-4,000 ppm). The final mean body weights of all dosed rat groups were 5%-10% lower than those of controls. Renal mineralization was dose related and judged to be minimal to mild at the lowest dose.

In the 13-week studies in mice, 7/10 males and 1/10 females that received 50,000 ppm hydrochlorothiazide died. The final mean body weights of mice that received 50,000 ppm were 11% lower than those of controls for males and females. Calculi were seen in the urinary bladder of mice that received hydrochlorothiazide at 12,500 ppm and above. Nephrosis occurred with dose-related incidences in mice receiving 12,500 ppm and above.

Based on these results, 2-year studies were conducted by feeding diets containing 0, 250, 500, or 2,000 ppm hydrochlorothiazide to groups of 50 male and 50 female rats for 105-106 weeks. Diets containing 0, 2,500, or 5,000 ppm hydrochlorothiazide were fed to groups of 50 male and 50 female mice for 103-104 weeks. Ten additional rats per sex and dose group were placed on study and killed at 1 year for blood-clotting studies and histopathologic examination.

Effects in the One-Year Studies: One of 10 female rats in the 1-year study group that received 2,000 ppm died with internal hemorrhage. In addition, evidence of hemorrhage was found in 11 of the 16 dosed female rats that died during the first year of the 2-year study. Hematologic analyses revealed no compound-related effects; however, activated partial thromboplastin times (APTTs) were highly variable and were lengthened in some dosed male rats. No effects on APTTs were seen for females, and no effects on prothrombin times or on the fibrinogen content of plasma were observed for dosed male or female rats. Nephropathy occurred in dosed and control rats, and the severity was judged to be greater in dosed male and high dose female rats. Increased incidences of mild focal renal mineralization were also seen in mid and high dose male rats and dosed female rats.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were 8%-25% lower than those of controls. Mean body weights of dosed and control mice were similar throughout the studies. No significant differences in survival were observed between rats or mice of either sex (rats--male: control, 18/50; low dose, 16/50; mid dose, 9/50; high dose, 11/50; female: 31/50; 26/50; 30/50; 27/50; mice--male: control, 43/50; low dose, 42/50; high dose, 43/50; female: 38/50; 40/50; 35/50). Survival of all groups of male rats was low because a large number of animals were killed in a moribund condition late in the study. The average daily feed consumption by dosed rats was 89%-94% that by controls. The average amount of hydrochlorothiazide consumed per day was approximately 11, 23, or 89 mg/kg for low, mid, or high dose rats. The average daily feed consumption by dosed mice was 100%-105% that by controls. The average amount of hydrochlorothiazide consumed per day was approximately 280 or 575 mg/kg for low dose or high dose mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy occurred in nearly all male and female rats, but the severity of this disease was greater in dosed rats, as evidenced by increases in renal cysts and epithelial hyperplasia of the renal pelvis in dosed rats shown in the following table. Mineralization was observed at increased incidences in dosed male and dosed female rats.

Changes associated with or secondary to renal injury were increased in dosed rats. These lesions included parathyroid hyperplasia, fibrous osteodystrophy of bone, and mineralization of multiple organs.

NUMBERS OF RATS WITH SELECTED RENAL LESIONS IN THE TWO-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE

Lesion	Male (ppm)				Female (ppm)			
	0	250	500	2,000	0	250	500	2,000
No. examined	50	49	50	50	50	50	49	50
Nephropathy	50	49	50	50	47	42	44	47
Cysts	2	19	21	18	0	3	4	3
Epithelial hyperplasia of the renal pelvis	6	21	26	23	0	4	2	3
Mineralization, kidney or multiple organs	1	19	27	27	10	40	39	40
Tubular cell adenoma	3	1	0	1	0	0	1	1

Adenomas or carcinomas (combined) of the Zymbal gland in male rats occurred in 1/50 control, 1/49 low dose, 2/50 mid dose, and 4/50 high dose animals. The historical incidence of Zymbal gland neoplasms in untreated F344/N male rats is 19/1,936 (1.0%), and the highest observed control group incidence is 4/50. This marginal increase was not considered to be chemically related.

The incidences of fibroadenomas of the mammary gland were decreased in dosed female rats (30/50; 12/50; 11/49; 5/50).

The incidence of hepatocellular neoplasms was increased in high dose male mice (adenomas or carcinomas, combined: control, 7/48; low dose, 10/49; high dose, 21/50). The historical incidence of hepatocellular adenomas or carcinomas (combined) is 609/2,032 (30%) in untreated controls.

Teratology: Hydrochlorothiazide produced no teratologic effects in the offspring of CD® rats or CD®-1 mice after gavage administration to pregnant females on day 6 through day 15 of gestation.

Genetic Toxicology: In the absence of exogenous metabolic activation, hydrochlorothiazide produced an equivocal increase in revertant colonies in *Salmonella typhimurium* strain TA98; no increase was observed in strains TA100, TA1535, or TA1537 with or without activation. Hydrochlorothiazide induced an increase in trifluorothymidine (Tft)-resistant cells in a mouse lymphoma L5178Y/TK$^{+/-}$ assay without exogenous metabolic activation; this assay was not performed with activation. In cultured CHO cells, hydrochlorothiazide induced sister chromatid exchanges (SCEs) in the presence and absence of exogenous metabolic activation but did not induce chromosomal aberrations. Hydrochlorothiazide did not increase the frequency of sex-linked recessive lethal mutations when administered by feeding or injection to adult male *Drosophila melanogaster*.

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

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of hydrochlorothiazide for male or female F344/N rats given feed containing 250, 500, or 2,000 ppm hydrochlorothiazide. There was *equivocal evidence of carcinogenic activity* of hydrochlorothiazide for male B6C3F$_1$ mice, based on increased incidences of hepatocellular neoplasms. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice given diets containing 2,500 or 5,000 ppm hydrochlorothiazide.

Chronic renal disease was more severe in rats administered hydrochlorothiazide, and increased incidences of secondary lesions (parathyroid hyperplasia, fibrous osteodystrophy, and mineralization in multiple organs) occurred in dosed rats.

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

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

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Dietary concentration			
0, 250, 500, or 2,000 ppm hydrochlorothiazide	0, 250, 500, or 2,000 ppm hydrochlorothiazide	0, 2,500, or 5,000 ppm hydrochlorothiazide	0, 2,500, or 5,000 ppm hydrochlorothiazide
Body weights in the 2-year study			
Dosed lower than controls	Dosed lower than controls	Dosed and controls similar	Dosed and controls similar
Survival rates in the 2-year study			
18/50; 16/50; 9/50; 11/50	31/50; 26/50; 30/50; 27/50	43/50; 42/50; 43/50	38/50; 40/50; 35/50
Nonneoplastic effects			
Chronic renal disease and secondary effects	Chronic renal disease and secondary effects	None	None
Neoplastic effects			
None	None	Hepatocellular neoplasms (7/48; 10/49; 21/50)	None
Level of evidence of carcinogenic activity			
No evidence	No evidence	Equivocal evidence	No evidence

Genetic toxicology

Salmonella (gene mutation)	Mouse L5178Y/TK$^{+/-}$ (Tft resistance)	CHO Cells in Vitro		Drosophila	
		SCE	Aberration	Sex-Linked Rec. Lethals	Reciprocal Translocation
Equivocal without S9; negative with S9	Positive without S9; no test with S9	Positive with and without S9	Negative with and without S9	Negative	No test

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 Hydrochlorothiazide is based on 13-week studies that began in April 1980 and ended in July 1980, the second 13-week studies that began in January 1981 and ended in April 1981, and 2-year studies that began in October 1981 and ended in October 1983 at SRI International (Menlo Park, California).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on hydrochlorothiazide on April 18, 1988, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF HYDROCHLOROTHIAZIDE

On April 18, 1988, the draft Technical Report on the toxicology and carcinogenesis studies of hydrochlorothiazide received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina.

Dr. J.R. Bucher, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (equivocal evidence of carcinogenic activity for male rats, no evidence of carcinogenic activity for female rats, some evidence of carcinogenic activity for male mice, no evidence of carcinogenic activity for female mice). Chronic renal disease was more severe in rats administered hydrochlorothiazide, and increased incidences of secondary lesions (parathyroid hyperplasia, fibrous osteodystrophy, and mineralization in multiple organs) occurred in dosed rats.

Dr. Hughes, a principal reviewer, agreed with the conclusions for female rats and mice. He stated that the conclusion for male rats should be changed to no evidence of carcinogenic activity because the incidence of Zymbal gland neoplasms in the high dose group was no greater than the highest incidence in any historical control group. In addition, 2-year studies by Lijinsky and Reuber (1987) showed no increases in neoplastic lesions in male or female rats given hydrochlorothiazide at 1,000 ppm. Dr. J. Huff, NIEHS, mentioned that the dietary concentrations used in those studies were one-half those used in the NTP studies. Dr. Hughes thought that, in view of the high historical control incidence (30%) of hepatocellular neoplasms in male mice, the conclusion should be changed to no evidence of carcinogenic activity. Dr. Bucher commented that there was a strong dose-related trend for the liver neoplasms and that the incidence in the high dose group was highly significant when compared with that in the controls, supporting the staff interpretation.

Dr. Sivak, the second principal reviewer, agreed with the conclusions.

Dr. Chinchilli, the third principal reviewer, agreed with the conclusions for female rats and male and female mice but felt that the conclusion for male rats should be changed to no evidence of carcinogenic activity. If a test had been employed which directly compared the current data with the historical control data and which yielded a more significant P value, he could have supported the conclusion. In response to Dr. Hughes and Dr. Chinchilli, Dr. Bucher agreed that the evidence in male mice was weakened when comparisons were drawn with the historical incidences. All the Zymbal gland neoplasms were observed on gross examination.

In other discussion, Dr. Ashby stated that the data from genotoxicology assays support the consideration that hydrochlorothiazide is nongenotoxic. Pertaining to the liver neoplasms in male mice, there was further discussion about the relative weight given to concurrent vs. historical control incidences. Dr. J. Haseman, NIEHS, stated that the NTP position is that the most important comparison is with the concurrent controls, whereas comparisons with historical controls are most useful for rare tumors and where there is a marginal increase, in helping to judge the appropriate level. With regard to the current study, he noted that the concurrent control incidence of liver neoplasms in male mice (7/48) was low relative to the historical control values but that the incidence in the high dose group (21/50) exceeds all but one of the incidences in the historical control data base. Dr. Hughes commented that he would have more confidence in the association of tumors with chemical administration if there were an adequate historical data base for the laboratory performing the hydrochlorothiazide studies. Dr. Huff indicated that the mean incidences in the two contemporary studies were not different from the incidence in the hydrochlorothiazide controls.

Dr. Hughes moved that the conclusion for male rats be changed to no evidence of carcinogenic activity, based on the incidence of Zymbal gland tumors in dosed rats being no greater than the top incidence in historical controls. Dr. Sivak seconded the motion, which was approved by five members (Dr. Capen, Dr. Gallo, Dr. Hughes, Dr. Popp, and Dr. Sivak), with three dissents (Dr. Chinchilli, Dr. Hooper, and Dr. Lijinsky) and one abstention (Dr. Ashby). Dr. Hughes moved that the conclusion for female rats, no evidence of carcinogenic activity, be accepted as written. Dr. Popp seconded the motion, which was approved by eight panelists, with one abstention (Dr. Ashby). Dr. Hughes moved that the conclusion for male mice be changed to equivocal evidence of carcinogenic activity, based on the variability of liver neoplasms in male mice and the lack of adequate historical control data from the study laboratory. Dr. Lijinsky seconded the motion, which was approved by five members (Dr. Capen, Dr. Hughes, Dr. Lijinsky, Dr. Popp, and Dr. Sivak), with three dissents (Dr. Chinchilli, Dr. Gallo, and Dr. Hooper) and one abstention (Dr. Ashby). Dr. Hughes moved that the conclusion for female mice, no evidence of carcinogenic activity, be accepted as written. Dr. Sivak seconded the motion, which was approved by eight panelists, with one abstention (Dr. Ashby).

I. INTRODUCTION

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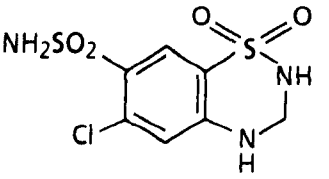

HYDROCHLOROTHIAZIDE

CAS No. 58-93-5

$C_7H_8ClN_3O_4S_2$ Molecular weight 297.7

Synonym: 6-chloro-3,4-dihydro-*2H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

Trade Names: Aquarius; Bremil; Chlorzide; Cidrex; Dichlorosal; Dichlotride; Diclotride; Direma; Disalunil; Esidrex; Esidrix; Fluvin; Hidroronol; Hydril; Hydro-Aquil; Hydro-Diuril; Hydrosaluric; Hydrothide; Hypothiazide; Ivaugan; Jen-Diril; Maschitt; Nefrix; Neo-Codema; Neoflumen; Oretic; Panurin; Ro-Hydrazide; Thiaretic; Thiuretic; Urodiazin; Vetidrex

Physical Properties, Production, and Use

Hydrochlorothiazide is a crystalline material with a melting point of 273°-275° C. It is soluble in dilute ammonia or sodium hydroxide and in methanol, ethanol, or acetone and is essentially insoluble in water (Merck, 1983).

Specific production data for hydrochlorothiazide were not found, but hydrochlorothiazide is available as a prescription drug by itself and in combination with other drugs. The combination of hydrochlorothiazide and triamterene is marketed under the trade name Dyazide. More prescriptions were written for Dyazide than for any other single prescription drug in the United States in 1984 and 1985 (Pharmacy Times, 1986). Hydrochlorothiazide is one of a number of benzothiadiazides that are used primarily as diuretic agents. They were first synthesized during attempts to develop inhibitors of carbonic anhydrase, but their diuretic action appears to be largely independent of this property (Gilman et al., 1985). Hydrochlorothiazide and other thiazide diuretics are used to lessen edema due to mild-to-moderate congestive heart failure or for chronic hepatic or renal disease. They are also widely used in the treatment of hypertension (Whelton, 1986). Effective oral doses in humans are 25-100 mg/day. A 75-mg dose results in a peak plasma concentration of about 375 ng/ml (Beermann and Groschinsky-Grind, 1977; Gilman et al., 1985).

Absorption, Distribution, Metabolism, and Excretion

In humans, about 70% of an orally administered therapeutic-level dose of hydrochlorothiazide is absorbed, primarily from the duodenum and upper jejunum (Beermann et al., 1976). Absorption is linear over the dose range 12.5-75 mg (Beermann, 1984). Absorption after a meal has been reported to be facilitated (Beermann and Groschinsky-Grind, 1978) or decreased (Barbhaiya et al., 1982). Approximately 40% of the absorbed hydrochlorothiazide binds to plasma proteins across the therapeutic dose range, and the drug accumulates in erythrocytes. The ratio of this uptake between blood corpuscles and plasma is about 3.5:1 (Beermann, 1984). Peak plasma levels are attained approximately 2-3 hours after dosing (Beermann and Groschinsky-Grind, 1978). The decline of plasma drug levels fits a two-compartment model with a terminal elimination phase of 9.5 hours. This relatively long half-life provides a fairly constant, long duration of action (12-24 hours) (Rahn, 1983). Renal clearance is over 300 ml/minute, indicating active secretion of the drug by the kidney (Beermann, 1984). Secretion is via the organic anion transport system in the proximal tubules and is

sensitive to inhibition by probenecid (Gilman et al., 1985). Urinary recovery of unchanged hydrochlorothiazide averages about 70% after oral administration and over 90% after intravenous administration. Biliary excretion is not thought to be significant (Beermann et al., 1976). Hydrochlorothiazide apparently does not undergo significant metabolism; however, an unidentified nonrenal excretion mechanism apparently is active in patients with severe renal failure (Welling, 1986). No information was found concerning absorption, distribution, metabolism, or excretion of hydrochlorothiazide in rodents.

Pharmacology

Hydrochlorothiazide increases the loss of sodium, chloride, potassium, and magnesium ions and of water in the urine (Gilman et al., 1985). The excretion of uric acid and calcium ions is decreased relative to that of sodium ions. Thiazide diuretics can increase urinary excretion of sodium ions up to 5%-10% of the filtered load (Rahn, 1983). The primary site of action of hydrochlorothiazide is on the distal convoluted tubule. Additional effects have been reported on resorption in the cortical and/or medullary collecting duct, and minor contributions to the diuretic effect may result from weak carbonic anhydrase inhibitory action in the proximal tubule (Wilson et al., 1983). Urine collected for 5 hours after male Sprague Dawley rats were given oral doses of 30 mg/kg contained a twelvefold increase in sodium ions and a threefold increase in potassium ions over controls (DeFelice et al., 1987). Plasma renin activity also was significantly increased in these animals. In studies of male Long Evans rats administered diets containing 0, 35, or 350 ppm hydrochlorothiazide for up to 10 days, both doses resulted in an initial diuresis and natriuresis (Walter and Shirley, 1986). The natriuresis abated after 1 day, but the diuresis continued throughout the studies. Micropuncture studies suggested that the return of sodium ion excretion to control levels was due to a reduction in the amount of sodium ions remaining in the filtrate at the end of the proximal tubule. This most likely reflected increased proximal tubule absorption of sodium ions and water due to volume depletion. It was suggested that the sustained water loss was due to a reduction in reabsorption of water in the collecting ducts.

The exact mechanism of the diuretic effect of hydrochlorothiazide is not fully understood. Bioelectric studies suggested a direct inhibition of sodium ion reabsorption in the toad bladder (Gilman et al., 1985), and studies of the effects of hydrochlorothiazide on ion movement in the isolated rabbit distal colon suggested specific inhibition of chloride absorption (Ferriola et al., 1986). In micropuncture studies, chloride transport in the medullary collecting duct of the rat kidney was almost completely inhibited by hydrochlorothiazide (Wilson et al., 1983). Garg and Narang (1987) examined the effect of hydrochlorothiazide on Na^+-K^+ ATPase activity in seven separate segments along the nephron of spontaneously hypertensive rats (SHR) and the normotensive control rats (WKY). Hydrochlorothiazide was administered at 15 mg/kg for 7 days by an osmotic minipump. Individual nephron segments then were dissected from the kidney, and ouabain-sensitive Na^+-K^+ ATPase activity was determined. Activity in the WKY strain was decreased by hydrochlorothiazide administration in the distal convoluted tubule and was increased in the cortical collecting duct. Hydrochlorothiazide decreased Na^+-K^+ ATPase activity in all but the proximal straight tubule and medullary collecting duct in the SHR strain.

Other recognized renal effects of hydrochlorothiazide include increased renin release (Griffing et al., 1983) and increased release of kallikrein (Overlack et al., 1982). Renin is an enzyme released from the juxtaglomerular apparatus, which is active in the formation of angiotensin I in the blood stream. Angiotensin I is converted to angiotensin II by angiotensin I-converting enzyme in the vasculature in the lung. One of the actions of angiotensin II is to stimulate secretion of aldosterone from the adrenal cortex. Aldosterone acts on the distal convoluted tubule and collecting duct to promote sodium ion reabsorption and potassium ion secretion (Melby, 1986). This action acts to temper the diuresis induced by hydrochlorothiazide. Prostaglandin biosynthesis is thought to be involved in the increase in renal renin release in response to loop diuretics such as furosemide (Gerber, 1983), but

the renal effects of hydrochlorothiazide do not appear to be mediated by prostaglandins (Williams et al., 1982; Wilson, 1986).

Kallikrein is a protease purportedly released from the kidney and is involved in the formation of vasoactive kinins, including the vasodilator bradykinin. Urinary kallikrein activity was increased in hypertensive volunteers after 2 weeks of daily dosing with 50 mg hydrochlorothiazide followed by 2 weeks of daily dosing with 100 mg hydrochlorothiazide (Overlack et al., 1982). This action may be important in the efficacy of hydrochlorothiazide therapy for hypertension.

Clinical experience in hypertensive patients has shown that hydrochlorothiazide has two major effects. One is an initial decrease in cardiac output due to volume depletion from the diuresis. The second is a fall in peripheral resistance which occurs after a few weeks of therapy. The reasons for this gradual reduction in peripheral resistance remain under investigation (Struyker-Boudier et al., 1983; Wilson, 1986).

Toxicity

Recognized side effects that have been associated with the use of hydrochlorothiazide include hypokalemia with resultant muscle cramps, cardiac arrhythmia, hyperglycemia, and hyperlipidemia (Chrysant et al., 1983). A variety of hypersensitivity reactions have also been reported. Electrolyte imbalances, in particular hypokalemia and hypomagnesemia, may be involved in increased incidences of sudden death in patients with preexisting electrocardiographic abnormalities (Kolata, 1982). Results of the large Multiple Risk Factor Intervention Trial, a 10-year, multicenter study of factors involved in heart disease, indicated that high dose hydrochlorothiazide therapy (100 mg/day) was associated with greater incidences of sudden death in patients with both high blood pressure and electrocardiographic abnormalities. The involvement of hypokalemia and hypomagnesemia in this observation remains a point of controversy (Freis, 1986; Hollifield, 1986; Kuller et al., 1986).

One electrolyte change that occurs with long-term hydrochlorothiazide therapy in humans is increased calcium ion retention; hypercalcemia occasionally results (Christensson et al., 1977). A related finding is an association of hydrochlorothiazide treatment with hyperparathyroidism (Paloyan et al., 1969). It has been suggested that thiazides cause a primary hyperparathyroidism, and the reduced calcium ion excretion and increased potassium ion loss seen with these diuretics may, at least in part, be secondary to increased parathyroid hormone secretion (Pickleman et al., 1969; Klimiuk et al., 1981). Pickleman et al. (1969) gave 20 dogs daily doses of 50-200 mg hydrochlorothiazide for up to 9 months; all dogs administered hydrochlorothiazide had enlarged and hyperactive parathyroid glands.

Thiazide diuretics also induce a transient increase in serum cholesterol and triglyceride levels, raising the possibility that long-term treatment may contribute to atherosclerosis (Flamenbaum, 1983; Ballantyne and Ballantyne, 1983), although the importance of these transient increases has been disputed (Freis, 1986). Hypertensive individuals receiving 50 mg hydrochlorothiazide per day for 4 weeks had increased concentrations of total plasma cholesterol, of high density, low density, and very low density lipoproteins, and of triglycerides. Increased plasma levels of fasting glucose and insulin were also observed (Johnson et al., 1986). Sarva et al. (1985) dosed Syrian golden hamsters daily with 1, 2, or 4 mg/kg hydrochlorothiazide by gavage for 6 months. At 6 months, they observed increased total cholesterol, triglyceride, and high density lipoprotein cholesterol levels. As noted in the Johnson et al. (1986) study, glucose intolerance is a frequently encountered side effect of long-term thiazide therapy (Amery et al., 1978) and may be associated with hypokalemia (Flamenbaum, 1983), but the mechanism for this effect is not understood. Other diuretics have similar effects on glucose tolerance.

Immunologic reactions to hydrochlorothiazide therapy were reported, including cases of severe allergic pneumonitis (Beaudry and Laplante, 1973), a photoallergic dermatitis resembling subacute cutaneous lupus erythematosus (Reed et al., 1985), and several types of hematologic dyscrasias (Swanson and Cook, 1977). Neutropenia was reported in several patients with a

pattern of onset which suggested a toxic depression of the bone marrow. On the other hand, thrombocytopenia also was reported with hydrochlorothiazide therapy and with other thiazides (Nordqvist et al., 1959; Swanson and Cook, 1977) and appears to be immunologically mediated. In one person, a specific IgM antibody was identified as an antiplatelet factor associated with hydrochlorothiazide-induced thrombocytopenia (Eisner and Crowell, 1971).

The LD_{50} of orally administered hydrochlorothiazide to an unspecified strain of mice was 3,080 mg/kg (Barnes and Eltherington, 1965). Lijinsky and Reuber (1987) fed diets containing 0 or 1,000 ppm hydrochlorothiazide to groups of 24 male and 24 female rats for 2 years. The incidence and severity of chronic progressive nephropathy was increased in the dosed rats, as were lesions secondary to chronic renal disease and polyarteritis and mural thrombosis. No increases in neoplastic lesions were seen in dosed rats.

Reproductive Toxicity

Hydrochlorothiazide has been evaluated for use in lessening sodium and water retention in conditions of preeclampsia during pregnancy. Although prophylactic use of hydrochlorothiazide apparently does not prevent preeclampsia, some benefit may be derived when hydrochlorothiazide is administered during the second trimester to pregnant women with underlying hypertension (Welt et al., 1981). Limited epidemiologic evaluations have not demonstrated fetotoxic effects attributed to the use of hydrochlorothiazide during pregnancy (Gjonnaess, 1968; Heinonen et al., 1977). Hydrochlorothiazide is known to cross the placenta and to accumulate in amniotic fluid to levels fivefold higher than those in maternal blood (Beermann et al., 1980). In contrast, breast feeding by a mother on long-term hydrochlorothiazide therapy did not result in detectable levels in her infant's blood (Miller et al., 1982).

When given by subcutaneous injection to two pregnant female Wistar rats at a dose of 250 mg/kg on gestation days 10 and 11, hydrochlorothiazide did not appear to cause fetal malformations. Nine normal pups were born; three were resorbed (Maren and Ellison, 1971). This is the only published report on reproductive effects of hydrochlorothiazide found in the literature. Because of this lack of information, the NTP performed teratologic evaluations of hydrochlorothiazide by administering the drug to pregnant CD® rats and CD®-1 mice on gestational days 6-15. A summary of these studies, which also revealed no teratologic effects, is presented in Appendix H.

Genetic Toxicology

In the *Salmonella typhimurium* gene mutation assay, hydrochlorothiazide demonstrated equivocal mutagenicity in strain TA98 in the absence of S9; no increase in revertant colonies was observed in strains TA100, TA1535, or TA1537 treated with up to 10 mg/plate hydrochlorothiazide with or without S9 from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver (Mortelmans et al., 1986; Table 26). The results of the Salmonella tests reported by other laboratories, using lower or unspecified doses of hydrochlorothiazide, were negative (Minnich et al., 1976; Waskell, 1978; Ishidate et al., 1981). Bignami et al. (1974) reported a significant increase in *p*-fluorophenylalanine (PFP)-resistant colonies of *Aspergillus nidulans* after exposure in a spot test to paper saturated with hydrochlorothiazide (a pharmaceutical preparation); this resistance was attributed to nondisjunctional events induced by chemical exposure. Hydrochlorothiazide did not induce sex-linked recessive lethal mutations in adult male *Drosophila melanogaster* when administered by feeding or injection (Valencia et al., 1985; Table 30). In cytogenetic tests with cultured Chinese hamster ovary cells, hydrochlorothiazide (43-1,300 µg/ml) induced sister chromatid exchanges in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 but did not induce chromosomal aberrations at doses up to 1,000 µg/ml without S9 and 2,600 µg/ml with S9 (Galloway et al., 1987; Tables 28 and 29). Ishidate et al. (1981) also detected no significant increase in chromosomal aberrations in Chinese hamster lung cells exposed at doses up to 500 µg/ml hydrochlorothiazide with and without S9 but did report induction of polyploidy in 12% of the cells.

I. INTRODUCTION

Study Rationale

Hydrochlorothiazide was nominated for study by the Bureau of Drugs, Food and Drug Administration, after a review of available long-term toxicity and carcinogenicity data for drugs used in hypertensive therapy. The absence of adequate animal studies and the potential for hydrochlorothiazide to form *N*-nitroso derivatives (Gold and Mirvish, 1977) formed the basis for the nomination. Two-year dietary studies of hydrochlorothiazide with and without sodium nitrite in F344 rats have recently been reported (Lijinsky and Reuber, 1987). The feed route of administration also was chosen for the present studies because oral exposure is the primary route of administration in humans.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF HYDROCHLOROTHIAZIDE

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

FIFTEEN-DAY STUDIES

FIRST THIRTEEN-WEEK STUDIES

SECOND THIRTEEN-WEEK STUDIES

ONE-YEAR STUDIES

TWO-YEAR STUDIES

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

GENETIC TOXICOLOGY

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF HYDROCHLOROTHIAZIDE

Hydrochlorothiazide (USP grade, unformulated) was obtained in two lots from Ciba Pharmaceutical Company (Summit, New Jersey) (Table 1). The study material was certified by the supplier as USP grade. Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, Missouri). MRI reports on the analyses performed in support of the hydrochlorothiazide studies are on file at NIEHS.

Both lots of the study chemical were identified as hydrochlorothiazide by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Infrared and nuclear magnetic resonance spectra were consistent with the structure and with the spectrum of USP-standard hydrochlorothiazide (representative spectra are presented in Figures 1 and 2); no literature references were found. The ultraviolet/visible spectrum was consistent with the literature spectrum (Siek et al., 1976) and with the spectrum of a USP standard. The material in these lots was a white, fluffy, microcrystalline powder.

Purity of both lots was determined by elemental analysis, Karl Fischer water analysis, titration under nitrogen in *n*-butylamine of the two sulfonamide groups with 0.1 N sodium methoxide dissolved in toluene, thin-layer chromatography, and high-performance liquid chromatography. Thin-layer chromatographic analysis was performed on silica gel plates with visualization at 254 nm, under visible light, and with four consecutive spray reagents: concentrated hydrochloric acid followed by heat at 100° C for 10 minutes (lot no. M3153 only), 1% sodium nitrite in 1% sulfuric acid, 5% aqueous ammonium sulfamate, and 1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 80% acetone. The two solvent systems used included ethyl acetate: toluene (80:20) (system 1) or isopropyl alcohol: concentrated ammonium hydroxide (80:20) (system 2). High-performance liquid chromatography was performed with a μBondapak C_{18} column, detection at 280 nm, and a solvent system of aqueous 1% acetic acid:1% acetic acid in methanol. A solvent ratio of 80:20 was used for lot no. M3153, and ratios of 100:0 (system 1) and 80:20 (system 2) were used for lot no. U-2975.

Cumulative data indicated that lot no. U-2975 was greater than 98% pure and consistent with USP specifications. Results of elemental analysis of carbon, hydrogen, nitrogen, chlorine, and sulfur agreed with the theoretical values. Water content was 0.33%. Titration of the two sulfonamide groups indicated a dry weight purity of 99.1%. Thin-layer chromatography by system 1 indicated one trace and one slight trace impurity; system 2 did not detect any impurities. High-performance liquid chromatography, system 1, detected two impurities with a combined area 0.07% of the major peak area; system 2 detected four impurities with a combined area 1.59% of the major peak area.

Cumulative data indicated that lot no. M3153 was greater than 98% pure and consistent with USP specifications. Results of elemental analysis of carbon, hydrogen, nitrogen, and chlorine

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

Fifteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	One-Year Studies	Two-Year Studies
Lot Numbers				
U-2975	U-2975	U-2975	U-2975	U-2975; M3153
Date of Initial Use				
8/29/79	4/15/80	1/6/81	10/13/81	U-2975--10/9/81; M3153--12/19/82
Supplier				
Ciba Pharmaceutical Co. (Summit, NJ)	Ciba Pharmaceutical Co. (Summit, NJ)	Ciba Pharmaceutical Co. (Summit, NJ)	Ciba Pharmaceutical Co. (Summit, NJ)	Ciba Pharmaceutical Co. (Summit, NJ)

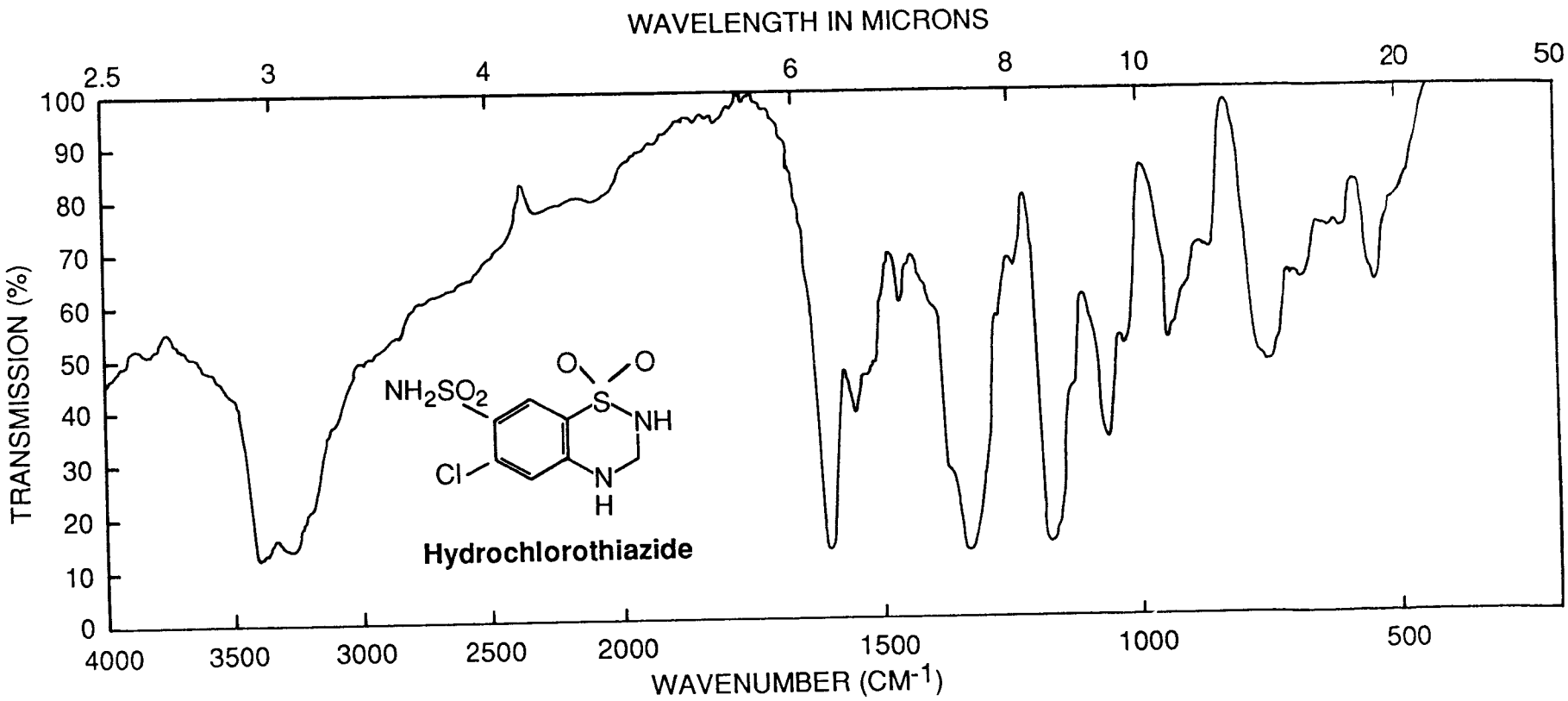

FIGURE 1. INFRARED ABSORPTION SPECTRUM OF HYDROCHLOROTHIAZIDE (LOT NO. U-2975)

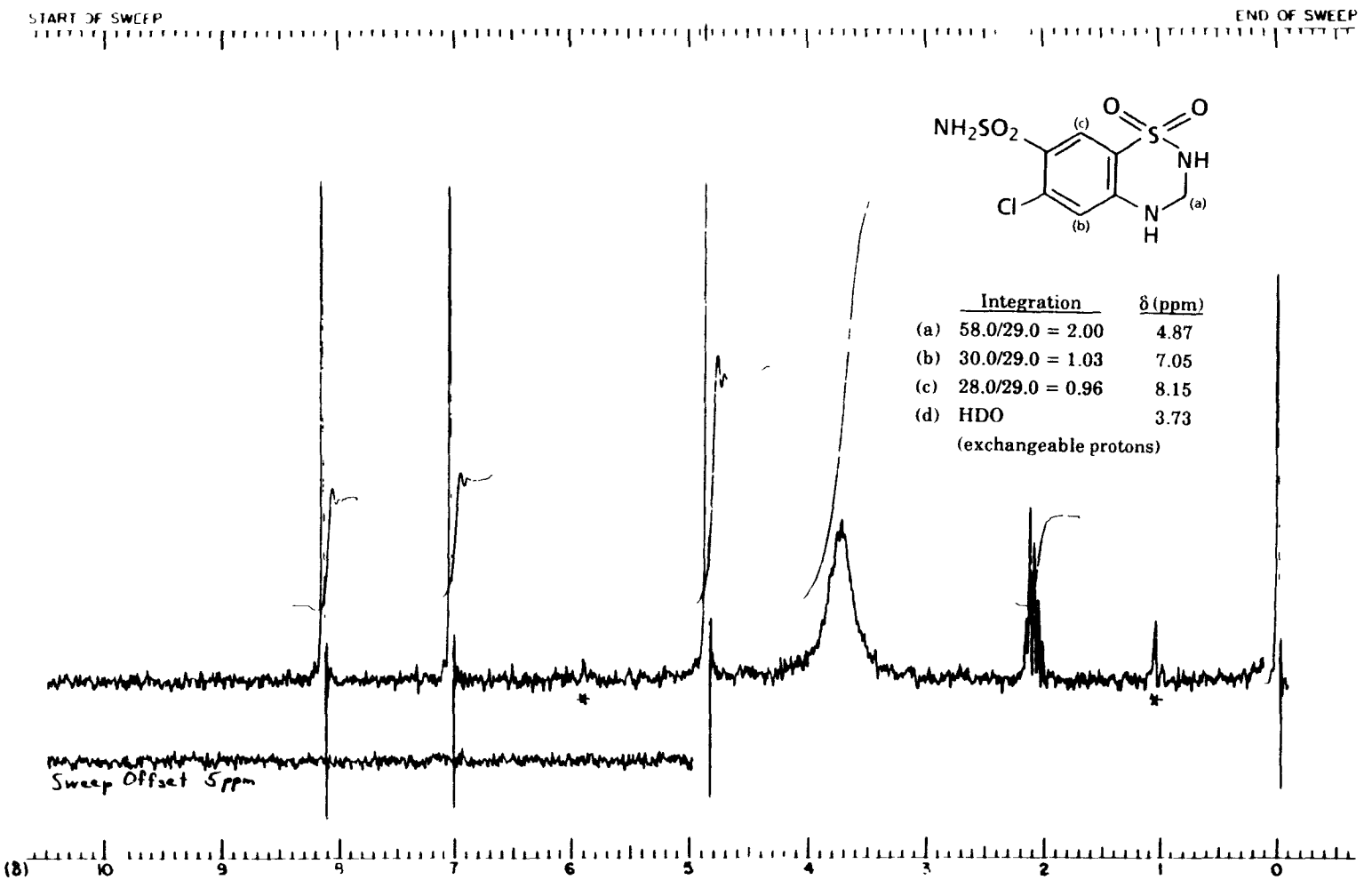

FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF HYDROCHLOROTHIAZIDE (LOT NO. U-2975)

agreed with the theoretical values; sulfur was high. Water content was 0.29%. Titration of the two sulfonamide groups indicated a dry weight purity of 98.4%. Thin-layer chromatography by both systems indicated only the major spot. High-performance liquid chromatography detected one impurity with an area 1.3% of the major peak area.

Major peak comparison of the two lots by high-performance liquid chromatography with a 68:32 solvent ratio indicated that the hydrochlorothiazide content of both lots of the study material was identical.

Stability studies monitored by high-performance liquid chromatography, with the same column as described before but with a 70:30 solvent ratio, indicated that hydrochlorothiazide is stable as the bulk chemical when stored protected from light for at least 2 weeks at temperatures up to 60° C. Periodic reanalysis of the bulk chemical by infrared spectroscopy, high-performance liquid chromatography, titration in *n*-butylamine with sodium methoxide, and other methods such as nuclear magnetic resonance or ultraviolet spectroscopy indicated no notable degradation of the bulk chemical during the studies.

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

Formulated diets were made by preparing a hydrochlorothiazide/feed premix by hand and blending with feed in a twin-shell blender for 15 minutes (Table 2). Formulated diets at a concentration of 1,010 ppm were shown to be stable for 2 weeks in the dark at 5° C, but losses of 6% at 25° C and 23.5% at 45° C occurred. Losses equivalent to 10% of the target concentrations of hydrochlorothiazide in formulated diets after 7 days under animal room conditions were observed at the study laboratory. As a result, feed was replaced twice per week during the 13-week, 1-year, and 2-year studies.

Periodic analysis of formulated diet mixtures of hydrochlorothiazide was conducted at the study laboratory and the analytical chemistry laboratory. Feed samples were extracted with acetonitrile:hydrochloric acid (99:1), and extracts were analyzed by high-performance liquid

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

Fifteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	One-Year Studies	Two-Year Studies
Preparation				
Premix prepared by weighing chemical into a beaker and mixing with an equal amount of feed; additional feed was added until one-third of the feed was used. Premix was mixed with remaining feed in an 8-qt twin-shell V blender for 5 min with the intensifier bar followed by 10 min without the intensifier bar	Same as 15-d studies except either an 8-qt or 16-qt blender used	Same as first 13-wk studies	Same as 15-d studies except a 1-ft^3 blender used	Same as 1-y studies
Maximum Storage Time				
14 d	14 d	14 d	24 d	24 d
Storage Conditions				
4° C or 5° C in the dark	5° C in the dark	5° C in the dark	5° C in the dark	5° C in the dark

chromatography with a Waters RCM-100 C_{18} column, a solvent system of 1% aqueous acetic acid: 1% acetic acid in acetonitrile (75:25), and ultraviolet detection at 280 nm. The method was modified during the studies by using centrifugation to clarify the extract, and the detection wavelength was changed to 265 nm.

Formulated diets were analyzed once during the first 13-week studies and three times during the second 13-week studies. The homogeneity of formulated diet preparations was determined with samples taken from different locations within the blender. The results ranged from 92% to 107% of the target concentrations (Table 3). During the 2-year studies, the formulated diets were analyzed at approximately 8-week intervals. Because 65/67 formulated diets analyzed were within ±10% of the target concentrations, the diets were estimated to have been formulated within specifications 97% of the time throughout the 2-year studies (Table 4). Referee analyses were performed periodically by the analytical chemistry laboratory. Good agreement was generally found between the study and analytical chemistry laboratories (Table 5).

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

Date Mixed	Concentration of Hydrochlorothiazide in Feed (ppm)		Determined as a Percent of Target
	Target	Determined (a)	
04/10/80	3,125	(b) 3,345	107
	3,125	(c) 3,227	103
	3,125	(d) 3,194	102
	50,000	(b) 52,703	105
	50,000	(c) 46,596	93
	50,000	(d) 46,353	93
	3,125	3,305	106
	6,250	6,465	103
	12,500	12,793	102
	25,000	24,350	97
	50,000	53,572	107
12/18/80	1,000	974	97
	2,000	1,916	96
	4,000	(d) 3,806	95
	4,000	(b) 3,823	96
	4,000	(c) 3,960	99
01/05/81	250	241	96
	250	(d) 258	103
	250	(b) 243	97
	500	(c) 524	105
02/18/81	250	256	102
	500	518	104
	1,000	973	97
	2,000	1,837	92
	4,000	3874	97

(a) Results of duplicate analysis
(b) Sample taken from upper left section of blender
(c) Sample taken from upper right section of blender
(d) Sample taken from bottom of blender

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

Date Mixed	Concentration of Hydrochlorothiazide in Feed for Target Concentration (ppm) (a)				
	250	500	2,000	2,500	5,000
10/01/81	239	458	1,847	2,297	(b) 4,241
12/02/81	251	501	1,982	2,430	4,866
02/10/82	231	472	2,132	2,711	5,034
04/07/82	236	448	1,934	2,402	4,644
06/16/82	249	483	(c) 1,792	2,297	4,627
	--	--	(d) 1,922	--	--
08/11/82	(e) 181	473	2,080	2,570	4,860
08/15/82	(f) 258	--	--	--	--
10/06/82	(c) 156	(c) 475	(c) 2,080	(c) 2,610	(c) 4,970
	(d) 232	(d) 519	(d) 1,990	(d) 2,530	(d) 5,070
12/01/82	249	505	1,990	2,360	4,920
01/12/83	233	480	1,880	--	--
02/23/83	241	502	1,960	2,520	5,000
04/06/83	241	478	1,910	--	--
05/04/83	242	508	2,010	--	--
06/15/83	250	504	1,946	2,446	4,721
07/28/83	228	479	2,011	--	--
09/07/83	260	550	2,130	2,696	5,264
Mean (ppm)	238	491	1,982	2,478	4,841
Standard deviation	18.0	25.7	83.3	142.6	275.7
Coefficient of variation (percent)	7.6	5.2	4.2	5.8	5.7
Range (ppm)	181-260	448-550	1,847-2,132	2,297-2,711	4,241-5,264
Number of samples	15	15	15	11	11

(a) Results of duplicate analysis
(b) Out of specifications; used in the studies.
(c) Sample reanalyzed; not included in the mean.
(d) Results of reanalysis; included in the mean.
(e) Out of specifications; not used in the studies.
(f) Remix; not included in the mean.

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

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory (a)	Referee Laboratory (b)
10/01/81	250	239	180
06/16/82	250	249	253
12/01/82	500	505	491
05/04/83	2,000	2,010	2,060

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

II. MATERIALS AND METHODS

FIFTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories. Rats were 6-7 weeks old when placed on study, and mice were 6-8 weeks old. Groups of five males and five females were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm hydrochlorothiazide for 15 consecutive days. Rats and mice were observed twice per day and were weighed on days 0, 7, and 14. Clinical signs were recorded once per day. Feed consumption was measured after 7 and 14 days. A necropsy was performed on all animals. A partial histopathologic examination was performed on animals in the 50,000-ppm groups. Details of animal maintenance and tissues examined are presented in Table 6.

FIRST THIRTEEN-WEEK STUDIES

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

Four-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 observed for 20 days (rats) or 19 days (mice). Animals were distributed to weight classes and assigned to cages and then to groups according to tables of random numbers. Groups of 10 males and 10 females were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm hydrochlorothiazide.

Animals were housed five per cage. Formulated or control diets and water were available ad libitum. Further experimental details are summarized in Table 6.

Animals were observed twice per day; moribund animals were killed. Feed consumption was measured twice per week. 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 except those excessively autolyzed or cannibalized. Tissues and groups examined histologically are listed in Table 6.

SECOND THIRTEEN-WEEK STUDIES

A second set of thirteen-week studies was conducted to assess the cumulative toxic effects of repeated exposure to hydrochlorothiazide at lower doses than were used in the first 13-week studies and to determine the concentrations to be used in the 2-year studies.

Six- to eight-week-old male and female F344/N rats were obtained from Charles River Breeding Laboratories, observed for 25 days, and distributed to weight classes. Animals were assigned to cages and then to groups according to tables of random numbers. Groups of 10 animals of each sex received diets containing 0, 250, 500, 1,000, 2,000, or 4,000 ppm hydrochlorothiazide.

Animals were housed five per cage. Formulated or control diets and water were available ad libitum. Further experimental details are summarized in Table 6.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Histopathologic examinations were performed on control and high dose groups. Liver weights were determined at necropsy. Tissues examined are listed in Table 6.

ONE-YEAR STUDIES

One-year studies were conducted to evaluate toxic effects and to determine if hydrochlorothiazide interfered with blood clotting. Four- to five-week-old male and female F344/N rats were obtained from Charles River Breeding Laboratories, observed for 20 days, and distributed to weight classes. Animals were assigned to cages and then to groups according to tables of random numbers. Groups of 10 animals of each sex received diets containing 0, 250, 500, or 2,000 ppm hydrochlorothiazide for 52 weeks. Animals were housed five per cage. Formulated or control diets and water were available ad libitum. Further experimental details are summarized in Table 6.

All animals were observed once per day and weighed once per week for 13 weeks and once per

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

Fifteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	One-Year Studies	Two-Year Studies
EXPERIMENTAL DESIGN				
Size of Study Groups				
5 males and 5 females of each species	10 males and 10 females of each species	10 male and 10 female rats	10 male and 10 female rats	50 males and 50 females of each species
Doses				
0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm hydrochlorothiazide in feed	Same as 15-d studies	0, 250, 500, 1,000, 2,000, or 4,000 ppm hydrochlorothiazide in feed	0, 250, 500, or 2,000 ppm hydrochlorothiazide in feed	Rats--0, 250, 500, or 2,000 ppm hydrochlorothiazide in feed; mice--0, 2,500, or 5,000 ppm
Date of First Dose				
8/29/79	Rats--4/16/80; mice--4/15/80	1/6/81	10/13/81	Rats--10/13/81; mice--10/9/81
Date of Last Dose				
9/12/79	Rats--7/16/80; mice--7/15/80	4/7/81	10/12/82-10/15/82	Rats--2,000 ppm, 10/18/83; 500 ppm, 10/21/83; 250 ppm, 10/24/83; mice--5,000 ppm, 10/4/83; 2,500 ppm, 10/7/83
Duration of Dosing				
15 consecutive d	13 wk	13 wk	52 wk	Rats--105-106 wk; mice--103-104 wk
Type and Frequency of Observation				
Observed 2 × d; weighed initially and 1 × wk thereafter; feed consumption measured 1 × wk	Observed 2 × d; weighed initially and 1 × wk thereafter; feed consumption measured 2 × wk	Same as first 13-wk studies	Observed 1 × d; weighed initially, 1 × wk for 13 wk, and then 1 × mo; feed consumption measured 1 wk/mo	Same as 1-y studies
Necropsy, Histologic Examinations, and Supplemental Studies				
Necropsy performed on all animals; kidneys and thymus examined histologically for high dose rats and kidneys, liver, pancreas, and spleen examined for high dose mice	Necropsy performed on all animals; the following tissues were examined histologically for animals dying before the scheduled kill, all controls, all high dose rats, all high dose female mice, and 25,000-ppm male mice: adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur or sternebrae or vertebrae including marrow, gallbladder (mice), gross lesions and tissue masses, heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/	Necropsy performed on all animals; the tissues examined histologically for control and high dose groups were the same as in 13-wk studies. Kidneys examined in the 250-, 500-, 1,000-, and 2,000-ppm groups. Liver weights recorded at necropsy	Necropsy and histologic exams performed on all animals; the following tissues were examined: adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands,	Necropsy performed on all animals; the following tissues examined histologically for animals that died before week 90, control and high dose groups, and male rat 250- and 500-ppm groups: same as 1-y studies except that the gallbladder was examined in mice and vertebrae and preputial and clitoral glands were examined in rats only. Epididymis and preputial or clitoral glands were saved after 4/9/82. Adrenal glands, bone, kidneys, liver, mammary gland, nasal cavity, pancreas, parathyroids, pituitary gland, thyroid

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

Fifteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	One-Year Studies	Two-Year Studies
Necropsy, Histologic Examinations, and Supplemental Studies (Continued)				
	uterus, salivary glands, skin, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Kidneys examined in the 3,125-, 6,250-, 12,500-, and 25,000-ppm groups of male rats; urinary bladder examined in the 25,000-ppm groups of rats; bone marrow, kidneys, and urinary bladder examined in the 3,125-, 6,250-, and 12,500-ppm groups of mice and in the 25,000-ppm group of female mice		skin, spleen, sternebrae and vertebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. Blood for hematologic analysis and plasma clotting time and bone marrow smears taken at necropsy	gland, trachea, and uterus examined from 250- and 500-ppm female rat groups; adrenal glands, bone marrow, gross lesions, kidneys, liver, salivary glands, spleen, and testes examined for the 2,500-ppm group of male mice; gross lesions, heart, kidneys, pituitary gland, and uterus examined for the 2,500-ppm group of female mice
ANIMALS AND ANIMAL MAINTENANCE				
Strain and Species				
F344/N rats; $B6C3F_1$ mice	F344/N rats; $B6C3F_1$ mice	F344/N rats	F344/N rats	F3444/N rats; $B6C3F_1$ mice
Animal Source				
Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)
Study Laboratory				
SRI International	SRI International	SRI International	SRI International	SRI International
Method of Animal Identification				
Ear punch	Ear clip	Ear clip	Ear punch	Ear punch
Time Held Before Study				
13 d	Rats--20 d; mice--19 d	25 d	20 d	Rats--20 d; mice--16 d
Age When Placed on Study				
Rats--6-7 wk; mice--6-8 wk	Rats--7 wk; mice--8-9 wk	6-8 wk	7-8 wk	7-8 wk
Age When Killed				
Rats--8-9 wk; mice--8-10 wk	Rats--20 wk; mice--21-22 wk	19-21 wk	59-60 wk	113-114 wk
Necropsy Dates				
9/13/79	Rats--7/17/80; mice--7/16/80	4/8/81	10/12/82-10/15/82	Rats--10/25/83-11/4/83; mice--10/11/83-10/21/83
Method of Animal Distribution				
Assigned from weight classes to groups according to a table of random numbers	Animals distributed to weight classes and then assigned to cages by one table of random numbers and to groups by another table of random numbers	Same as first 13-wk studies	Same as first 13-wk studies	Same as first 13-wk studies

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

Fifteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	One-Year Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)				
Feed				
Rodent Laboratory Chow 5001® (Ralston® Purina Co., St. Louis, MO); available ad libitum	NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 15-d studies	Same as first 13-wk studies	Same as first 13-wk studies
Bedding				
Hardwood chips (Pressed Wood, Inc.)	Ab-Sorb-Dri (Lab Products, Inc., Rochelle Park, NJ)	Same as first 13-wk studies	Same as first 13-wk studies	Same as first 13-wk studies
Water				
Automatic watering system (Systems Engineering, Napa, CA, and SRI International, Menlo Park, CA); deionized, filtered, UV-sterilized city water available ad libitum	Same as 15-d studies	Same as 15-d studies	Same as 15-d studies	Same as 15-d studies
Cages				
Drawer-type polycarbonate (Lab Products, Inc., Rochelle Park, NJ)	Same as 15-d studies	Same as 15-d studies	Same as 15-d studies	Same as 15-d studies
Cage Filters				
Nonwoven polyester (Lab Products, Rochelle Park, NJ, or Research Equipment Co., Bryan, TX)	Nonwoven polyester (Lab Products, Rochelle Park, NJ)	Same as first 13-wk studies	Nonwoven fiber filters (Snow Filtration, Cincinnati, OH)	Same as 1-y studies
Animals per Cage				
5	5	5	5	5
Other Chemicals on Study in the Same Room				
None	None	None	None	None
Animal Room Environment				
Temp--68°-78.8° F; hum--50%-65%; fluorescent light 12 h/d; 12-15 room air changes/h	Temp--70°-76° F; hum--41%-72%; fluorescent light 12 h/d; 13 room air changes/h	Temp--72°-76° F; hum--36%-65%; fluorescent light 12 h/d; 13 room air changes/h	Temp--mean 73.6° F, range 64°-78° F; hum--mean, 50.8% (range, 20%-82%); fluorescent light 12 h/d; 11-17 room air changes/h	Rats--temp: mean, 73.7° F (range, 64°-80° F); hum: mean, 52.0% (range, 20%-89%); fluorescent light 12 h/d; 11-17 room air changes/h; mice--temp: mean, 74.2° F (range, 66°-80° F); hum: mean, 53.9% (range, 20%-93%); fluorescent light 12 h/d; 13.5-18 room air changes/h

month thereafter. Clinical observations were recorded once per week for the first 13 weeks of the studies and once per month thereafter. Feed consumption was measured once per month. Animals surviving to the end of the studies were humanely killed. A necropsy and histologic examination were performed on all animals. Tissues examined are listed in Table 6.

Blood was collected from the heart of rats before death and examined for differential blood count and erythrocyte morphology. Plasma for clotting studies was obtained from cardiac blood. Bone marrow was removed from the femur within 10 minutes of killing the animal. Smears were prepared, fixed, and then stained with Wright's and Giemsa stains.

Plasma for clotting studies was frozen in a dry ice-methanol bath and mailed frozen to the NTP. The samples were thawed and analyzed for prothrombin time, activated partial thromboplastin time, and fibrinogen content according to methods described in Appendix I.

TWO-YEAR STUDIES

Study Design

Diets containing 0, 250, 500, or 2,000 ppm hydrochlorothiazide were fed to groups of 50 male and 50 female rats for 105-106 weeks. Diets containing 0, 2,500, or 5,000 ppm hydrochlorothiazide were fed to groups of 50 male and 50 female mice for 103-104 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 under a contract to the Carcinogenesis Program. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats were shipped to the study laboratory at 4-5 weeks of age and mice at 5-6 weeks of age. Rats were quarantined at the study laboratory for 20 days and mice for 16 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 7-8 weeks of age. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix E).

Animal Maintenance

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

Clinical Examinations and Pathology

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

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examination of tissues of female rats and of mice of each sex was performed according to the "inverse pyramid" design (McConnell, 1983a,b). That is, complete histopathologic examinations (Table 6) were performed on all high dose and control animals and on lower dose animals dying through month 21 of the study. In addition, histopathologic examinations were performed on all grossly visible lesions in all dose groups. Potential target organs for chemically related neoplastic and nonneoplastic effects were identified from the short-term studies or the literature and were determined by examination of the

pathology data; these target organs/tissues in the lower dose groups were examined histopathologically. If mortality in the highest dose group exceeded that in the control group by 15%, complete histopathologic examinations were performed on all animals in the second highest dose group in addition to those in the high dose group.

When the pathology evaluation was completed, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

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

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

Statistical Methods

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

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

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

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data: life table tests, incidental tumor analysis,

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

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

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

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

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

GENETIC TOXICOLOGY

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

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

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

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

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

All doses within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 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 a "equivocal" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

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

Chinese Hamster Ovary Cytogenetics Assays: Testing was performed as reported by Galloway et al. (1985, 1987) and is described briefly below. Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, Texas). Chemicals were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine (BrdU)-substituted

DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of the study chemical; the high dose was limited by toxicity or solubility but did not exceed 5 mg/ml.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing the study chemical was removed and replaced with fresh medium plus BrdU and colcemid, and incubation was continued for 2 more hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no study chemical; incubation proceeded for an additional 26 hours, with colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

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

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

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 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.

Drosophila Protocol: The assays for gene mutation and chromosomal translocation induction were performed as described by Valencia et al. (1985). Study chemicals were supplied as coded aliquots from Radian Corporation (Austin, Texas). Initially, study chemicals were assayed in the sex-linked recessive lethal (SLRL) test by feeding to adult Canton-S wild-type males that were no more than 24 hours old. If no response was obtained, the chemical was retested by injection into adult males. If either route of administration produced a positive result, the chemical was assayed for induction of reciprocal translocations (RTs) by using the same method of exposure. If, because of the physical nature of the chemical, feeding experiments were not possible, injection was selected as the method of study chemical administration, and a positive result was followed by an RT test.

Toxicity tests attempted to set concentrations of study chemical at a level that would produce

30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL test, exposure by feeding was done by allowing Canton-S males (10-20 flies per vial) to feed for 72 hours on a solution of the study chemical in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were given a solution of the chemical dissolved in 0.7% saline or peanut oil and allowed 24 hours to recover. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days; sample sperm from successive matings were treated as successively earlier postmeiotic stages. F_1 heterozygous females were allowed to mate with their siblings and then were placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. At least two experiments were performed for each study chemical, resulting in the testing of some 5,000 treated and 5,000 control chromosomes. The only exceptions occurred when the results of the first experiment were clearly positive (induced frequency of recessive lethal mutations equal to or greater than 1%); then, the second trial was run.

Recessive lethal data were analyzed by the normal test (Margolin et al., 1983). A test result was considered to be positive if the P value was less than 0.01 and the mutation frequency in the tested group was greater than 0.10% or if the P value was less than 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or (b) the frequency in the treatment group was greater than 0.10% but the P value was between 0.10 and 0.05. A result was considered to be negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

For the RT test, the exposure regimen was the same as that for the SLRL test except that small mass matings were used (10 males and 20 females). Exposed males were mated to bw;st or bw;e females for 3 days and discarded. The females were transferred to fresh medium every 3-4 days for a period of about 3 weeks to produce a total of six broods. The results of the SLRL test were used to narrow the germ-cell stage most likely to be affected by the chemical; for example, if earlier germ-cell stages seemed to exhibit increased sensitivity, mating of the males was continued and translocation tests were carried out from the offspring derived from these earlier germ cell stages. F_1 males were mated individually to bw;st females and the progeny were examined for missing classes, which indicate the occurrence of a translocation in the parental male. Suspected RTs were retested. The translocation data were analyzed according to the conditional binomial (Kastenbaum and Bowman, 1970).

III. RESULTS

RATS

FIFTEEN-DAY STUDIES

FIRST THIRTEEN-WEEK STUDIES

SECOND THIRTEEN-WEEK STUDIES

ONE-YEAR STUDIES

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

MICE

FIFTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

GENETIC TOXICOLOGY

III. RESULTS: RATS

FIFTEEN-DAY STUDIES

All rats lived to the end of the studies (Table 7). Body weight gains of male rats receiving diets containing 25,000 or 50,000 ppm hydrochlorothiazide were lower than that of controls; body weight gains of females did not appear to be related to compound exposure. Feed consumption by rats administered 25,000 or 50,000 ppm during week 1 was lower than that by controls. Thymic hemorrhage of slight-to-moderate severity was observed grossly in dosed rats (3/5 males and 2/5 females at 50,000 ppm; 2/5 males and 1/5 females at 25,000 ppm; 1/5 males and 1/5 females at 12,500 ppm).

TABLE 7. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FIFTEEN-DAY FEED STUDIES OF HYDROCHLOROTHIAZIDE

Concentration (ppm)	Survival (a)	Mean Body Weight (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 1	Week 2
MALE							
0	5/5	151 ± 5	198 ± 9	+47 ± 9		13.4	14.9
3,125	5/5	143 ± 2	188 ± 4	+45 ± 4	95	13.8	14.1
6,250	5/5	141 ± 7	186 ± 6	+45 ± 2	94	13.2	14.4
12,500	5/5	136 ± 9	182 ± 5	+46 ± 7	92	13.8	12.7
25,000	5/5	145 ± 2	182 ± 5	+37 ± 3	92	11.9	15.3
50,000	5/5	(e) 139	180 ± 5	+41	91	8.5	15.5
FEMALE							
0	5/5	117 ± 3	140 ± 4	+23 ± 2		8.7	11.0
3,125	5/5	107 ± 2	130 ± 2	+23 ± 1	93	9.4	10.4
6,250	5/5	114 ± 5	124 ± 4	+10 ± 3	89	8.2	7.7
12,500	5/5	111 ± 3	132 ± 2	+21 ± 3	94	8.6	10.6
25,000	5/5	110 ± 1	129 ± 2	+19 ± 2	92	8.7	11.1
50,000	5/5	106 ± 3	128 ± 1	+22 ± 2	91	8.7	10.9

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

FIRST THIRTEEN-WEEK STUDIES

All rats lived to the end of the studies (Table 8). The final mean body weights of dosed rats were 7%-16% lower than those of the controls. Feed consumption was similar for dosed and control rats. No compound-related clinical signs were observed, and there was no evidence of wet bedding. Mineralization in the kidney was observed in all dosed rats; this was most prominent at the corticomedullary junction. The severity was dose related. Tubular cell degeneration and necrosis were observed in one male and five females in the 50,000-ppm groups.

White crystals were found in the stomach of three males in the 6,250-ppm group and in one male in the 25,000-ppm group. The urinary bladder of one male in the 25,000-ppm group contained calculi. Similar calculi found in mice were determined to be relatively pure hydrochlorothiazide.

Because a no-effect level for mineralization was not achieved, additional 13-week studies were conducted at lower dietary concentrations.

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

Concentration (ppm)	Survival (a)	Mean Body Weight (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 7	Week 13
MALE							
0	10/10	150 ± 2	347 ± 5	+197 ± 5		17	16
3,125	10/10	158 ± 3	306 ± 6	+148 ± 4	88	16	15
6,250	10/10	154 ± 3	303 ± 4	+149 ± 3	87	15	14
12,500	10/10	155 ± 4	303 ± 4	+148 ± 3	87	15	15
25,000	10/10	147 ± 3	295 ± 9	+148 ± 7	85	15	14
50,000	10/10	153 ± 3	293 ± 3	+140 ± 3	84	15	14
FEMALE							
0	10/10	115 ± 2	193 ± 2	+78 ± 2		9	(e) 6
3,125	10/10	113 ± 2	178 ± 2	+65 ± 2	92	8	9
6,250	10/10	117 ± 2	180 ± 4	+63 ± 2	93	10	8
12,500	10/10	116 ± 2	175 ± 2	+59 ± 1	91	9	9
25,000	10/10	114 ± 2	169 ± 2	+55 ± 3	88	10	9
50,000	10/10	115 ± 3	172 ± 4	+57 ± 2	89	9	8

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.
(e) Bedding material mixed with feed; actual feed consumption probably greater.

SECOND THIRTEEN-WEEK STUDIES

All rats lived to the end of the studies (Table 9). The final mean body weights of all dosed groups were 5%-10% lower than those of the controls. Feed consumption by dosed and control rats was similar. No clinical signs were considered chemically related. Liver weight to body weight ratios for dosed and control rats were comparable (Table 10). Mineralization in the kidney was seen in 4/10 males and 5/10 females at 250 ppm, 8/10 males and 8/10 females at 500 ppm, and 10/10 males and 10/10 females at 1,000, 2,000, and 4,000 ppm. The severity increased as the dose increased. At the lowest dose, the severity was minimal to mild in rats of each sex.

Dose Selection Rationale: Because of the incidence and severity of mineralization in the kidney, dietary concentrations selected for rats for the 1-year and 2-year studies were 250, 500, and 2,000 ppm hydrochlorothiazide. Three doses were selected because it could not be predicted if the mineralization seen at 250 and 500 ppm would exacerbate the nephropathy that occurs in aging F344 rats. If the mineralization in the kidney was not life threatening, the higher dose (2,000 ppm) was considered appropriate.

TABLE 9. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE SECOND THIRTEEN-WEEK FEED STUDIES OF HYDROCHLOROTHIAZIDE

Concentration (ppm)	Survival (a)	Mean Body Weight (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 7	Week 13
MALE							
0	10/10	164 ± 4	359 ± 5	+195 ± 4		17	18
250	10/10	165 ± 3	335 ± 5	+170 ± 4	93	17	17
500	10/10	162 ± 2	322 ± 6	+160 ± 6	90	16	16
1,000	10/10	163 ± 3	330 ± 7	+167 ± 6	92	17	16
2,000	10/10	161 ± 3	327 ± 4	+166 ± 4	91	17	16
4,000	10/10	166 ± 3	340 ± 6	+174 ± 5	95	17	16
FEMALE							
0	10/10	127 ± 1	210 ± 2	+83 ± 2		12	12
250	10/10	128 ± 2	197 ± 2	+69 ± 1	94	12	11
500	10/10	127 ± 2	194 ± 2	+67 ± 2	92	12	11
1,000	10/10	126 ± 2	191 ± 2	+65 ± 2	91	12	10
2,000	10/10	125 ± 2	191 ± 2	+66 ± 2	91	11	11
4,000	10/10	126 ± 2	190 ± 2	+64 ± 2	90	11	11

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

TABLE 10. ANALYSIS OF LIVER WEIGHTS OF RATS IN THE SECOND THIRTEEN-WEEK FEED STUDIES OF HYDROCHLOROTHIAZIDE (a)

Concentration (ppm)	Number Weighed	Necropsy Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Necropsy Body Weight (mg/g)
MALE				
0	10	349 ± 5.2	10,313 ± 264	29.6 ± 0.54
250	10	(b) 323 ± 5.2	9,810 ± 208	30.3 ± 0.40
500	10	(b) 310 ± 5.3	9,219 ± 349	29.7 ± 0.79
1,000	10	(b) 316 ± 7.1	9,436 ± 384	29.9 ± 0.89
2,000	10	(b) 309 ± 3.1	(c) 9,090 ± 161	29.4 ± 0.44
4,000	10	(b) 323 ± 5.9	10,058 ± 339	31.1 ± 0.74
FEMALE				
0	10	200 ± 2.1	6,878 ± 228	34.3 ± 1.14
250	10	(b) 186 ± 1.7	(b) 5,751 ± 213	31.0 ± 1.12
500	10	(b) 185 ± 2.3	(b) 5,624 ± 215	30.5 ± 1.16
1,000	10	(b) 181 ± 1.8	(c) 5,894 ± 278	32.6 ± 1.41
2,000	10	(b) 177 ± 2.0	(b) 5,323 ± 234	30.0 ± 1.20
4,000	10	(b) 177 ± 2.0	(b) 5,636 ± 256	31.9 ± 1.34

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

ONE-YEAR STUDIES

One of 10 females that received 2,000 ppm died before the end of the studies. This animal showed evidence of hemorrhage in the stomach, duodenum, and pleural cavity and around the nose and mouth. Nephropathy occurred in dosed and control rats, but the average severity of this spontaneous disease was judged to be greater in dosed male and high dose female rats. Increased incidences of mild focal renal mineralization were seen in mid and high dose male rats and dosed female rats. No compound-related hematologic effects were observed (Table 11).

In addition to the 1 female rat that died in the 1-year study group, 16 dosed female rats in the 2-year study groups died by week 52. No control female rats died during this period. Of these 16 rats, 11 showed evidence of internal hemorrhage. Because of this, blood-clotting tests were performed on the 1-year study animals (Table 12). Measures of prothrombin times and fibrinogen content appeared similar to control values in dosed male and female rats. Activated partial thromboplastin times were variable and appeared prolonged for certain dosed male rats and were unchanged for dosed female rats. No statistically significant changes in activated partial thromboplastin times were noted.

TABLE 11. ANALYSIS OF HEMATOLOGIC DATA FOR RATS IN THE ONE-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE (a)

	Concentration (ppm)			
	0	250	500	2,000
MALE				
Number examined	9	9	9	8
Erythrocytes (10^6/mm^3)	7.94 ± 0.062	8.07 ± 0.096	8.02 ± 0.083	7.93 ± 0.168
Hemoglobin (g/dl)	14.4 ± 0.20	14.6 ± 0.22	14.7 ± 0.22	14.5 ± 0.29
Hematocrit (percent)	40.6 ± 0.24	41.2 ± 0.52	41.4 ± 0.44	41.4 ± 0.707
Mean corpuscular volume (μ^3)	51.4 ± 0.47	51.2 ± 0.15	51.9 ± 0.26	52.4 ± 0.33
Mean corpuscular hemoglobin (pg)	18.3 ± 0.24	18.2 ± 0.15	18.2 ± 0.15	18.1 ± 0.12
Mean corpuscular hemoglobin concentration (percent)	35.3 ± 0.37	35.6 ± 0.18	35.6 ± 0.24	35.0 ± 0.19
Platelets (1,000/mm^3)	472 ± 64.7	534 ± 26.2	521 ± 31.5	458 ± 28.9
Leukocytes (1,000/mm^3)	2.9 ± 0.43	2.8 ± 0.29	3.8 ± 0.60	3.5 ± 0.46
Segmented neutrophils (percent)	28.1 ± 3.01	20.7 ± 1.79	29.9 ± 2.75	21.9 ± 2.99
Bands (percent)	0.2 ± 0.15	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.12
Lymphocytes (percent)	69.7 ± 2.98	76.9 ± 1.80	66.8 ± 3.20	75.0 ± 3.18
Monocytes (percent)	1.6 ± 0.34	1.7 ± 0.41	2.4 ± 0.65	2.1 ± 0.45
Eosinophils (percent)	0.4 ± 0.34	0.8 ± 0.32	0.9 ± 0.39	1.0 ± 0.25
FEMALE				
Number examined (b)	10	9	7	6
Erythrocytes (10^6/mm^3)	7.07 ± 0.260	7.65 ± 0.098	7.32 ± 0.091	7.46 ± 0.168
Hemoglobin (g/dl)	13.9 ± 0.44	14.7 ± 0.17	14.5 ± 0.12	14.6 ± 0.29
Hematocrit (percent)	38.8 ± 1.50	41.4 ± 0.40	40.3 ± 0.46	41.3 ± 0.96
Mean corpuscular volume (μ^3)	54.9 ± 0.18	54.3 ± 0.58	55.3 ± 0.29	55.3 ± 0.21
Mean corpuscular hemoglobin (pg)	19.9 ± 0.18	(c) 19.1 ± 0.26	19.9 ± 0.14	19.7 ± 0.21
Mean corpuscular hemoglobin concentration (percent)	36.1 ± 0.35	35.4 ± 0.18	36.1 ± 0.26	35.3 ± 0.21
Platelets (1,000/mm^3)	(d) 436 ± 56.3	533 ± 34.8	332 ± 86.3	385 ± 53.7
Leukocytes (1,000/mm^3)	1.9 ± 0.13	2.1 ± 0.29	(e) 2.2 ± 0.27	2.2 ± 0.15
Segmented neutrophils (percent)	17.4 ± 2.01	23.0 ± 1.44	21.3 ± 3.98	21.2 ± 2.37
Bands (percent)	0.0 ± 0.00	0.1 ± 0.11	0.1 ± 0.14	0.2 ± 0.17
Lymphocytes (percent)	79.7 ± 2.26	74.4 ± 1.92	76.6 ± 4.19	76.3 ± 2.75
Monocytes (percent)	1.3 ± 0.45	1.9 ± 0.61	1.9 ± 0.40	1.5 ± 0.72
Eosinophils (percent)	1.5 ± 0.43	0.7 ± 0.24	(c) 0.1 ± 0.14	0.8 ± 0.31

(a) Mean ± standard error; P values vs. the controls by Dunnett's test (Dunnett, 1955).
(b) Except as noted
(c) $P<0.05$
(d) Nine animals were examined.
(e) One value recorded as <1 but used as 1.0 for statistical calculations

TABLE 12. ANALYSIS OF BLOOD CLOTTING TESTS FOR RATS IN THE ONE-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE (a)

Concentration (ppm)	Number Examined	Prothrombin Time (seconds)	Activated Partial Thromboplastin Time (seconds)	Fibrinogen Concentration (mg/dl)
MALE				
0	10	15.1 ± 0.13	30.5 ± 1.18	215 ± 5.3
250	8	15.4 ± 0.19	51.7 ± 9.91	222 ± 8.1
500	7	15.5 ± 0.26	51.1 ± 7.91	222 ± 4.6
2,000	6	15.5 ± 0.30	44.2 ± 10.60	218 ± 12.9
FEMALE				
0	8	14.1 ± 0.19	36.5 ± 3.05	159 ± 3.0
250	6	13.2 ± 0.31	40.4 ± 5.44	147 ± 6.1
500	6	14.2 ± 0.25	41.9 ± 5.04	164 ± 6.7
2,000	5	14.3 ± 0.54	33.2 ± 5.30	171 ± 8.0

(a) Mean ± standard error; no significant differences vs. the controls by Dunnett's test (Dunnett, 1955).

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of dosed male rats were 9%-21% lower than those of controls after week 1 (Table 13 and Figure 3). Mean body weights of dosed female rats were 8%-25% lower than those of controls after week 3. The average daily feed consumption per rat by low, mid, and high dose rats was 89%, 92%, or 90% that by the controls for males and 92%, 94%, or 93% for females (Tables F1 and F2). The average amount of hydrochlorothiazide consumed per day was approximately 10, 21, or 82 mg/kg for low, mid, or high dose male rats and 12, 24, or 96 mg/kg for low, mid, or high dose female rats. No compound-related clinical signs were observed.

TABLE 13. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE

Weeks on Study	Control		250 ppm			500 ppm			2,000 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE											
0	178	50	179	101	50	178	100	50	177	99	50
1	215	50	196	91	50	194	90	50	190	88	50
2	245	50	219	89	50	217	89	50	212	87	50
3	270	50	241	89	50	240	89	50	238	88	50
4	289	50	260	90	50	259	90	50	257	89	50
5	305	50	273	90	50	271	89	50	269	88	50
6	319	50	284	89	50	282	88	50	281	88	50
7	333	50	296	89	50	293	88	50	293	88	50
8	345	50	307	89	50	303	88	50	304	88	50
9	354	50	315	89	50	313	88	50	315	89	50
10	363	50	323	89	50	320	88	50	322	89	50
11	373	50	331	89	50	326	87	50	330	88	50
12	377	50	333	88	50	328	86	50	331	88	50
13	384	50	339	88	50	334	87	50	337	88	50
15	398	50	350	88	50	342	86	50	347	87	50
19	419	50	367	88	50	362	86	50	363	87	50
23	437	49	377	86	50	371	85	50	374	86	50
27	452	49	394	87	50	388	86	49	390	86	50
31	467	49	405	87	50	401	86	49	404	87	50
36	476	49	414	87	50	408	86	49	413	87	50
39	483	49	419	87	50	414	86	49	417	86	50
44	490	49	426	87	50	421	86	49	424	87	50
49	500	49	434	87	50	431	86	49	433	87	50
53	507	49	447	88	50	443	87	49	444	88	50
57	513	49	450	88	50	445	87	49	442	86	50
61	516	49	453	88	50	448	87	49	453	88	50
67	516	48	451	87	50	446	86	49	454	88	49
71	518	47	435	84	50	442	85	49	452	87	47
75	514	46	448	87	47	440	86	47	451	88	45
79	508	46	440	87	44	432	85	47	440	87	44
83	503	43	429	85	43	423	84	46	437	87	42
87	496	38	421	85	40	416	84	40	423	85	41
91	482	35	414	86	37	418	87	31	398	83	39
96	467	31	393	84	31	390	84	24	388	83	30
99	461	26	379	82	27	383	83	19	378	82	28
103	458	21	360	79	23	381	83	13	370	81	17
FEMALE											
0	135	50	133	99	50	133	99	50	134	99	50
1	150	50	140	93	50	138	92	50	138	92	50
2	161	50	149	93	50	148	92	50	146	91	50
3	173	50	160	92	50	160	92	50	156	90	50
4	182	50	167	92	50	168	92	50	166	91	50
5	189	50	173	92	50	174	92	50	172	91	50
6	195	50	177	91	50	179	92	50	176	90	50
7	201	50	182	91	50	183	91	50	181	90	50
8	205	50	187	91	50	187	91	50	186	91	50
9	209	50	190	91	50	192	92	50	191	91	50
10	212	50	193	91	50	195	92	50	192	91	50
11	216	50	195	90	50	197	91	50	197	91	50
12	216	50	193	89	50	195	90	50	195	90	50
13	219	50	196	89	50	198	90	50	196	89	50
15	226	50	199	88	50	200	88	50	199	88	50
19	232	50	207	89	50	207	89	50	205	88	50
23	238	50	208	87	50	208	87	48	206	87	50
27	247	50	216	87	49	219	89	48	215	87	49
31	256	50	221	86	49	224	88	48	223	87	48
36	264	50	225	85	47	227	86	48	224	85	48
39	270	50	228	84	47	229	85	47	227	84	48
44	277	50	231	83	45	235	85	47	233	84	47
49	290	50	240	83	43	242	83	47	240	83	47
53	299	50	249	83	42	251	84	47	250	84	45
57	314	50	252	80	41	258	82	47	253	81	45
61	326	50	265	81	41	261	80	47	262	80	45
67	338	50	277	82	41	282	83	46	275	81	44
71	347	50	288	83	41	290	84	46	286	82	44
75	356	49	300	84	41	300	84	44	296	83	44
79	364	49	303	83	41	310	85	43	300	82	44
83	366	49	307	84	41	307	84	43	306	84	42
87	365	48	304	83	39	305	84	43	303	83	42
91	365	46	295	81	38	303	83	40	299	82	40
96	366	39	297	81	32	282	77	38	289	79	35
99	365	37	290	79	30	283	78	35	287	79	34
103	366	33	277	76	29	275	75	32	275	75	31

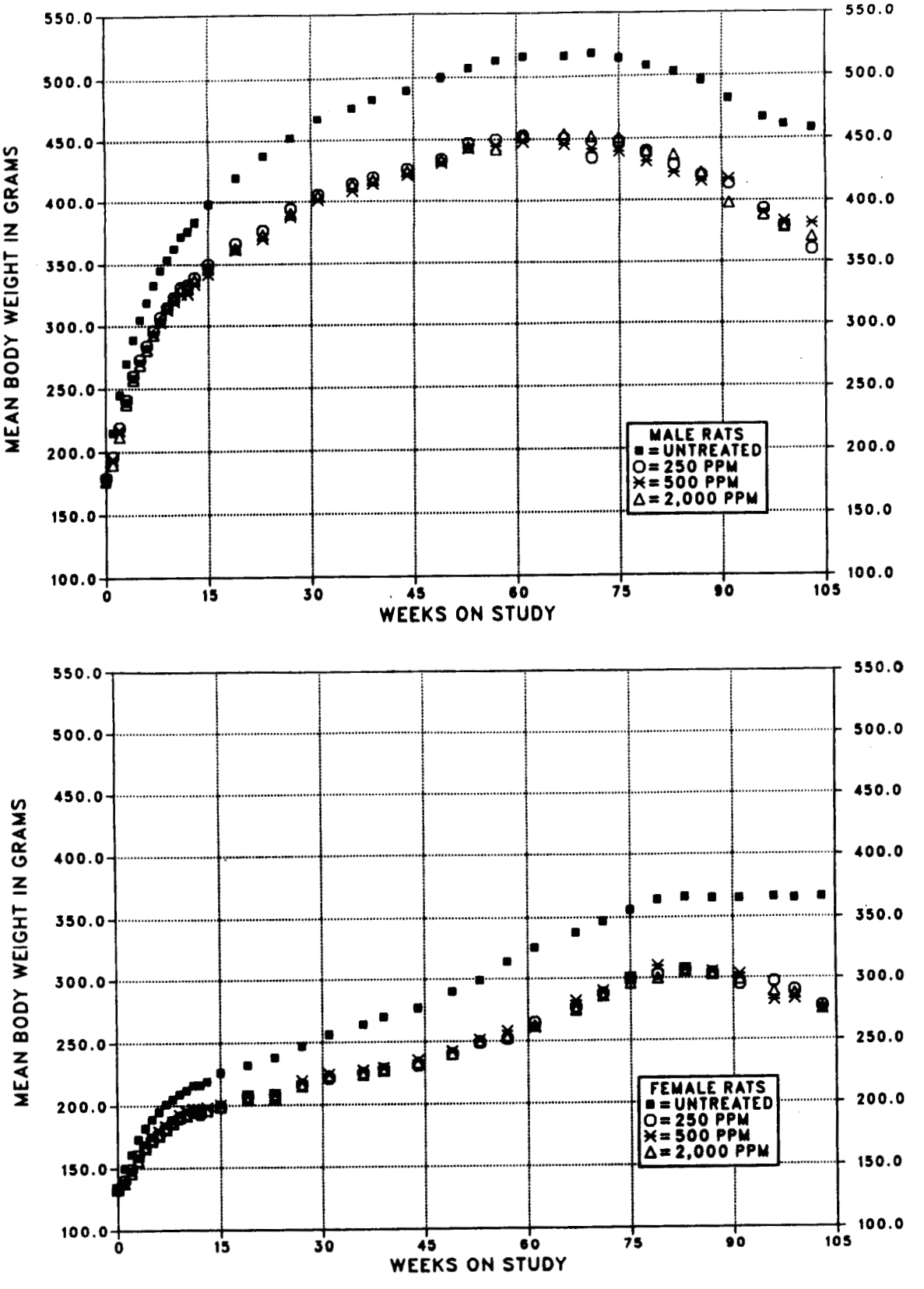

FIGURE 3. GROWTH CURVES FOR RATS FED DIETS CONTAINING HYDROCHLOROTHIAZIDE FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats fed diets containing hydrochlorothiazide at the concentrations used in these studies and for controls are shown in Table 14 and in the Kaplan and Meier curves in Figure 4. No significant differences in survival were observed between any groups of either sex. The number of male rats that were killed in a moribund condition was very high in all groups compared with the number of male rats that died naturally.

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 kidney, parathyroids, bone, multiple organs, Zymbal gland, hematopoietic system, lung, nasal cavity, brain, mammary gland, and uterus.

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 14. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE

	Control	250 ppm	500 ppm	2,000 ppm
MALE (a)				
Animals initially in study	50	50	50	50
Natural deaths	7	11	15	11
Moribund kills	25	28	28	28
Animals surviving until study termination	18	(b) 16	(b) 9	11
Survival P values (c)	0.471	0.905	0.114	0.386
FEMALE (a)				
Animals initially in study	50	50	50	50
Natural deaths	9	16	7	10
Moribund kills	11	10	16	13
Animals surviving until study termination	(b) 31	(b) 25	(b) 30	27
Survival P values (c)	0.714	0.255	0.785	0.467

(a) Termination period: weeks 106-107
(b) Animals dying or killed in a moribund condition during the termination period were combined, for statistical purposes, with those killed at termination.
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

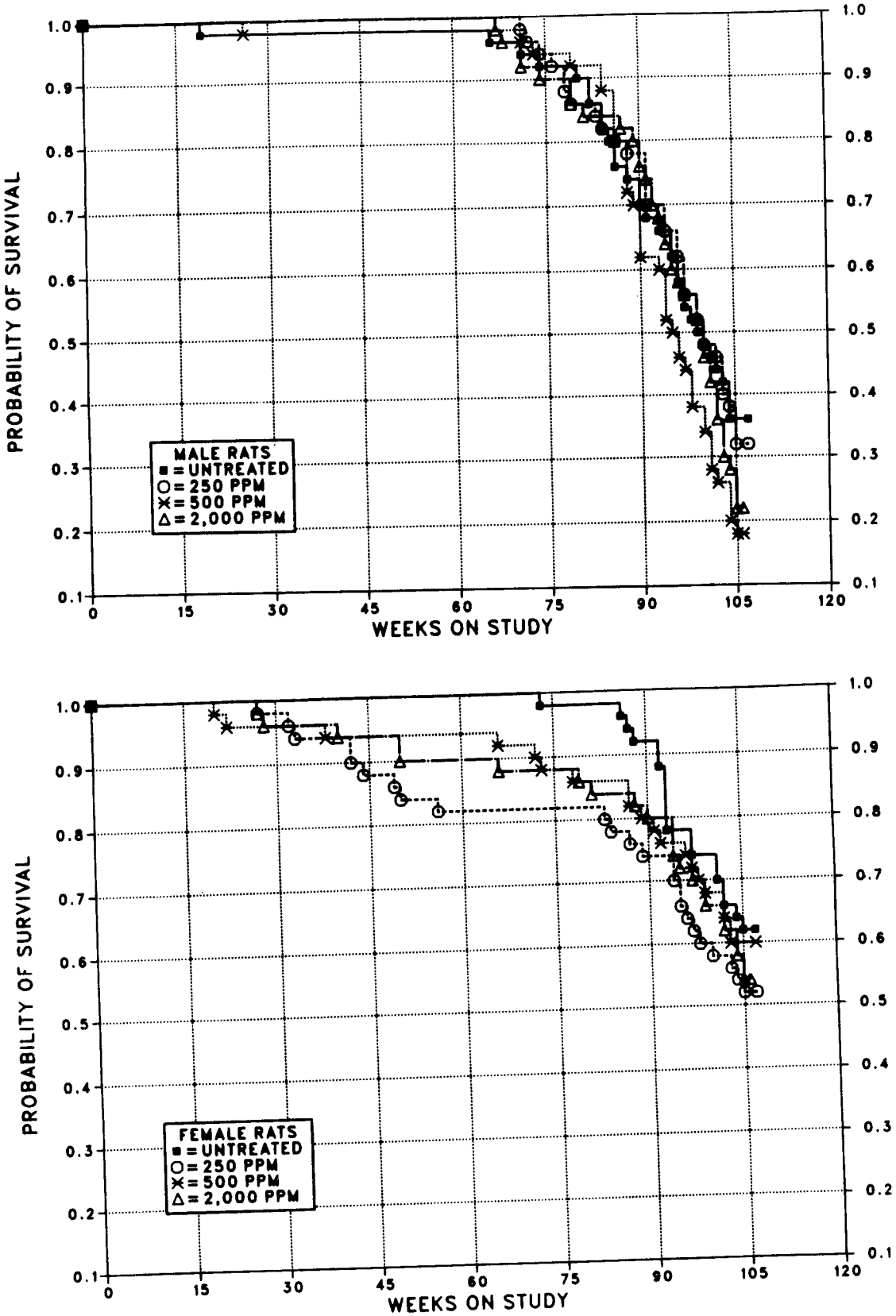

FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING HYDROCHLOROTHIAZIDE FOR TWO YEARS

Kidney: Chronic renal disease (nephropathy) was present in all groups of male and female rats, but the severity of nephropathy was increased in dosed groups relative to controls (Table 15). The nephropathy was characterized by degeneration and regeneration of tubular epithelium, tubular dilatation and atrophy, thickening of basement membranes, glomerulosclerosis, and variable interstitial fibrosis and chronic inflammation. The incidences of cysts in the renal cortex and hyperplasia of the transitional epithelium overlying the renal pelvis were also increased in dosed rats. These changes occur in kidneys with more advanced nephropathy, and the increased incidences reflect the more severe renal disease observed in the dosed animals. Tubular cell adenomas were observed in 1/49 mid dose and 1/50 high dose female rats. The historical incidence of renal tubular cell neoplasms in untreated female F344/N rats is 4/1,928 (0.2%), and the highest observed incidence is 1/49.

Parathyroids, Bone, and Multiple Organs: A spectrum of changes characteristic of renal secondary hyperparathyroidism occurred in dosed rats. Parathyroid hyperplasia was observed at increased incidences in dosed rats (male: control, 7/50; low dose, 20/49; mid dose, 30/50; high dose, 28/49; female: 0/50; 12/47; 11/49; 10/49). Fibrous osteodystrophy of the bone was increased in dosed male and female rats (male: 2/50; 18/49; 23/50; 22/50; female: 0/50; 9/50; 9/49; 5/50). Mineralization in multiple organs was observed at increased incidences in dosed rats (male: 1/50; 19/49; 26/50; 20/50; female: 0/50; 6/50; 6/49; 5/50).

TABLE 15. NUMBERS OF RATS WITH SELECTED RENAL LESIONS IN THE TWO-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE

Lesion	Male (ppm)				Female (ppm)			
	0	250	500	2,000	0	250	500	2,000
No. examined	50	49	50	50	50	50	49	50
Nephropathy	50	49	50	50	47	42	44	47
Cysts	2	19	21	18	0	3	4	3
Mineralization, kidney or multiple organs	1	19	27	27	10	40	39	40
Epithelial hyperplasia of the renal pelvis	6	21	26	23	0	4	2	3
Tubular cell hyperplasia	0	0	1	0	0	0	0	0
Tubular cell adenoma	3	1	0	1	0	0	1	1

Zymbal Gland: Adenomas or carcinomas (combined) occurred in 1/50 control, 1/49 low dose, 2/50 mid dose, and 4/50 high dose male rats (Table 16). The historical incidence of Zymbal gland neoplasms in untreated male F344/N rats is 19/1,936 (1.0%), and the highest observed incidence is 4/50.

Hematopoietic System: The incidence of mononuclear cell leukemia in mid dose female rats was significantly greater than that in the controls; the incidences of mononuclear cell leukemia in low and mid dose male rats were lower than that in controls (Table 17).

Lung, Nasal Cavity, and Brain: The incidences of hemorrhage were increased in female rats in the lung (control, 1/50; low dose, 9/30; mid dose, 4/26; high dose, 7/50), nasal cavity (0/49; 4/50; 2/48; 2/49), and brain (0/50; 1/14; 1/11; 5/50).

TABLE 16. ZYMBAL GLAND TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE (a)

	Control	250 ppm	500 ppm	2,000 ppm
Adenoma				
Overall Rates	0/50 (0%)	0/49 (0%)	1/50 (2%)	2/50 (4%)
Carcinoma				
Overall Rates	1/50 (2%)	1/49 (2%)	1/50 (2%)	2/50 (4%)
Adenoma or Carcinoma (b)				
Overall Rates	1/50 (2%)	1/49 (2%)	2/50 (4%)	4/50 (8%)
Adjusted Rates	2.9%	4.3%	5.9%	17.1%
Terminal Rates	0/18 (0%)	0/16 (0%)	0/9 (0%)	1/11 (9%)
Week of First Observation	93	103	84	71
Life Table Tests	P=0.077	P=0.752N	P=0.470	P=0.162
Incidental Tumor Tests	P=0.122	P=0.725N	P=0.607	P=0.259

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table A3 (footnotes). The estimated doses in milligrams per kilogram per day are given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.
(b) Historical incidence in NTP studies (mean ± SD): 19/1,936 (1% ± 2%)

TABLE 17. MONONUCLEAR CELL LEUKEMIA IN RATS IN THE TWO-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE

	Control	250 ppm	500 ppm	2,000 ppm
MALE (a)				
Overall Rates	33/50 (66%)	21/49 (43%)	16/50 (32%)	25/50 (50%)
Adjusted Rates	80.6%	55.5%	76.8%	74.9%
Terminal Rates	11/18 (61%)	4/16 (25%)	6/9 (67%)	6/11 (55%)
Week of First Observation	71	71	79	71
Life Table Tests	P=0.464	P=0.062N	P=0.086N	P=0.350N
Incidental Tumor Tests	P=0.430N	P=0.018N	P=0.001N	P=0.101N
FEMALE (b)				
Overall Rates	6/50 (12%)	5/50 (10%)	14/49 (29%)	10/50 (20%)
Adjusted Rates	16.0%	15.3%	36.7%	28.3%
Terminal Rates	3/31 (10%)	2/26 (8%)	7/29 (24%)	5/27 (19%)
Week of First Observation	86	84	72	88
Life Table Tests	P=0.185	P=0.619N	P=0.036	P=0.161
Incidental Tumor Tests	P=0.170	P=0.625N	P=0.041	P=0.144

(a) Historical incidence of leukemia in NTP studies (mean ± SD): 636/1,936 (33% ± 15%)
(b) Historical incidence of leukemia in NTP studies (mean ± SD): 383/1,983 (19% ± 7%)

Mammary Gland: Fibroadenomas in female rats occurred with a significant negative trend; the incidences in the dosed groups were significantly lower than that in the controls (Table 18).

Uterus: The incidence of endometrial stromal polyps in low dose female rats was significantly lower than that in controls (Table 19).

TABLE 18. MAMMARY GLAND FIBROADENOMAS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE (a)

	Control	250 ppm	500 ppm	2,000 ppm
Overall Rates	30/50 (60%)	12/50 (24%)	11/49 (22%)	5/50 (10%)
Adjusted Rates	72.5%	37.7%	34.8%	17.6%
Terminal Rates	20/31 (65%)	7/26 (27%)	9/29 (31%)	4/27 (15%)
Week of First Observation	87	56	92	104
Life Table Tests	P<0.001N	P=0.007N	P<0.001N	P<0.001N
Incidental Tumor Tests	P<0.001N	P=0.003N	P<0.001N	P<0.001N

(a) Historical incidence in NTP studies (mean ± SD): 589/1,983 (30% ± 10%)

TABLE 19. UTERINE ENDOMETRIAL STROMAL POLYPS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE (a)

	Control	250 ppm	500 ppm	2,000 ppm
Overall Rates	18/50 (36%)	6/50 (12%)	10/49 (20%)	11/50 (22%)
Adjusted Rates	46.9%	19.9%	27.8%	29.0%
Terminal Rates	12/31 (39%)	4/26 (15%)	5/29 (17%)	4/27 (15%)
Week of First Observation	88	50	87	66
Life Table Tests	P=0.419N	P=0.023N	P=0.111N	P=0.191N
Incidental Tumor Tests	P=0.384N	P=0.009N	P=0.141N	P=0.165N

(a) Historical incidence in NTP studies (mean ± SD): 420/1,966 (21% ± 8%)

FIFTEEN-DAY STUDIES

All mice lived to the end of the studies (Table 20). The final mean body weights of male mice that received 6,250, 12,500, 25,000, or 50,000 ppm were 13%, 10%, 11%, or 14% lower than that of controls. The final mean body weights of dosed and control female mice were similar. Feed consumption by dosed mice was similar to that by control mice. No compound-related clinical signs were observed. Calculi were seen in the urinary bladder of 2/5 male and 2/5 female mice receiving 50,000 ppm and in 1/5 male and 1/5 female mice receiving 25,000 ppm.

TABLE 20. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE FIFTEEN-DAY FEED STUDIES OF HYDROCHLOROTHIAZIDE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 1	Week 2
MALE							
0	5/5	25.6 ± 1.6	30.0 ± 0.7	+4.4 ± 1.0		4.5	3.9
3,125	5/5	28.4 ± 1.3	28.6 ± 0.9	+0.2 ± 0.7	95.3	4.5	3.7
6,250	5/5	25.8 ± 1.0	26.0 ± 0.8	+0.2 ± 0.2	86.7	4.0	4.1
12,500	5/5	27.0 ± 1.1	27.0 ± 1.3	0.0 ± 0.3	90.0	4.7	4.0
25,000	5/5	27.8 ± 1.4	26.6 ± 2.3	−1.2 ± 1.0	88.7	4.2	3.6
50,000	5/5	23.2 ± 1.5	25.8 ± 1.2	+2.6 ± 1.9	86.0	4.2	3.9
FEMALE							
0	5/5	20.0 ± 0.7	21.4 ± 0.8	+1.4 ± 0 5		4.2	3.2
3,125	5/5	19.4 ± 0.4	20.6 ± 0.7	+1.2 ± 0.4	96.3	3.9	3.7
6,250	5/5	20.6 ± 0.7	21.2 ± 0.7	+0.6 ± 0.5	99.1	4.2	3.2
12,500	5/5	19.4 ± 0.2	19.8 ± 0.6	+0.4 ± 0.4	92.5	4.5	3.3
25,000	5/5	21.0 ± 0.6	21.4 ± 0.5	+0.4 ± 0.2	100.0	4.2	3.2
50,000	5/5	21.0 ± 0.5	21.2 ± 0.7	+0.2 ± 0.5	99.1	3.5	4.5

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

THIRTEEN-WEEK STUDIES

Seven of 10 males and 1/10 females that received 50,000 ppm hydrochlorothiazide, 2/10 males that received 25,000 ppm, 3/10 females that received 12,500 ppm, and 1/10 males and 3/10 females that received 3,125 ppm died before the end of the studies (Table 21). Histopathologic evaluation of several of these animals was prevented by cannibalism and/or autolysis. Histopathologic evidence of pneumonia was found in several groups at the termination of the studies. The final mean body weights of mice that received 50,000 ppm was 11% lower than those of controls for males and females. Feed consumption by dosed and control mice was comparable. Compound-related effects included increased incidences of nephrosis (tubular cell degeneration and necrosis), gross observations of calculi in the urinary bladder, and inflammation and/or epithelial hyperplasia of the urinary bladder (Table 22). The calculi found in one urinary bladder were collected and analyzed and found to consist of relatively pure hydrochlorothiazide. Nephrosis was characterized by tubular cell degeneration and necrosis.

Dose Selection Rationale: Based on the incidence and severity of kidney and urinary bladder lesions, dietary concentrations of hydrochlorothiazide selected for mice for the 2-year studies were 2,500 ppm and 5,000 ppm. Mortality data were not used for dose selection because of uncertainty concerning the possible contribution of pneumonia to the deaths observed at the lower doses.

TABLE 21. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF HYDROCHLOROTHIAZIDE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 7	Week 13
MALE							
0	10/10	23.3 ± 0.4	(e) 31.6 ± 1.0	+8.3 ± 1.1		4.0	(e) 2.3
3,125	(f) 9/10	23.2 ± 0.7	35.4 ± 0.9	+12.0 ± 0.6	112.0	4.3	3.9
6,250	10/10	23.4 ± 0.5	35.5 ± 0.8	+12.1 ± 0.5	112.3	4.1	3.8
12,500	10/10	22.6 ± 0.5	34.1 ± 0.9	+11.5 ± 0.5	107.9	4.1	3.9
25,000	(g) 8/10	22.3 ± 0.5	32.9 ± 0.8	+10.3 ± 0.9	104.1	4.2	4.2
50,000	(h) 3/10	23.7 ± 0.5	28.0 ± 1.3	+4.0 ± 0.8	88.6	3.2	3.8
FEMALE							
0	10/10	18.7 ± 0.4	27.1 ± 0.6	+8.4 ± 0.4		3.7	3.7
3,125	(i) 7/10	18.9 ± 0.3	26.5 ± 0.6	+7.5 ± 0.4	97.8	3.6	3.9
6,250	10/10	19.0 ± 0.4	26.8 ± 0.6	+7.8 ± 0.5	98.9	3.7	3.5
12,500	(j) 7/10	17.8 ± 0.3	20.6 ± 0.7	+3.0 ± 0.5	76.0	3.8	2.7
25,000	10/10	19.0 ± 0.3	24.7 ± 0.7	+5.7 ± 0.5	91.1	3.4	3.6
50,000	(k) 9/10	19.0 ± 0.4	24.1 ± 0.7	+5.2 ± 0.6	88.9	4.2	4.2

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.
(e) An obstructed feeder during the last week of the study resulted in a loss of approximately 4.5 g body weight for one cage of mice, accounting for the higher weight gain in the dosed groups.
(f) Week of death: 7
(g) Week of death: all 12
(h) Week of death: 1,2,2,4,12,12,12
(i) Week of death: all 8
(j) Week of death: all 13
(k) Week of death: 4

TABLE 22. INCIDENCES OF MICE WITH SELECTED LESIONS OF THE URINARY BLADDER AND KIDNEY IN THE THIRTEEN-WEEK FEED STUDIES OF HYDROCHLOROTHIAZIDE

Site/Lesion	Male (ppm)					Female (ppm)				
	0	6,250	12,500	25,000	50,000	0	6,250	12,500	25,000	50,000
Urinary bladder										
Calculi (a)	0/10	0/10	3/10	2/10	7/10	0/10	0/10	4/10	9/10	6/10
Inflammation and/or epithelial hyperplasia	0/10	0/10	3/10	2/8	2/3	0/10	2/9	7/8	10/10	6/9
Kidney										
Nephrosis	0/10	0/10	2/10	2/8	3/3	0/10	0/10	5/8	9/10	9/9

(a) Gross observation

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of dosed male mice were generally within 5% of those of the controls (Table 23 and Figure 5). Mean body weights of high dose and control female mice were similar until week 92, after which the weights of the high dose group were 5%-8% lower. The average daily feed consumption per mouse by low dose or high dose mice was 103% or 105% that by the controls for males and 103% or 100% for females (Tables F3 and F4). The average amount of hydrochlorothiazide consumed per day was approximately 265 or 550 mg/kg for low dose or high dose male mice and 300 or 600 mg/kg for low dose or high dose female mice. No compound-related clinical signs were seen.

TABLE 23. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE

Weeks on Study	Control		2,500 ppm			5,000 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	21.5	50	21.5	100	50	21.0	98	50
1	23.3	50	22.9	98	50	22.8	98	50
2	24.5	50	24.0	98	50	24.0	98	50
3	25.4	50	24.8	98	50	24.4	96	50
4	26.4	50	26.0	98	50	25.4	96	50
5	27.0	50	26.7	99	50	26.5	98	50
6	28.2	50	27.5	98	50	27.4	97	50
7	28.9	50	28.4	98	50	28.3	98	50
8	29.8	50	29.1	98	50	28.8	97	50
9	30.4	50	29.6	97	50	29.2	96	50
10	30.9	50	30.0	97	50	29.8	96	50
11	30.3	50	29.5	97	50	29.5	97	50
12	30.7	50	29.8	97	50	29.2	95	50
13	32.0	50	30.8	96	50	30.9	97	50
16	32.8	50	31.5	96	50	31.6	96	50
20	34.2	50	33.3	97	50	33.1	97	50
24	35.5	50	34.8	98	50	35.0	99	50
28	36.6	50	35.8	98	50	36.3	99	50
32	37.3	50	36.8	99	50	37.8	101	50
37	37.8	50	36.7	97	50	37.4	99	50
40	37.7	50	37.5	99	50	37.7	100	50
45	38.0	49	38.1	100	50	38.5	101	50
49	38.1	47	38.6	101	50	39.2	103	50
54	38.7	47	39.1	101	50	40.3	104	50
58	39.0	47	38.8	99	50	40.2	103	49
62	38.7	47	38.6	100	50	40.3	104	49
68	39.6	47	39.4	99	48	40.1	101	49
72	39.7	47	39.6	100	47	40.0	101	49
76	39.9	47	39.8	100	46	39.8	100	49
80	39.6	47	40.3	102	45	40.0	101	48
84	39.1	46	40.1	103	45	38.5	98	46
88	38.7	46	39.4	102	43	37.1	96	46
92	39.0	46	39.7	102	43	37.2	95	45
96	38.5	46	39.0	101	43	36.4	95	43
100	37.2	45	38.6	104	43	36.6	98	43
104	37.9	44	38.5	102	42	35.1	93	43
FEMALE								
0	17.9	50	18.2	102	50	17.6	98	50
1	19.2	50	19.1	99	50	18.7	97	50
2	19.8	50	19.8	100	50	19.3	97	50
3	20.8	50	20.6	99	50	20.2	97	50
4	21.4	50	21.5	100	50	21.0	98	50
5	21.9	50	21.8	100	50	21.7	99	50
6	22.4	50	22.5	100	50	22.3	100	50
7	23.0	50	23.1	100	50	23.0	100	50
8	23.5	50	23.6	100	50	23.2	99	50
9	24.0	50	24.2	101	50	23.8	99	50
10	24.7	50	24.7	100	50	24.0	97	50
11	24.3	50	24.2	100	50	24.2	100	50
12	24.6	50	24.2	98	50	24.2	98	50
13	25.2	50	25.1	100	50	25.1	100	50
16	25.8	50	25.1	97	50	25.2	98	50
20	27.4	50	26.9	98	50	26.7	97	50
24	28.5	50	28.4	100	50	28.0	98	49
28	29.9	50	29.0	97	50	28.9	97	49
32	30.4	50	30.4	100	50	30.5	100	49
37	31.0	50	30.3	98	50	30.4	98	49
40	31.3	50	31.2	100	50	31.3	100	49
45	32.4	50	31.9	98	50	31.6	98	49
49	33.8	50	33.3	99	50	32.9	97	49
54	34.2	50	33.9	99	50	33.5	98	49
58	35.1	49	34.9	99	50	34.1	97	49
62	36.5	48	36.3	99	50	36.2	99	49
68	38.9	48	38.5	99	50	38.7	99	49
72	39.6	48	39.7	100	50	40.0	101	49
76	41.4	48	40.6	98	50	40.2	97	49
80	41.5	48	41.6	100	49	41.1	99	48
84	41.5	48	41.0	99	48	41.3	100	45
88	41.8	45	40.6	97	47	40.8	98	45
92	41.3	44	39.1	95	45	39.4	95	43
96	40.1	44	40.3	100	44	37.2	93	42
100	39.5	42	38.7	98	43	36.4	92	39
104	40.3	38	39.0	97	41	37.1	92	35

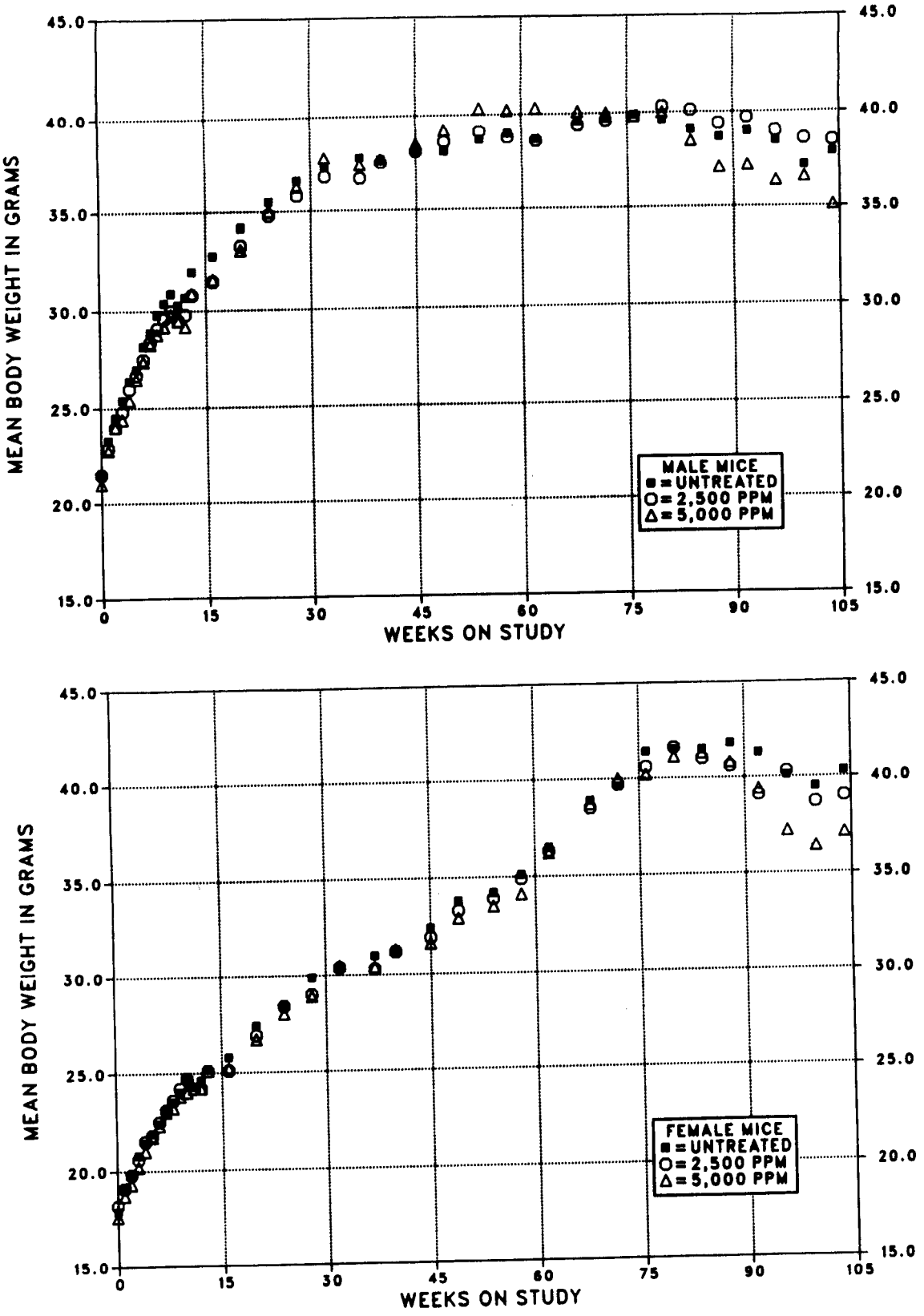

FIGURE 5. GROWTH CURVES FOR MICE FED DIETS CONTAINING HYDROCHLOROTHIAZIDE FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female mice fed diets containing hydrochlorothiazide at the concentrations used in these studies and for controls are shown in Table 24 and in the Kaplan and Meier curves in Figure 6. 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 mice with neoplastic or nonneoplastic lesions of the liver.

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 24. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF HYDROCHLOROTHIAZIDE

	Control	2,500 ppm	5,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Natural deaths	5	4	2
Moribund kills	2	3	5
Animals missing	0	1	0
Animals surviving until study termination	43	42	43
Survival P values (b)	1.000	0.982	1.000
FEMALE (a)			
Animals initially in study	50	50	50
Natural deaths	10	6	11
Moribund kills	3	5	4
Animals surviving until study termination	(c) 38	(c) 40	35
Survival P values (b)	0.529	0.786	0.602

(a) Termination period: weeks 105-106
(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.
(c) Animals dying or killed in a moribund condition during the termination period were combined, for statistical purposes, with those killed at termination.

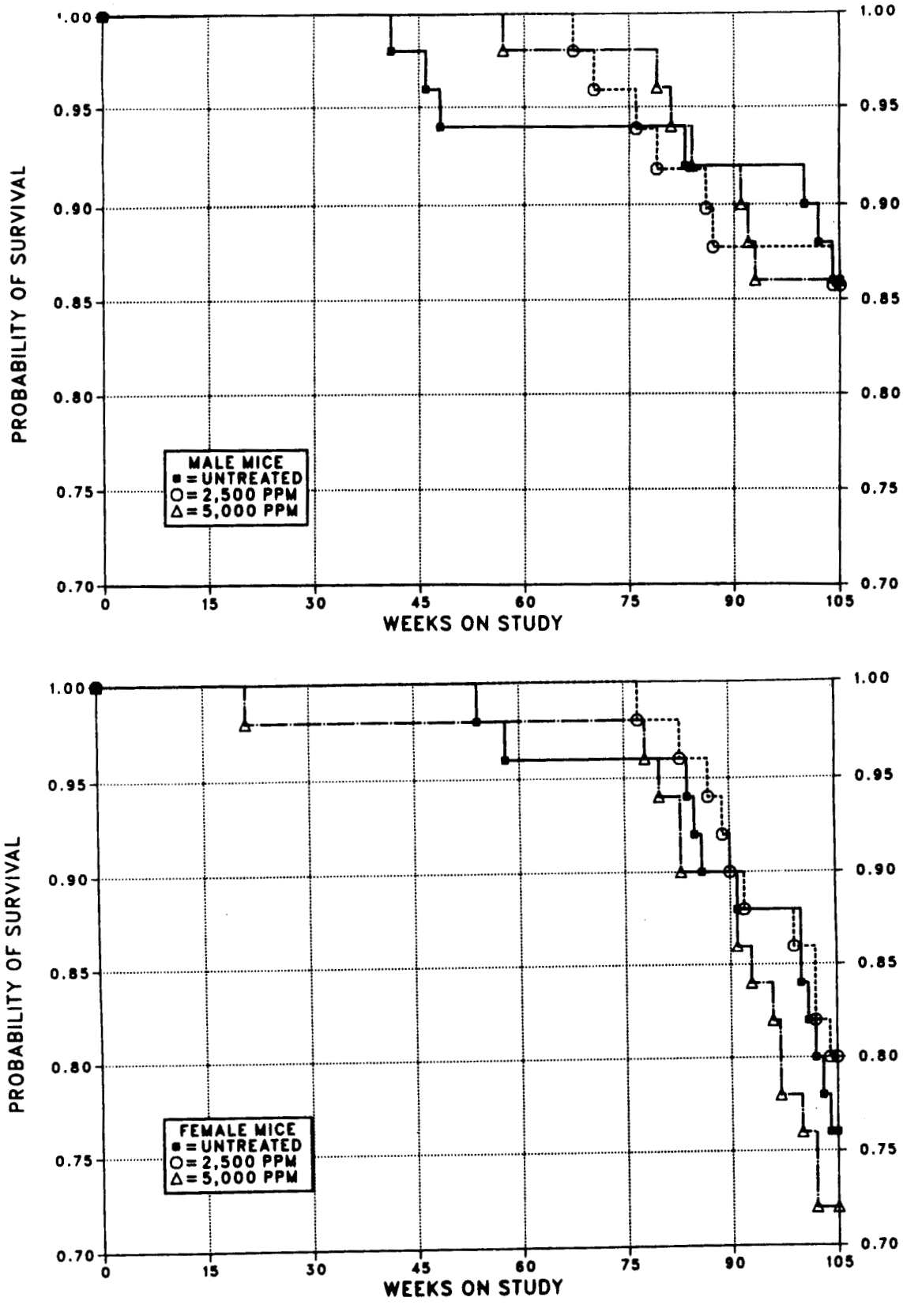

FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING HYDROCHLOROTHIAZIDE FOR TWO YEARS

Liver: Hepatocellular adenomas and hepatocellular adenomas or carcinomas (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 25). Hepatocellular adenomas generally consisted of well-differentiated hepatocytes arranged in branching cords, one or two cell layers thick. The normal lobular architecture was partially or completely effaced, and the neoplastic hepatocytes were more basophilic, eosinophilic, or vacuolated than the adjoining tissue. Hepatocellular carcinomas usually exhibited a more heterogenous growth pattern than did the adenomas and often contained areas in which the neoplastic cells were arranged in trabeculae, four to six cell layers thick. The hepatocytes were often pleomorphic and had enlarged vesicular nuclei with multiple nucleoli. Metastasis to the lung was seen in two control and two high dose males.

TABLE 25. HEPATOCELLULAR TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *HYDROCHLOROTHIAZIDE* (a)

	Control	2,500 ppm	5,000 ppm
Adenoma			
Overall Rates	3/48 (6%)	8/49 (16%)	14/50 (28%)
Adjusted Rates	7.0%	18.5%	30.9%
Terminal Rates	3/43 (7%)	7/42 (17%)	12/43 (28%)
Week of First Observation	105	79	79
Life Table Tests	P=0.003	P=0.095	P=0.004
Incidental Tumor Tests	P=0.008	P=0.148	P=0.012
Carcinoma			
Overall Rates	4/48 (8%)	4/49 (8%)	9/50 (18%)
Adjusted Rates	9.1%	9.5%	19.6%
Terminal Rates	3/43 (7%)	4/42 (10%)	6/43 (14%)
Week of First Observation	104	105	91
Life Table Tests	P=0.082	P=0.630	P=0.123
Incidental Tumor Tests	P=0.092	P=0.562	P=0.161
Adenoma or Carcinoma (b)			
Overall Rates	7/48 (15%)	10/49 (20%)	21/50 (42%)
Adjusted Rates	15.9%	23.1%	43.7%
Terminal Rates	6/43 (14%)	9/42 (21%)	16/43 (37%)
Week of First Observation	104	79	79
Life Table Tests	P=0.001	P=0.282	P=0.003
Incidental Tumor Tests	P=0.004	P=0.323	P=0.009

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table C3. The estimated doses in milligrams per kilograms per day are given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.
(b) Historical incidence in NTP studies (mean ± SD): 609/2,032 (30% ± 8%)

Hydrochlorothiazide was tested at doses up to 10 mg/plate in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in a preincubation protocol with and without Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Table 26). No increase in revertant colonies was observed in strains TA100, TA1535, or TA1537; however, in three trials performed with strain TA98 in the absence of S9, a small but repeatable dose-related increase in revertant colonies was recorded. Hydrochlorothiazide was therefore judged to exhibit equivocal activity in the Salmonella assay. In the mouse lymphoma L5178Y/TK$^{+/-}$ assay for induction of trifluorothymidine resistance, hydrochlorothiazide produced a positive response in the absence of exogenous metabolic activation at doses of 500-1,200 µg/ml; it was not tested with S9 (Table 27). Hydrochlorothiazide induced sister chromatid exchanges but not chromosomal aberrations in cultured Chinese hamster ovary cells in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 (Tables 28 and 29). No induction of sex-linked recessive lethal mutations was detected in adult male *Drosophila melanogaster* following administration of 10,000 ppm hydrochlorothiazide by feeding or injection (Table 30).

TABLE 26. MUTAGENICITY OF HYDROCHLOROTHIAZIDE IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (μg/plate)	Revertants/Plate (b)					
		−S9		+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	156 ± 4.8	135 ± 2.2	154 ± 4.1	126 ± 1.0	153 ± 6.7	153 ± 6.3
	100	142 ± 3.8	126 ± 2.7	159 ± 7.0	130 ± 12.8	156 ± 6.1	149 ± 3.6
	333	132 ± 1.5	137 ± 4.2	158 ± 4.4	118 ± 7.2	140 ± 7.7	136 ± 3.9
	1,000	139 ± 2.3	142 ± 7.5	161 ± 1.7	117 ± 11.0	135 ± 7.6	139 ± 0.9
	3,333	132 ± 7.5	143 ± 6.4	169 ± 9.0	126 ± 3.8	143 ± 13.9	144 ± 11.1
	10,000	111 ± 14.9	152 ± 1.7	200 ± 7.3	141 ± 5.5	150 ± 9.0	141 ± 8.4
	Trial summary	Negative	Negative	Equivocal	Negative	Negative	Negative
	Positive control (c)	1,213 ± 44.1	1,030 ± 13.1	1,111 ± 30.6	838 ± 21.2	992 ± 8.5	675 ± 13.2
TA1535	0	30 ± 5.9	32 ± 3.2	15 ± 1.0	13 ± 1.8	14 ± 1.9	7 ± 1.5
	100	35 ± 3.2	28 ± 1.8	16 ± 1.8	13 ± 3.2	13 ± 1.5	11 ± 2.0
	333	26 ± 3.6	21 ± 2.7	15 ± 3.5	10 ± 2.2	15 ± 0.7	10 ± 1.0
	1,000	20 ± 2.0	22 ± 2.6	13 ± 0.9	10 ± 2.1	12 ± 0.3	8 ± 1.7
	3,333	23 ± 1.7	21 ± 4.7	11 ± 1.7	12 ± 1.0	10 ± 1.5	11 ± 0.6
	10,000	14 ± 5.2	20 ± 1.2	15 ± 3.5	11 ± 0.7	13 ± 3.3	11 ± 1.3
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (c)	871 ± 28.3	726 ± 22.2	87 ± 3.6	66 ± 2.5	88 ± 4.6	58 ± 3.3

Strain	Dose (μg/plate)	−S9			+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 1	Trial 2
TA1537	0	4 ± 1.5	8 ± 1.8	7 ± 2.1	7 ± 0.3	8 ± 2.3	12 ± 1.8	11 ± 4.0
	100	8 ± 1.2	6 ± 1.7	9 ± 2.0	11 ± 3.2	9 ± 2.0	10 ± 1.9	12 ± 1.7
	333	6 ± 1.5	9 ± 1.5	8 ± 1.0	13 ± 1.7	11 ± 1.5	8 ± 2.3	14 ± 3.4
	1,000	7 ± 1.2	7 ± 0.6	6 ± 1.2	9 ± 0.9	8 ± 0.9	11 ± 1.7	10 ± 3.1
	3,333	8 ± 1.0	10 ± 0.9	11 ± 0.7	14 ± 1.7	13 ± 2.3	14 ± 0.6	14 ± 4.1
	6,667			7 ± 1.3				
	10,000	10 ± 1.5	19 ± 1.2	9 ± 3.8	12 ± 1.2	14 ± 3.2	15 ± 2.6	14 ± 0.9
	Trial summary	Negative	Equivocal	Negative	Negative	Negative	Negative	Negative
	Positive control (c)	600 ± 14.5	99 ± 19.5	195 ± 12.8	82 ± 5.5	72 ± 6.2	86 ± 6.7	60 ± 7.7
TA98	0	15 ± 1.3	19 ± 1.3	19 ± 1.0	29 ± 2.2	32 ± 4.1	30 ± 1.2	31 ± 3.1
	100	21 ± 0.9	17 ± 1.7	16 ± 0.6	28 ± 2.0	35 ± 1.2	34 ± 4.1	36 ± 0.7
	333	18 ± 1.8	23 ± 2.8	25 ± 3.0	32 ± 0.9	33 ± 0.7	32 ± 1.2	37 ± 2.0
	1,000	25 ± 4.5	22 ± 2.3	21 ± 3.2	39 ± 2.5	37 ± 2.7	31 ± 5.2	40 ± 5.7
	3,333	24 ± 2.7	30 ± 4.4	24 ± 0.3	36 ± 3.5	41 ± 2.1	32 ± 2.7	35 ± 1.5
	6,667			31 ± 1.9				
	10,000	26 ± 1.7	39 ± 1.9	32 ± 3.1	41 ± 0.9	38 ± 1.8	34 ± 1.5	39 ± 4.6
	Trial summary	Equivocal	Weakly positive	Equivocal	Negative	Negative	Negative	Negative
	Positive control (c)	1,406 ± 9	870 ± 38.4	1,270 ± 42.2	1,119 ± 39.4	800 ± 24.3	891 ± 43.4	554 ± 18.9

(a) Study performed at EG&G Mason Research Institute. The detailed protocol is presented by Mortelmans et al. (1986). Cells and study compound or solvent (dimethyl sulfoxide) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 μg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-o-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

TABLE 27. MUTAGENICITY OF HYDROCHLOROTHIAZIDE IN MOUSE L5178Y LYMPHOMA CELLS (a,b)

Compound	Concentration (µl/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
-S9					
Trial 1					
Acetone (d)		78.8 ± 5.7	100.0 ± 3.7	99.5 ± 1.9	42.8 ± 3.1
Hydrochlorothiazide	62.5	56.7 ± 2.3	69.0 ± 9.5	75.7 ± 8.7	45.0 ± 7.1
	(e) 125	49.0 ± 1.0	55.5 ± 6.5	78.0 ± 0.0	53.0 ± 1.0
	250	64.7 ± 3.7	65.3 ± 5.0	74.7 ± 5.8	38.7 ± 0.9
	500	61.3 ± 3.0	51.0 ± 4.0	169.0 ± 16.0	(f) 92.0 ± 9.0
	(e) 750	54.5 ± 7.5	17.0 ± 5.0	423.5 ± 8.5	(f) 265.5 ± 41.5
	(g) 1,000	45.0 ± 2.0	7.0 ± 1.0	470.5 ± 20.5	(f) 347.0 ± 1.0
Methyl methanesulfonate	5	38.3 ± 2.2	24.3 ± 5.2	574.7 ± 14.5	(f) 505.7 ± 14.8
Trial 2					
Acetone (e)		79.5 ± 5.5	100.0 ± 7.0	95.5 ± 9.5	40.0 ± 1.0
Hydrochlorothiazide	(e) 200	67.0 ± 1.0	72.0 ± 3.0	68.5 ± 3.5	34.5 ± 1.5
	(e) 400	46.0 ± 2.0	48.0 ± 4.0	69.5 ± 11.5	50.5 ± 10.5
	500	64.3 ± 4.8	43.3 ± 4.1	92.0 ± 19.4	48.3 ± 11.3
	800	48.0 ± 4.4	21.0 ± 1.5	212.7 ± 23.5	(f) 149.3 ± 20.8
	1,000	54.3 ± 5.6	13.0 ± 2.5	484.0 ± 53.3	(f) 304.0 ± 47.6
	(g) 1,200	27.5 ± 8.5	2.5 ± 0.5	488.5 ± 14.5	(f) 672.5 ± 233.5
Methyl methanesulfonate	(e) 5	62.0 ± 13.0	60.5 ± 11.5	383.0 ± 24.0	(f) 214.0 ± 33.0

(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; the average for the three tests is presented in the table. All tests were performed in the absence of exogenous metabolic activation (-S9). 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 non-selective 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 the average of four tests.

(e) Data presented are the average of two tests.

(f) 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.

(g) Data presented are the average of two tests. The dose in one test was lethal.

TABLE 28. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY HYDROCHLOROTHIAZIDE (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: Positive								
Dimethyl sulfoxide		50	1,022	427	0.42	8.5	25.7	
Hydrochlorothiazide	43	50	1,047	525	0.50	10.5	25.7	123.5
	130	50	1,019	496	0.49	9.9	25.7	116.5
	430	50	1,026	987	0.96	19.7	25.7	231.8
Mitomycin C	0.005	25	511	786	1.54	31.4	25.7	369.4
Trial 2--Summary: Positive								
Dimethyl sulfoxide		50	1,034	427	0.41	8.5	25.7	
Hydrochlorothiazide	500	50	1,036	1,033	1.00	20.7	25.7	243.5
	600	50	1,027	1,387	1.35	27.7	25.7	325.9
	800	50	1,025	1,267	1.24	25.3	25.7	297.6
	1,000	0						
Mitomycin C	0.001	50	1,044	589	0.56	11.8	25.7	138.8
	0.01	5	101	203	2.01	40.6	25.7	477.6
+S9 (d)--Summary: Positive								
Dimethyl sulfoxide		50	1,028	414	0.4	8.3	25.7	
Hydrochlorothiazide	130	50	1,045	554	0.53	11.1	25.7	133.7
	430	50	1,037	434	0.42	8.7	25.7	104.8
	1,300	50	1,038	510	0.49	10.2	25.7	122.9
Cyclophosphamide	0.3	50	1,035	600	0.58	12.0	25.7	144.6
	2	5	105	107	1.02	21.4	25.7	257.8

(a) Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) as described in (c) or (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 in treated culture expressed as a percent of the SCEs/cell in the control culture
(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. Then cells were 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 29. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY HYDROCHLOROTHIAZIDE (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
Trial 1--Harvest time: 21 h (d)					Harvest time: 10.5 h				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	100	0	0	0.0		100	0	0.00	0.0
	100	0	0	0.0					
Hydrochlorothiazide					Hydrochlorothiazide				
260	100	1	0.01	1.0	2,080	100	0	0.00	0.0
520	100	0	0.00	0.0	2,340	100	0	0.00	0.0
780	100	3	0.03	3.0	2,600	100	1	0.01	1.0
1,040	0								
Summary: Negative					Summary: Negative				
Mitomycin C					Cyclophosphamide				
0.062	50	20	0.40	28.0	50	50	12	0.24	20.0
Trial 2--Harvest time: 10.5 h									
Dimethyl sulfoxide									
	100	0	0.00	0.0					
	100	0	0.00	0.0					
Hydrochlorothiazide									
800	100	2	0.02	2.0					
900	100	2	0.02	2.0					
1,000	100	4	0.04	4.0					
1,250	0								
Summary: Negative									
Mitomycin C									
0.5	50	21	0.42	20.0					

(a) Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is presented by Galloway et al. (1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) as indicated in (b) or (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, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) 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 (dimethyl sulfoxide) 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.
(d) Because of significant chemical-induced cell cycle delay, incubation time prior to addition of colcemid was lengthened to provide sufficient metaphases at harvest.

TABLE 30. INDUCTION OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN DROSOPHILA BY HYDROCHLOROTHIAZIDE (a)

Route of Exposure	Dose (ppm)	Incidence of Deaths (percent)	Incidence of Sterility (percent)	No. of Lethals/No. of X Chromosomes Tested			Overall Total (b)
				Mating 1	Mating 2	Mating 3	
Feeding	10,000	0	0	0/2,158	2/2,151	0/2,101	2/6,410 (0.03%)
	0			1/2,353	2/1,959	1/1,846	4/6,158 (0.06%)
Injection	10,000	0	8	2/2,158	0/2,033	0/1,426	2/5,617 (0.04%)
	0			3/2,305	0/2,139	2/1,913	5/6,357 (0.08%)

(a) Study performed at Bowling Green State University. A detailed protocol of the sex-linked recessive lethal assay is presented in Valencia et al. (1985). Exposure by feeding was done by allowing 24-hour-old Canton-S males to feed for 3 days on a solution of the study chemical dissolved in 5% sucrose. In the injection experiments, 24-hour-old Canton-S males were treated with a solution of the chemical dissolved in 0.7% saline and allowed 24 hours to recover. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were crossed to their siblings and placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters; no clusters were found. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were not significant at the 5% level (Margolin et al., 1983).
(b) Combined total of number of lethal mutations/number of X chromosomes tested for three mating trials

IV. DISCUSSION AND CONCLUSIONS

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Hydrochlorothiazide, a widely used diuretic, was evaluated in 15-day, 13-week, and 2-year toxicity studies. In the 15-day studies, with dietary concentrations of up to 50,000 ppm, no rats or mice died before the end of the studies; body weight gains of dosed animals were lower than those of the controls, although not clearly dose related. Chemically related clinical signs, including evidence of diuresis, were not evident during the studies. No compound-related microscopic pathologic effects were seen in the 15-day studies, although signs of increased hemorrhage were noted upon gross examination of dosed rats and calculi were seen in the urinary bladder of some of the mice in the two highest dose groups.

In the 13-week studies, the concentrations of hydrochlorothiazide in feed ranged up to 50,000 ppm, and again no rats died during the studies. However, several mice that received 3,125 ppm or more died. The cause of these deaths could not be determined, but it was thought unlikely that the deaths were related to exposure to hydrochlorothiazide, with the possible exception of those in the high dose groups. In three dose groups, deaths of several mice occurred in the same cage during the same week. Many of these animals were autolyzed or cannibalized, which prevented a thorough examination. Diagnoses of pneumonia were made for animals killed at the end of the studies, and it is possible that this disease contributed to the pattern of early deaths. Body weight gains of dosed rats and female mice were generally lower than those of controls but were not consistently dose related.

The kidney was the major target of toxicity in the short-term studies in both rats and mice. In the 13-week studies, mineralization was observed at the corticomedullary junction in rats in all dosed groups, and severe nephropathy was seen in one male and five female rats in the 50,000-ppm groups. The severity of the mineralization was dose related and ranged from minimal to moderate. Because mineralization was observed at all doses, the 13-week studies were repeated in rats with 4,000 ppm as the highest dose. All rats survived to the end of the second 13-week studies, body weight gains were lower than those of the controls, and mineralization of the kidney was again observed for all dosed groups (250 ppm and above). The lack of consistent dose-related effects on body weight gain and the presence of mineralization of the kidney at all doses suggested that the doses used in these studies were above that needed to produce a maximal pharmacologic effect and that the mineralization could be associated with the increased calcium retention that has been observed with the use of thiazide diuretics (Christensson et al., 1977). Increased calcium retention could result from a primary hyperparathyroidism as suggested by Pickleman et al. (1969), but the parathyroid glands were not obviously enlarged in the current short-term studies.

In mice, the incidences of kidney tubular cell degeneration and necrosis and inflammation and/or epithelial hyperplasia and calculi in the urinary bladder increased in males and females at 12,500 ppm and above. The calculi were similar to those noted in mice in the 15-day studies and were also seen in the urinary bladder in one male rat in the 25,000-ppm group in the first 13-week studies. The calculi from one mouse urinary bladder were collected, analyzed, and found to contain hydrochlorothiazide. It is not clear why calculi developed in some animals and not in others or why they were not confined to the highest dose groups. White crystals were noted in the stomachs of some rats exposed to hydrochlorothiazide at concentrations as low as 6,250 ppm.

Dose selections for the 2-year studies were based primarily on the severity of the kidney lesions observed in the 13-week studies. Three dietary concentrations over an eightfold range (250-2,000 ppm) were selected for rats because of uncertainty over the potential for these lesions to worsen the nephropathy that is a common cause of death of F344 rats. A 1-year interim kill for rats was added to the 2-year studies to assess the progression of kidney lesions and to evaluate if they were severe enough to compromise completion of the 2-year studies.

During the first year of the 2-year study, 11 of the 16 dosed female rats that died showed evidence of internal hemorrhage; consequently, hematologic evaluation and blood-clotting tests were performed on the rats killed at 1 year. Thrombocytopenia has been reported as an idiosyncratic response to hydrochlorothiazide

therapy in humans (Swanson and Cook, 1977), but platelet levels for dosed rats were not different from those for controls in the 1-year study animals. Plasma concentrations of prothrombin and fibrinogen were also unchanged from those of controls, and activated partial thromboplastin times for plasma were highly variable but were not significantly longer for dosed rats. Chronic nephropathy was judged more severe in dosed rats than in controls at 1 year, but the lesion did not appear to be life threatening. No other lesions appeared to be chemically related.

During the 2-year studies, body weights of dosed rats were reduced to the same extent in all dosed groups of males and females, suggesting that the effect on body weight was secondary to a pharmacologic effect, even at the low dose of 250 ppm. The average amount of hydrochlorothiazide consumed per day by rats ranged from 10 mg/kg to approximately 100 mg/kg and by mice, from approximately 250 to 600 mg/kg. For humans, maximally effective diuretic doses are in the range of 1 to 1.5 mg/kg (Gilman et al., 1985).

The survival rate of female rats was in the range generally found in 2-year studies, but survival of all groups of male rats was low and was lower in dosed groups than in controls. Survival of the control male rats in this study (36%) was similar to that seen in the concurrent studies of furosemide (34%) (NTP, 1989), performed at the same laboratory, and may reflect, at least in part, an aggressive moribund-kill program at this laboratory. An increasing number of deaths occurred after week 75 in all male groups, with little difference in the pattern of deaths between the groups thereafter.

Chronic renal disease was present in all groups of male and female rats, and severity was increased in dosed groups. Secondary signs of chronic renal disease, including parathyroid hyperplasia, mineralization in multiple organs, and fibrous osteodystrophy, were also increased in dosed groups. No other nonneoplastic lesions in rats appeared to be related to hydrochlorothiazide exposure.

Proliferative lesions were noted in the Zymbal (auditory sebaceous) gland in dosed male rats and in the hematopoietic system in dosed female rats. Zymbal gland neoplasms occurred in one control, one low dose, two mid dose, and four high dose male rats. These incidences represent a marginal increase in a neoplasm that historically occurs at a rate of 1%. The occurrence of this neoplasm was not considered related to hydrochlorothiazide exposure. Similarly, the marginal increase in the incidence of mononuclear cell leukemia in mid dose female rats was not considered to be due to hydrochlorothiazide exposure because of the lack of an increased incidence in the high dose group and the fact that the incidences in the control and low dose groups are lower than the mean historical incidence for this lesion. In contrast, mammary gland neoplasms in control female rats occurred at twice the historical incidence. The incidences in all dosed female groups were somewhat lower than the mean historical incidence, resulting in a negative trend. These decreased incidences may be in part related to the reduced body weights of dosed rats compared with those of controls (Rao et al., 1987).

The results in rats are quite similar to those of a study reported by Lijinsky and Reuber (1987), in which hydrochlorothiazide was given (with or without sodium nitrite) to groups of 24 male and 24 female F344 rats at dietary concentrations of 0 or 1,000 ppm for 104 weeks. Hydrochlorothiazide administration was associated with an increase in the incidence and severity of nephropathy and increased incidences of parathyroid hyperplasia; osteitis fibrosa; and calcification of the aorta, other arteries, mucosa of the glandular stomach, muscle of the forestomach, heart, and pulmonary alveoli. Two parathyroid adenomas were found in the 24 dosed male rats. Several kidney tubular cell neoplasms were observed in the dosed groups (two in males and one in females receiving hydrochlorothiazide; three in males and one in females receiving hydrochlorothiazide and sodium nitrite). None was observed in controls. These incidences of tubular cell neoplasms of the kidney were not significantly increased, in concordance with observations of a renal tubular cell neoplasm in one mid and one high dose female rat in the current studies. Differences in results between the Lijinsky and Reuber study and the current studies were limited to an absence of increased cardiac thrombosis and polyarteritis in the current studies.

IV. DISCUSSION AND CONCLUSIONS

For mice, the doses selected for the 2-year studies were higher than those selected for rats, and the doses per body weight were much higher than for rats. Nonetheless, these doses (2,500 and 5,000 ppm) produced no obvious diuresis or other clinical signs and had negligible effects on body weights. Survival was also not affected by hydrochlorothiazide exposure and was typical of that usually seen in 2-year studies. The doses were chosen based on the observation of increased nephropathy and urinary bladder lesions at 12,500 ppm and higher in the 13-week studies. No increases in nonneoplastic lesions in the kidney, urinary bladder, or any other organs were attributed to hydrochlorothiazide administration in the 2-year studies in mice.

No neoplastic lesions in female mice were attributed to hydrochlorothiazide exposure. In male mice, incidences of hepatocellular neoplasms were dose related and were increased in dosed groups over that in controls (control, 7/48; low dose, 10/49; high dose, 21/50; see Table 25). However, the incidence of hepatocellular neoplasms in control male mice is low compared with that seen in contemporary studies in the NTP historical data base (Table C4), and the total incidence of neoplasms seen in male mice in this study (26%) is lower than the average incidence in controls in all NTP studies (30%). These factors tend to diminish the strength of the statistical association of hydrochlorothiazide exposure with liver neoplasms in male mice in this study.

Evidence for the genetic toxicity of hydrochlorothiazide is limited to an equivocal response in one Salmonella assay and positive responses in assays for induction of sister chromatid exchanges in Chinese hamster ovary cells, *p*-fluorophenylalanine (PFP)-resistant colonies in Aspergillus, and trifluorothymidine-resistant colonies in L5178Y mouse lymphoma cells. Results of Salmonella tests of hydrochlorothiazide have been generally negative; the equivocal response in strain TA98 reported by the NTP represents a summary conclusion for one weak positive and two equivocal trials conducted in the absence of S9. In the PFP-resistance assay with Aspergillus (Bignami et al., 1974), a single dose was applied as a "a small sterile triangle of absorbent paper...saturated with a solution of the drug." The lack of a positive control and a limited presentation of the data decrease the ability to evaluate this positive result. Hydrochlorothiazide was negative in assays for induction of chromosomal aberrations in cultured mammalian cells and for sex-linked recessive lethal mutations in germ cells of male Drosophila. In view of the very limited evidence for genotoxicity and the apparent absence of significant conversion to reactive metabolites, in vivo mutagenicity by hydrochlorothiazide seems unlikely.

The experimental and tabulated data for the NTP Technical Report on hydrochlorothiazide were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix J, 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 *no evidence of carcinogenic activity** of hydrochlorothiazide for male or female F344/N rats given feed containing 250, 500, or 2,000 ppm hydrochlorothiazide. There was *equivocal evidence of carcinogenic activity* of hydrochlorothiazide for male $B6C3F_1$ mice, based on increased incidences of hepatocellular neoplasms. There was *no evidence of carcinogenic activity* for female $B6C3F_1$ mice given diets containing 2,500 or 5,000 ppm hydrochlorothiazide.

Chronic renal disease was more severe in rats administered hydrochlorothiazide, and increased incidences of secondary lesions (parathyroid hyperplasia, fibrous osteodystrophy, and mineralization in multiple organs) occurred in dosed rats.

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

V. REFERENCES

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APPENDIX A

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

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

	Untreated Control	Low Dose	Mid Dose	High Dose
Animals initially in study	50	50	50	50
Animals necropsied	50	49	50	50
Animals examined histopathologically	50	49	50	50
INTEGUMENTARY SYSTEM				
*Multiple organs	(50)	(49)	(50)	(50)
Fibrous histiocytoma			1 (2%)	
*Skin	(50)	(49)	(50)	(50)
Squamous cell papilloma		1 (2%)		1 (2%)
Squamous cell carcinoma			1 (2%)	1 (2%)
Basal cell tumor	1 (2%)			1 (2%)
Trichoepithelioma	1 (2%)		2 (4%)	1 (2%)
Sebaceous adenoma	1 (2%)			
Keratoacanthoma	6 (12%)	1 (2%)	1 (2%)	3 (6%)
*Subcutaneous tissue	(50)	(49)	(50)	(50)
Fibroma	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Fibrosarcoma	1 (2%)			
Fibrous histiocytoma		1 (2%)		
Neurilemoma, malignant	2 (4%)			
RESPIRATORY SYSTEM				
#Nasal cavity	(50)	(49)	(50)	(50)
Squamous cell carcinoma			1 (2%)	
#Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)			2 (4%)
C-cell carcinoma, metastatic				1 (2%)
Neurilemoma, metastatic	1 (2%)			
HEMATOPOIETIC SYSTEM				
*Multiple organs	(50)	(49)	(50)	(50)
Leukemia, mononuclear cell	33 (66%)	21 (43%)	16 (32%)	25 (50%)
#Spleen	(50)	(49)	(49)	(50)
Fibrosarcoma		1 (2%)		
CIRCULATORY SYSTEM				
*Vertebra	(50)	(49)	(50)	(50)
Hemangiosarcoma	1 (2%)			
DIGESTIVE SYSTEM				
*Palate	(50)	(49)	(50)	(50)
Squamous cell carcinoma	1 (2%)			
*Tongue	(50)	(49)	(50)	(50)
Squamous cell papilloma			1 (2%)	
#Salivary gland	(50)	(49)	(49)	(49)
Neurilemoma, malignant	1 (2%)			
#Liver	(50)	(49)	(50)	(50)
Neoplastic nodule	1 (2%)			2 (4%)
Hepatocellular carcinoma			1 (2%)	
Neurilemoma, metastatic	1 (2%)			
#Forestomach	(50)	(48)	(50)	(50)
Squamous cell papilloma	1 (2%)			
URINARY SYSTEM				
#Kidney	(50)	(49)	(50)	(50)
Tubular cell adenoma	3 (6%)	1 (2%)		1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
ENDOCRINE SYSTEM				
#Anterior pituitary	(50)	(49)	(50)	(50)
Carcinoma, NOS		1 (2%)		
Adenoma, NOS	9 (18%)	7 (14%)	6 (12%)	4 (8%)
#Adrenal cortex	(50)	(49)	(50)	(50)
Carcinoma, NOS	1 (2%)			
Adenoma, NOS	1 (2%)			
#Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma	15 (30%)	21 (43%)	16 (32%)	21 (42%)
Pheochromocytoma, malignant	3 (6%)	1 (2%)	1 (2%)	1 (2%)
#Thyroid	(50)	(49)	(50)	(50)
Follicular cell adenoma			1 (2%)	
Follicular cell carcinoma	1 (2%)			
C-cell adenoma	5 (10%)	6 (12%)	3 (6%)	3 (6%)
C-cell carcinoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
#Pancreatic islets	(50)	(49)	(49)	(50)
Islet cell adenoma	2 (4%)			
Islet cell carcinoma	1 (2%)		1 (2%)	
REPRODUCTIVE SYSTEM				
*Mammary gland	(50)	(49)	(50)	(50)
Adenoma, NOS		1 (2%)		
Adenocarcinoma, NOS		2 (4%)		
Fibroadenoma		1 (2%)		1 (2%)
*Preputial gland	(50)	(49)	(50)	(50)
Carcinoma, NOS	4 (8%)	2 (4%)	3 (6%)	2 (4%)
Adenoma, NOS	2 (4%)	3 (6%)	5 (10%)	4 (8%)
#Testis	(50)	(49)	(50)	(50)
Interstitial cell tumor	46 (92%)	45 (92%)	48 (96%)	46 (92%)
NERVOUS SYSTEM				
#Brain	(50)	(49)	(50)	(50)
Carcinoma, NOS, invasive		1 (2%)		
Granular cell tumor, benign	1 (2%)			
Astrocytoma		1 (2%)		
SPECIAL SENSE ORGANS				
*Zymbal gland	(50)	(49)	(50)	(50)
Carcinoma, NOS	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Adenoma, NOS			1 (2%)	2 (4%)
MUSCULOSKELETAL SYSTEM				
*Bone	(50)	(49)	(50)	(50)
Osteochondroma				1 (2%)
BODY CAVITIES				
*Abdominal cavity	(50)	(49)	(50)	(50)
Mesothelioma, NOS	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Neurilemoma, malignant	1 (2%)			
*Tunica vaginalis	(50)	(49)	(50)	(50)
Mesothelioma, NOS	1 (2%)	1 (2%)	2 (4%)	1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
ALL OTHER SYSTEMS				
*Multiple organs	(50)	(49)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Cranial cavity				
Carcinoma, NOS, metastatic				1
ANIMAL DISPOSITION SUMMARY				
Animals initially in study	50	50	50	50
Natural death	7	11	15	11
Moribund sacrifice	25	28	28	28
Terminal sacrifice	18	11	7	11
TUMOR SUMMARY				
Total animals with primary tumors**	50	49	50	48
Total primary tumors	154	124	116	130
Total animals with benign tumors	49	47	50	47
Total benign tumors	98	91	87	92
Total animals with malignant tumors	45	27	24	32
Total malignant tumors	53	31	26	33
Total animals with secondary tumors##	1	1		2
Total secondary tumors	2	1		2
Total animals with tumors-- uncertain benign or malignant	2	1	2	4
Total uncertain tumors	3	2	3	5

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

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

ANIMAL NUMBER	01 13	03 30	03 32	03 38	02 21	00 01	00 06	00 02	03 37	02 25	02 26	03 39	04 46	00 08	02 29	01 19	02 27	01 12	01 16	00 05	01 10	01 17	04 22	02 24	03 34
WEEKS ON STUDY	01 99	06 66	07 01	07 74	08 00	08 82	08 82	08 84	08 84	08 85	08 86	08 86	08 88	09 00	09 00	09 91	09 93	09 95	09 95	09 96	09 96	09 97	09 97	09 98	09 99
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell tumor																									
Trichoepithelioma																									
Sebaceous adenoma																									
Keratoacanthoma																X									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Fibrosarcoma																									
Neurilemoma, malignant																								X	
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Neurilemoma, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma				X																					
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neurilemoma, malignant														X											
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Neurilemoma, metastatic																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma										X															
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																			X		X				
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
* Animals necropsied

: 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	043	035	033	040	007	036	045	003	004	009	011	014	015	018	020	022	023	028	031	041	044	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	100	101	102	103	104	104	104	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Basal cell tumor																									X	1
Trichoepithelioma																		X								1
Sebaceous adenoma																								X		1
Keratoacanthoma								X							X				X		X			X		6
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma		X													X											2
Fibrosarcoma																			X							1
Neurilemoma, malignant						X																				2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X															X		2
Neurilemoma, metastatic						X																				1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	−	+	−	+	+	+	+	+	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+	40
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma																										1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neurilemoma, malignant																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule											X															1
Neurilemoma, metastatic						X																				1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma																		X								3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

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

ANIMAL NUMBER	0 1 3	0 3 0	0 3 2	0 3 8	0 2 1	0 0 1	0 0 6	0 0 2	0 3 7	0 2 5	0 2 6	0 3 9	0 4 6	0 0 8	0 2 9	0 1 9	0 2 7	0 1 2	0 1 6	0 0 5	0 1 0	0 1 7	0 4 2	0 2 4	0 3 4
WEEKS ON STUDY	0 1 9	0 6 6	0 7 1	0 7 4	0 8 0	0 8 2	0 8 2	0 8 4	0 8 4	0 8 5	0 8 6	0 8 6	0 8 8	0 9 0	0 9 0	0 9 1	0 9 3	0 9 5	0 9 5	0 9 6	0 9 6	0 9 7	0 9 7	0 9 8	0 9 9
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS		X															X					X			
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									X
Adenoma, NOS																									
Pheochromocytoma												X				X					X		X	X	
Pheochromocytoma, malignant															X										
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
C-cell adenoma																									
C-cell carcinoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																					X		X		
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	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	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																			
Adenoma, NOS												X													
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor, benign																									
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																	X								
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
Hemangiosarcoma	X																								
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS																									
Neurilemoma, malignant																						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
Leukemia, mononuclear cell			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	043	035	033	040	007	036	045	003	004	009	011	014	015	018	020	022	023	028	031	041	044	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	100	101	102	103	104	104	104	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS			X			X			X						X	X	X									9
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS																										1
Adenoma, NOS																						X				1
Pheochromocytoma				X				X	X				X			X		X	X		X			X	X	15
Pheochromocytoma, malignant	X																						X			3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell carcinoma											X															1
C-cell adenoma	X			X			X	X																	X	5
C-cell carcinoma										X											X					2
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																										2
Islet cell carcinoma				X																						1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*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	46
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
Carcinoma, NOS							X					X									X					4
Adenoma, NOS									X																	2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor, benign																									X	1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
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	*50
Hemangiosarcoma																										1
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS										X																1
Neurilemoma, malignant																										1
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
Leukemia, mononuclear cell	X	X	X	X	X			X					X	X	X	X		X	X	X		X	X	X		33

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

ANIMAL NUMBER	044	043	004	005	040	049	028	020	018	023	027	016	033	039	048	006	024	010	011	022	025	035	031	041	015
WEEKS ON STUDY	071	072	074	076	078	078	079	083	084	086	088	091	091	091	091	094	094	096	096	097	097	097	099	099	100
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma			X																						
Keratoacanthoma						X																			
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Fibroma							X																		
Fibrous histiocytoma												X													
												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	–	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS												X							X		X				
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma					X			X				X	X	X				X			X		X		
Pheochromocytoma, malignant																						X			
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
C cell adenoma							X																		
C-cell carcinoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS											X														
Adenocarcinoma, NOS																									
Fibroadenoma																									X
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor		X		X	X	X	X	X	X	X	X	X	X	X		X	X	X		X	X		X	X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																					X				
Adenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, invasive																									
Astrocytoma											X														
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS						X																			
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS						X																			
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell	X		X	X			X	X	X	X	X					X	X	X	X					X	

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

ANIMAL NUMBER	046	017	001	012	037	050	038	045	047	003	014	026	029	002	007	008	009	013	019	021	030	032	034	036	042	TOTAL TISSUES TUMORS
WEEKS ON STUDY	100	102	103	103	103	104	105	105	105	106	106	106	106	107	107	107	107	107	107	107	107	107	107	107	107	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Squamous cell papilloma																										1
Keratoacanthoma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Fibroma																		X								3
Fibrous histiocytoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrosarcoma																X										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	−	+	+	+	−	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	43
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	48
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tubular cell adenoma																									X	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS					X																					1
Adenoma, NOS							X					X				X				X						7
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma	X			X		X		X		X			X		X		X	X		X	X			X	X	21
Pheochromocytoma, malignant																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C cell adenoma					X	X	X									X								X		6
C cell carcinoma																X										1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Adenoma, NOS																										1
Adenocarcinoma, NOS												X											X			2
Fibroadenoma																										1
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	45
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
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	*49
Carcinoma, NOS														X												2
Adenoma, NOS		X															X							X		3
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS, invasive					X																					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	*49
Carcinoma, NOS					X																					1
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Mesothelioma, NOS																										1
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Mesothelioma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Leukemia, mononuclear cell	X			X	X	X											X	X			X	X				21

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE: MID DOSE

ANIMAL NUMBER	044	016	005	007	024	029	018	019	020	041	003	006	017	048	032	009	013	014	026	042	001	035	045	050	002
WEEKS ON STUDY	026	071	073	079	084	084	086	086	086	086	088	088	088	088	089	090	090	090	090	093	094	094	094	094	095
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Trichoepithelioma																									
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma		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 papilloma														X											
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma						X																			
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS					X				X	X								X							
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma															X	X		X					X		X
Pheochromocytoma, malignant																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma									X																
C-cell adenoma																									
C-cell carcinoma											X														
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	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	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		
Adenoma, NOS			X																		X			X	
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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																				
Adenoma, NOS																									X
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS																									
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
Fibrous histiocytoma	X																								
Leukemia, mononuclear cell				X			X	X					X				X					X		X	

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

ANIMAL NUMBER	021	028	023	012	036	049	004	040	031	039	043	030	011	022	025	046	008	010	015	027	033	034	037	038	047	TOTAL:
WEEKS ON STUDY	096	096	097	098	098	098	100	000	001	101	101	102	104	104	104	105	106	106	106	106	106	106	106	106	106	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	*50
Squamous cell carcinoma														X												1
Trichoepithelioma			X								X															2
Keratoacanthoma		X																								1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	*50
Fibroma																X					X					2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	–	–	+	+	+	+	–	+	+	–	–	–	+	+	+	+	+	+	+	–	+	+	+	+	42
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																										1
Salivary gland	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																X		X								6
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma					X	X		X	X			X	X	X	X					X			X		X	16
Pheochromocytoma, malignant										X																1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																										1
C cell adenoma	X		X							X																3
C cell carcinoma																										1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell carcinoma																								X		1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	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	48
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
Carcinoma, NOS																			X							3
Adenoma, NOS		X																			X					5
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
Adenoma, NOS																										1
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS																X										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
Fibrous histiocytoma																										1
Leukemia, mononuclear cell	X							X				X						X	X			X	X	X	X	16

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

ANIMAL NUMBER	0022	0033	0025	0039	0049	0028	0048	0030	0037	0010	0026	0036	0047	0005	0035	0043	0001	0003	0002	0050	0038	0014	0021	0040	0017
WEEKS ON STUDY	067	068	071	071	074	079	079	081	087	089	090	090	091	092	092	093	094	094	095	095	096	097	099	099	100
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Squamous cell carcinoma																					X				
Basal cell tumor														X											
Trichoepithelioma																									
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									X
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X		X																	
C-cell carcinoma, metastatic																									X
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	+	−	−	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																	X								
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS						X						X										X			
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma						X				X		X	X		X				X		X		X		X
Pheochromocytoma, malignant																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C cell adenoma																	X								
C cell carcinoma																									X
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
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
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preput ial/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																									
Adenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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			
Adenoma, NOS				X																			X		
MUSCULOSKELETAL SYSTEM																									
Bone	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	+
Osteochondroma																									
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS																	X								X
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS																									X
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Histiocytic sarcoma							X																		
Leukemia, mononuclear cell			X		X	X		X		X	X	X	X	X	X	X	X	X	X	X	X				
Cranial cavity																									
Carcinoma, NOS, metastatic																						X			

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

ANIMAL NUMBER	032	044	045	046	004	007	011	018	023	024	027	006	009	015	008	012	013	016	019	020	029	031	034	041	042	TOTAL:
WEEKS ON STUDY	100	100	101	101	102	102	102	103	103	103	104	105	105	105	106	106	106	106	106	106	106	106	106	106	106	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma													X													1
Squamous cell carcinoma																										1
Basal cell tumor																										1
Trichoepithelioma																			X							1
Keratoacanthoma							X			X							X									3
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										2
C-cell carcinoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	+	36
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule														X												2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma																					X					1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS							X																			4
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma		X		X	X	X			X		X	X			X						X		X	X	X	21
Pheochromocytoma, malignant								X																		1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma																					X	X				3
C-cell carcinoma																										1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma										X																1
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	46
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
Carcinoma, NOS											X														X	2
Adenoma, NOS																	X			X	X			X		4
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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
Adenoma, NOS																										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	*50
Osteochondroma																	X									1
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS																										2
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Histiocytic sarcoma																										1
Leukemia, mononuclear cell	X			X										X	X	X	X	X		X	X					25
Cranial cavity																										
Carcinoma, NOS, metastatic																										1

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

	Control	250 ppm	500 ppm	2,000 ppm
Skin: Basal Cell Tumor, Trichoepithelioma, or Sebaceous Adenoma				
Overall Rates (a)	3/50 (6%)	0/49 (0%)	2/50 (4%)	2/50 (4%)
Adjusted Rates (b)	16.7%	0.0%	10.0%	11.5%
Terminal Rates (c)	3/18 (17%)	0/16 (0%)	0/9 (0%)	1/11 (9%)
Week of First Observation	106		97	92
Life Table Tests (d)	P=0.521	P=0.138N	P=0.633	P=0.671N
Incidental Tumor Tests (d)	P=0.547	P=0.138N	P=0.628N	P=0.674
Cochran-Armitage Trend Test (d)	P=0.612			
Fisher Exact Test (d)		P=0.125N	P=0.500N	P=0.500N
Skin: Keratoacanthoma				
Overall Rates (a)	6/50 (12%)	1/49 (2%)	1/50 (2%)	3/50 (6%)
Adjusted Rates (b)	29.8%	2.2%	4.0%	18.2%
Terminal Rates (c)	5/18 (28%)	0/16 (0%)	0/9 (0%)	1/11 (9%)
Week of First Observation	91	78	96	102
Life Table Tests (d)	P=0.617N	P=0.069N	P=0.183N	P=0.436N
Incidental Tumor Tests (d)	P=0.551N	P=0.062N	P=0.135N	P=0.377N
Cochran-Armitage Trend Test (d)	P=0.486N			
Fisher Exact Test (d)		P=0.059N	P=0.056N	P=0.243N
Subcutaneous Tissue: Fibroma				
Overall Rates (a)	2/50 (4%)	3/49 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	9.5%	10.8%	23.0%	3.8%
Terminal Rates (c)	1/18 (6%)	1/16 (6%)	1/9 (11%)	0/11 (0%)
Week of First Observation	101	79	105	100
Life Table Tests (d)	P=0.390N	P=0.482	P=0.474	P=0.556N
Incidental Tumor Tests (d)	P=0.344N	P=0.421	P=0.634N	P=0.462N
Cochran-Armitage Trend Test (d)	P=0.319N			
Fisher Exact Test (d)		P=0.490	P=0.691	P=0.500N
Subcutaneous Tissue: Fibroma or Fibrosarcoma				
Overall Rates (a)	3/50 (6%)	3/49 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	14.8%	10.8%	23.0%	3.8%
Terminal Rates (c)	2/18 (11%)	1/16 (6%)	1/9 (11%)	0/11 (0%)
Week of First Observation	101	79	105	100
Life Table Tests (d)	P=0.304N	P=0.637	P=0.603	P=0.396N
Incidental Tumor Tests (d)	P=0.263N	P=0.585	P=0.628N	P=0.316N
Cochran-Armitage Trend Test (d)	P=0.227N			
Fisher Exact Test (d)		P=0.651	P=0.500N	P=0.309N
Hematopoietic System: Mononuclear Cell Leukemia				
Overall Rates (a)	33/50 (66%)	21/49 (43%)	16/50 (32%)	25/50 (50%)
Adjusted Rates (b)	80.6%	55.5%	76.8%	74.9%
Terminal Rates (c)	11/18 (61%)	4/16 (25%)	6/9 (67%)	6/11 (55%)
Week of First Observation	71	71	79	71
Life Table Tests (d)	P=0.464	P=0.062N	P=0.086N	P=0.350N
Incidental Tumor Tests (d)	P=0.430N	P=0.018N	P=0.001N	P=0.101N
Cochran-Armitage Trend Test (d)	P=0.391N			
Fisher Exact Test (d)		P=0.017N	P<0.001N	P=0.078N
Kidney: Tubular Cell Adenoma				
Overall Rates (a)	3/50 (6%)	1/49 (2%)	0/50 (0%)	1/50 (2%)
Adjusted Rates (b)	11.4%	6.3%	0.0%	9.1%
Terminal Rates (c)	1/18 (6%)	1/16 (6%)	0/9 (0%)	1/11 (9%)
Week of First Observation	95	106		106
Life Table Tests (d)	P=0.486N	P=0.322N	P=0.197N	P=0.402N
Incidental Tumor Tests (d)	P=0.437N	P=0.296N	P=0.117N	P=0.316N
Cochran-Armitage Trend Test (d)	P=0.397N			
Fisher Exact Test (d)		P=0.316N	P=0.121N	P=0.309N

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

	Control	250 ppm	500 ppm	2,000 ppm
Anterior Pituitary Gland: Adenoma				
Overall Rates (a)	9/50 (18%)	7/49 (14%)	6/50 (12%)	4/50 (8%)
Adjusted Rates (b)	35.0%	29.7%	27.4%	12.3%
Terminal Rates (c)	4/18 (22%)	3/16 (19%)	1/9 (11%)	0/11 (0%)
Week of First Observation	66	91	84	79
Life Table Tests (d)	P=0.201N	P=0.420N	P=0.553N	P=0.209N
Incidental Tumor Tests (d)	P=0.125N	P=0.358N	P=0.337N	P=0.099N
Cochran-Armitage Trend Test (d)	P=0.116N			
Fisher Exact Test (d)		P=0.410N	P=0.288N	P=0.117N
Anterior Pituitary Gland: Adenoma or Carcinoma				
Overall Rates (a)	9/50 (18%)	8/49 (16%)	6/50 (12%)	4/50 (8%)
Adjusted Rates (b)	35.0%	32.7%	27.4%	12.3%
Terminal Rates (c)	4/18 (22%)	3/16 (19%)	1/9 (11%)	0/11 (0%)
Week of First Observation	66	91	84	79
Life Table Tests (d)	P=0.179N	P=0.520N	P=0.553N	P=0.209N
Incidental Tumor Tests (d)	P=0.102N	P=0.456N	P=0.337N	P=0.099N
Cochran-Armitage Trend Test (d)	P=0.098N			
Fisher Exact Test (d)		P=0.518N	P=0.288N	P=0.117N
Adrenal Gland Medulla: Pheochromocytoma				
Overall Rates (a)	15/50 (30%)	21/49 (43%)	16/50 (32%)	21/50 (42%)
Adjusted Rates (b)	59.3%	71.2%	67.1%	71.6%
Terminal Rates (c)	9/18 (50%)	9/16 (56%)	3/9 (33%)	5/11 (45%)
Week of First Observation	86	78	89	79
Life Table Tests (d)	P=0.097	P=0.137	P=0.101	P=0.052
Incidental Tumor Tests (d)	P=0.179	P=0.099	P=0.403	P=0.096
Cochran-Armitage Trend Test (d)	P=0.217			
Fisher Exact Test (d)		P=0.131	P=0.500	P=0.149
Adrenal Gland Medulla: Malignant Pheochromocytoma				
Overall Rates (a)	3/50 (6%)	1/49 (2%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	11.8%	3.2%	5.9%	5.6%
Terminal Rates (c)	1/18 (6%)	0/16 (0%)	0/9 (0%)	0/11 (0%)
Week of First Observation	90	97	101	103
Life Table Tests (d)	P=0.398N	P=0.305N	P=0.433N	P=0.359N
Incidental Tumor Tests (d)	P=0.324N	P=0.322N	P=0.281N	P=0.292N
Cochran-Armitage Trend Test (d)	P=0.367N			
Fisher Exact Test (d)		P=0.316N	P=0.309N	P=0.309N
Adrenal Gland Medulla: Pheochromocytoma or Malignant Pheochromocytoma				
Overall Rates (a)	18/50 (36%)	22/49 (45%)	17/50 (34%)	22/50 (44%)
Adjusted Rates (b)	66.2%	72.1%	69.2%	73.3%
Terminal Rates (c)	10/18 (56%)	9/16 (56%)	3/9 (33%)	5/11 (45%)
Week of First Observation	86	78	89	79
Life Table Tests (d)	P=0.146	P=0.252	P=0.171	P=0.107
Incidental Tumor Tests (d)	P=0.275	P=0.195	P=0.584N	P=0.200
Cochran-Armitage Trend Test (d)	P=0.304			
Fisher Exact Test (d)		P=0.243	P=0.500N	P=0.270
Thyroid Gland: C-Cell Adenoma				
Overall Rates (a)	5/50 (10%)	6/49 (12%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	22.4%	26.4%	13.6%	20.6%
Terminal Rates (c)	2/18 (11%)	2/16 (13%)	0/9 (0%)	2/11 (18%)
Week of First Observation	100	79	96	94
Life Table Tests (d)	P=0.390N	P=0.474	P=0.599N	P=0.529N
Incidental Tumor Tests (d)	P=0.239N	P=0.510	P=0.311N	P=0.375N
Cochran-Armitage Trend Test (d)	P=0.260N			
Fisher Exact Test (d)		P=0.486	P=0.357N	P=0.357N

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

	Control	250 ppm	500 ppm	2,000 ppm
Thyroid Gland: C-Cell Adenoma or Carcinoma				
Overall Rates (a)	7/50 (14%)	6/49 (12%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	32.1%	26.4%	15.7%	23.6%
Terminal Rates (c)	4/18 (22%)	2/16 (13%)	0/9 (0%)	2/11 (18%)
Week of First Observation	100	79	88	94
Life Table Tests (d)	P=0.395N	P=0.538N	P=0.542N	P=0.456N
Incidental Tumor Tests (d)	P=0.252N	P=0.505N	P=0.281N	P=0.302N
Cochran-Armitage Trend Test (d)	P=0.254N			
Fisher Exact Test (d)		P=0.516N	P=0.262N	P=0.262N
Pancreatic Islets: Islet Cell Adenoma or Carcinoma				
Overall Rates (a)	3/50 (6%)	0/49 (0%)	1/49 (2%)	0/50 (0%)
Adjusted Rates (b)	10.8%	0.0%	11.1%	0.0%
Terminal Rates (c)	0/18 (0%)	0/16 (0%)	1/9 (11%)	0/11 (0%)
Week of First Observation	96		106	
Life Table Tests (d)	P=0.198N	P=0.114N	P=0.462N	P=0.138N
Incidental Tumor Tests (d)	P=0.142N	P=0.089N	P=0.275N	P=0.058N
Cochran-Armitage Trend Test (d)	P=0.164N			
Fisher Exact Test (d)		P=0.125N	P=0.316N	P=0.121N
Mammary Gland: Adenoma, Fibroadenoma, or Adenocarcinoma				
Overall Rates (a)	0/50 (0%)	4/49 (8%)	0/50 (0%)	1/50 (2%)
Adjusted Rates (b)	0.0%	18.0%	0.0%	5.6%
Terminal Rates (c)	0/18 (0%)	2/16 (13%)	0/9 (0%)	0/11 (0%)
Week of First Observation		88		103
Life Table Tests (d)	P=0.630N	P=0.061	(e)	P=0.460
Incidental Tumor Tests (d)	P=0.591N	P=0.051	(e)	P=0.581
Cochran-Armitage Trend Test (d)	P=0.570N			
Fisher Exact Test (d)		P=0.056	(e)	P=0.500
Preputial Gland: Adenoma				
Overall Rates (a)	2/50 (4%)	3/49 (6%)	5/50 (10%)	4/50 (8%)
Adjusted Rates (b)	7.9%	16.1%	22.0%	36.4%
Terminal Rates (c)	1/18 (6%)	2/16 (13%)	1/9 (11%)	4/11 (36%)
Week of First Observation	86	102	73	106
Life Table Tests (d)	P=0.248	P=0.474	P=0.138	P=0.165
Incidental Tumor Tests (d)	P=0.306	P=0.447	P=0.187	P=0.153
Cochran-Armitage Trend Test (d)	P=0.382			
Fisher Exact Test (d)		P=0.490	P=0.218	P=0.339
Preputial Gland: Carcinoma				
Overall Rates (a)	4/50 (8%)	2/49 (4%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	17.2%	9.3%	16.0%	15.2%
Terminal Rates (c)	2/18 (11%)	1/16 (6%)	1/9 (11%)	1/11 (9%)
Week of First Observation	82	97	86	104
Life Table Tests (d)	P=0.496N	P=0.368N	P=0.620	P=0.516N
Incidental Tumor Tests (d)	P=0.416N	P=0.370N	P=0.563N	P=0.401N
Cochran-Armitage Trend Test (d)	P=0.370N			
Fisher Exact Test (d)		P=0.349N	P=0.500N	P=0.339N
Preputial Gland: Adenoma or Carcinoma				
Overall Rates (a)	6/50 (12%)	5/49 (10%)	8/50 (16%)	6/50 (12%)
Adjusted Rates (b)	24.3%	24.6%	35.7%	49.1%
Terminal Rates (c)	3/18 (17%)	3/16 (19%)	2/9 (22%)	5/11 (45%)
Week of First Observation	82	97	73	104
Life Table Tests (d)	P=0.380	P=0.542N	P=0.196	P=0.340
Incidental Tumor Tests (d)	P=0.486	P=0.563N	P=0.316	P=0.408
Cochran-Armitage Trend Test (d)	P=0.581			
Fisher Exact Test (d)		P=0.514N	P=0.387	P=0.620

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

	Control	250 ppm	500 ppm	2,000 ppm
Testis: Interstitial Cell Tumor				
Overall Rates (a)	46/50 (92%)	45/49 (92%)	48/50 (96%)	46/50 (92%)
Adjusted Rates (b)	97.8%	100.0%	100.0%	100.0%
Terminal Rates (c)	17/18 (94%)	16/16 (100%)	9/9 (100%)	11/11 (100%)
Week of First Observation	71	72	71	71
Life Table Tests (d)	P=0.228	P=0.547	P=0.034	P=0.191
Incidental Tumor Tests (d)	P=0.548N	P=0.630	P=0.347	P=0.647
Cochran-Armitage Trend Test (d)	P=0.557N			
Fisher Exact Test (d)		P=0.631N	P=0.339	P=0.642
Zymbal Gland: Adenoma or Carcinoma				
Overall Rates (a)	1/50 (2%)	1/49 (2%)	2/50 (4%)	4/50 (8%)
Adjusted Rates (b)	2.9%	4.3%	5.9%	17.1%
Terminal Rates (c)	0/18 (0%)	0/16 (0%)	0/9 (0%)	1/11 (9%)
Week of First Observation	93	103	84	71
Life Table Tests (d)	P=0.077	P=0.752N	P=0.470	P=0.162
Incidental Tumor Tests (d)	P=0.122	P=0.725N	P=0.607	P=0.259
Cochran-Armitage Trend Test (d)	P=0.084			
Fisher Exact Test (d)		P=0.747	P=0.500	P=0.181
All Sites: Benign Tumors				
Overall Rates (a)	49/50 (98%)	47/49 (96%)	50/50 (100%)	47/50 (94%)
Adjusted Rates (b)	100.0%	100.0%	100.0%	100.0%
Terminal Rates (c)	18/18 (100%)	16/16 (100%)	9/9 (100%)	11/11 (100%)
Week of First Observation	66	72	26	71
Life Table Tests (d)	P=0.300	P=0.506N	P=0.043	P=0.259
Incidental Tumor Tests (d)	P=0.177N	P=0.336N	P=0.500	P=0.189N
Cochran-Armitage Trend Test (d)	P=0.196N			
Fisher Exact Test (d)		P=0.492N	P=0.500	P=0.309N
All Sites: Malignant Tumors				
Overall Rates (a)	45/50 (90%)	27/49 (55%)	24/50 (48%)	32/50 (64%)
Adjusted Rates (b)	91.8%	72.3%	82.6%	88.0%
Terminal Rates (c)	14/18 (78%)	8/16 (50%)	6/9 (67%)	8/11 (73%)
Week of First Observation	19	71	71	71
Life Table Tests (d)	P=0.518N	P=0.015N	P=0.078N	P=0.219N
Incidental Tumor Tests (d)	P=0.226N	P<0.001N	P<0.001N	P=0.004N
Cochran-Armitage Trend Test (d)	P=0.182N			
Fisher Exact Test (d)		P<0.001N	P<0.001N	P=0.003N
All Sites: All Tumors				
Overall Rates (a)	50/50 (100%)	49/49 (100%)	50/50 (100%)	48/50 (96%)
Adjusted Rates (b)	100.0%	100.0%	100.0%	100.0%
Terminal Rates (c)	18/18 (100%)	16/16 (100%)	9/9 (100%)	11/11 (100%)
Week of First Observation	19	71	26	71
Life Table Tests (d)	P=0.312	P=0.547	P=0.057	P=0.262
Incidental Tumor Tests (d)	P=0.069N	(f)	(f)	P=0.347N
Cochran-Armitage Trend Test (d)	P=0.044N			
Fisher Exact Test (d)		P=1.000N	P=1.000	P=0.248N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is reported because no tumors were observed in the 500-ppm and control groups.
(f) No P value is reported because all animals in control, 250-ppm, and 500-ppm groups had tumors.

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

	Incidence in Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	(b) 19/1,936 (1.0%)
SD (c)	1.71%
Range (d)	
High	(e) 4/50
Low	0/50

(a) Data as of April 29, 1987 for studies of at least 104 weeks
(b) Includes nine squamous cell carcinomas and one ceruminous carcinoma; no benign tumors have been observed.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Second highest: 3/50; no other control group had more than one tumor.

TABLE A4b. HISTORICAL INCIDENCE OF LEUKEMIA IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

	Incidence in Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	636/1,936 (32.9%)
SD (b)	14.62%
Range (c)	
High	36/50
Low	5/50

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

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

	Untreated Control	Low Dose	Mid Dose	High Dose
Animals initially in study	50	50	50	50
Animals necropsied	50	49	50	50
Animals examined histopathologically	50	49	50	50
INTEGUMENTARY SYSTEM				
*Skin	(50)	(49)	(50)	(50)
Epidermal inclusion cyst		4 (8%)	1 (2%)	
Ulcer, NOS	1 (2%)			
Inflammation, acute focal	1 (2%)		1 (2%)	
Inflammation, chronic focal				2 (4%)
Erosion			1 (2%)	
Fibrosis, focal			1 (2%)	
Hyperplasia, NOS				1 (2%)
Hyperplasia, epithelial	1 (2%)		3 (6%)	3 (6%)
Hyperkeratosis	5 (10%)		1 (2%)	4 (8%)
*Subcutaneous tissue	(50)	(49)	(50)	(50)
Cyst, NOS			1 (2%)	1 (2%)
Edema, NOS		1 (2%)		
Hemorrhage		1 (2%)		
Inflammation, suppurative		2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic focal		2 (4%)		4 (8%)
Necrosis, fat				1 (2%)
RESPIRATORY SYSTEM				
#Nasal cavity	(50)	(49)	(50)	(50)
Foreign body, NOS	3 (6%)	2 (4%)	2 (4%)	7 (14%)
Hemorrhage	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Inflammation, acute focal	9 (18%)	8 (16%)	12 (24%)	13 (26%)
Inflammation, chronic focal		1 (2%)		2 (4%)
Infection, fungal	5 (10%)	2 (4%)	2 (4%)	1 (2%)
Hyperplasia, epithelial	1 (2%)			
#Trachea	(50)	(49)	(50)	(50)
Hemorrhage		1 (2%)		
#Tracheal submucosa	(50)	(49)	(50)	(50)
Inflammation, chronic focal	1 (2%)		1 (2%)	
#Tracheal gland	(50)	(49)	(50)	(50)
Cyst, NOS		2 (4%)		
Inflammation, acute focal	1 (2%)			
#Lung/bronchus	(50)	(49)	(50)	(50)
Fibrosis, focal	1 (2%)			
#Lung	(50)	(49)	(50)	(50)
Mineralization			1 (2%)	3 (6%)
Atelectasis		1 (2%)		
Congestion, NOS	4 (8%)	10 (20%)	8 (16%)	11 (22%)
Edema, NOS	1 (2%)	1 (2%)		2 (4%)
Hemorrhage	7 (14%)	5 (10%)	4 (8%)	8 (16%)
Inflammation, interstitial	1 (2%)		2 (4%)	
Inflammation, suppurative				1 (2%)
Inflammation, chronic focal	3 (6%)	6 (12%)	4 (8%)	4 (8%)
Crystals, NOS	2 (4%)	2 (4%)		
Pigmentation, NOS	1 (2%)	9 (18%)	3 (6%)	6 (12%)
Alveolar macrophages	1 (2%)	4 (8%)	3 (6%)	2 (4%)
Hyperplasia, alveolar epithelium	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Metaplasia, osseous	2 (4%)		2 (4%)	3 (6%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
HEMATOPOIETIC SYSTEM				
*Multiple organs	(50)	(49)	(50)	(50)
Hyperplasia, lymphoid				1 (2%)
Hematopoiesis				1 (2%)
#Bone marrow	(50)	(49)	(50)	(50)
Fibrosis	1 (2%)			
Atrophy, diffuse				1 (2%)
Myelofibrosis	1 (2%)		2 (4%)	1 (2%)
Hyperplasia, hematopoietic		1 (2%)		
Hyperplasia, granulocytic	1 (2%)		1 (2%)	1 (2%)
Hyperplasia, reticulum cell	2 (4%)		1 (2%)	1 (2%)
#Spleen	(50)	(49)	(49)	(50)
Abnormal curvature			1 (2%)	
Congestion, NOS	2 (4%)	4 (8%)	1 (2%)	4 (8%)
Edema, NOS				1 (2%)
Degeneration, NOS				1 (2%)
Necrosis, focal	1 (2%)	3 (6%)		
Infarct, NOS				1 (2%)
Hemosiderosis			1 (2%)	
Atrophy, NOS				1 (2%)
Atrophy, focal	6 (12%)	11 (22%)	7 (14%)	4 (8%)
Atrophy, diffuse		3 (6%)		
Depletion, lymphoid			1 (2%)	
Hyperplasia, reticulum cell			1 (2%)	
Hematopoiesis	7 (14%)	6 (12%)	5 (10%)	
#Lymph node	(50)	(49)	(50)	(50)
Cyst, NOS				1 (2%)
Congestion, NOS			1 (2%)	
Pigmentation, NOS				1 (2%)
Hyperplasia, plasma cell		1 (2%)	1 (2%)	
#Mandibular lymph node	(50)	(49)	(50)	(50)
Cyst, NOS	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Congestion, NOS	2 (4%)	1 (2%)	6 (12%)	5 (10%)
Edema, NOS				1 (2%)
Hyperplasia, plasma cell	4 (8%)	4 (8%)	3 (6%)	1 (2%)
#Thoracic lymph node	(50)	(49)	(50)	(50)
Cyst, NOS	1 (2%)	1 (2%)		
Edema, NOS				1 (2%)
Hemorrhage			1 (2%)	
Inflammation, acute focal				1 (2%)
Necrosis, focal				1 (2%)
Hyperplasia, NOS			1 (2%)	
Hyperplasia, plasma cell	1 (2%)			
Hyperplasia, reticulum cell	1 (2%)			
Hyperplasia, lymphoid	1 (2%)		1 (2%)	
#Hepatic lymph node	(50)	(49)	(50)	(50)
Congestion, NOS				1 (2%)
#Mesenteric lymph node	(50)	(49)	(50)	(50)
Cyst, NOS		1 (2%)		
Congestion, NOS		1 (2%)		1 (2%)
Edema, NOS	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Hemorrhage		1 (2%)	2 (4%)	2 (4%)
Inflammation, acute focal				1 (2%)
Histiocytosis		2 (4%)		
Hyperplasia, plasma cell		2 (4%)		
Hyperplasia, reticulum cell		1 (2%)		1 (2%)
Hyperplasia, lymphoid	1 (2%)			
Mastocytosis		1 (2%)		
#Renal lymph node	(50)	(49)	(50)	(50)
Cyst, NOS	1 (2%)	2 (4%)		
Edema, NOS				2 (4%)
Hemorrhage		1 (2%)	1 (2%)	
Pigmentation, NOS			2 (4%)	

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

	Untreated Control	Low Dose	Mid Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)				
#Brachial lymph node	(50)	(49)	(50)	(50)
Hemorrhage			1 (2%)	
#Inguinal lymph node	(50)	(49)	(50)	(50)
Edema, NOS			1 (2%)	
Hyperplasia, plasma cell			1 (2%)	
#Lung	(50)	(49)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)	
#Liver	(50)	(49)	(50)	(50)
Hematopoiesis	4 (8%)	2 (4%)	1 (2%)	
#Thymus	(40)	(43)	(42)	(36)
Congestion, NOS			2 (5%)	
Hemorrhage	1 (3%)		1 (2%)	
Involution, NOS	27 (68%)	36 (84%)	36 (86%)	29 (81%)
CIRCULATORY SYSTEM				
#Brain/meninges	(50)	(49)	(50)	(50)
Thrombosis, NOS		1 (2%)		
*Mediastinum	(50)	(49)	(50)	(50)
Thrombosis, NOS		1 (2%)		
Periarteritis	1 (2%)			1 (2%)
*Mammary gland	(50)	(49)	(50)	(50)
Thrombosis, NOS			1 (2%)	
#Mandibular lymph node	(50)	(49)	(50)	(50)
Lymphangiectasis				1 (2%)
#Heart	(50)	(49)	(50)	(50)
Mineralization	1 (2%)		1 (2%)	2 (4%)
Dilatation, NOS				1 (2%)
Thrombus, mural				1 (2%)
Hemorrhage	1 (2%)			1 (2%)
Inflammation, chronic focal	41 (82%)	48 (98%)	48 (96%)	43 (86%)
Necrosis, focal		1 (2%)		
#Heart/atrium	(50)	(49)	(50)	(50)
Dilatation, NOS	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Thrombosis, NOS	5 (10%)	4 (8%)	7 (14%)	7 (14%)
#Heart/ventricle	(50)	(49)	(50)	(50)
Thrombosis, NOS	1 (2%)	1 (2%)		
*Artery	(50)	(49)	(50)	(50)
Hypertrophy, NOS				1 (2%)
*Aorta	(50)	(49)	(50)	(50)
Dilatation, NOS			1 (2%)	1 (2%)
*Pulmonary artery	(50)	(49)	(50)	(50)
Mineralization	39 (78%)	21 (43%)	17 (34%)	19 (38%)
Inflammation, chronic focal			1 (2%)	
#Liver	(50)	(49)	(50)	(50)
Thrombosis, NOS		2 (4%)	1 (2%)	
*Mesentery	(50)	(49)	(50)	(50)
Periarteritis		1 (2%)		
DIGESTIVE SYSTEM				
*Tongue	(50)	(49)	(50)	(50)
Hemorrhage	1 (2%)			
#Salivary gland	(50)	(49)	(49)	(49)
Hemorrhage		1 (2%)		
Inflammation, acute focal			2 (4%)	
Inflammation, chronic focal			1 (2%)	1 (2%)
Basophilic cyto change	1 (2%)			
Atrophy, focal	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Hyperplasia, epithelial	1 (2%)			
Hyperplasia, intraductal			1 (2%)	1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
DIGESTIVE SYSTEM (Continued)				
#Liver	(50)	(49)	(50)	(50)
Hernia, NOS	1 (2%)			
Abnormal curvature	3 (6%)	2 (4%)	7 (14%)	1 (2%)
Cyst, NOS	1 (2%)		1 (2%)	
Congestion, NOS	4 (8%)	4 (8%)	5 (10%)	4 (8%)
Hemorrhage			2 (4%)	3 (6%)
Inflammation, chronic focal	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Degeneration, cystic	13 (26%)	13 (27%)	11 (22%)	14 (28%)
Peliosis hepatis	1 (2%)			
Necrosis, focal	7 (14%)	7 (14%)	5 (10%)	12 (24%)
Pigmentation, NOS				2 (4%)
#Liver/hepatocytes	(50)	(49)	(50)	(50)
Degeneration, NOS	1 (2%)		1 (2%)	2 (4%)
Cytoplasmic vacuolization	13 (26%)	6 (12%)	4 (8%)	7 (14%)
Basophilic cyto change	18 (36%)	20 (41%)	23 (46%)	18 (36%)
Eosinophilic cyto change	5 (10%)		1 (2%)	1 (2%)
Clear cell change	1 (2%)	2 (4%)	1 (2%)	1 (2%)
#Bile duct	(50)	(49)	(50)	(50)
Dilatation, NOS		1 (2%)		
Inflammation, suppurative				1 (2%)
Hyperplasia, focal	45 (90%)	40 (82%)	39 (78%)	40 (80%)
#Pancreas	(50)	(49)	(49)	(50)
Edema, NOS			2 (4%)	1 (2%)
Inflammation, chronic focal	6 (12%)	2 (4%)	2 (4%)	2 (4%)
Necrosis, focal	1 (2%)			
#Pancreatic duct	(50)	(49)	(49)	(50)
Calculus, unknown gross or micro		1 (2%)		
#Pancreatic acinus	(50)	(49)	(49)	(50)
Basophilic cyto change	2 (4%)			
Atrophy, focal	33 (66%)	27 (55%)	26 (53%)	26 (52%)
Atrophy, diffuse		1 (2%)	1 (2%)	
*Esophageal lumen	(50)	(49)	(50)	(50)
Inflammation, acute	1 (2%)			
#Esophagus	(50)	(49)	(50)	(50)
Hemorrhage	1 (2%)			
Hyperkeratosis	1 (2%)			
#Stomach	(50)	(48)	(50)	(50)
Mineralization			2 (4%)	
#Glandular stomach	(50)	(48)	(50)	(50)
Mineralization	3 (6%)	2 (4%)		3 (6%)
Cyst, NOS				1 (2%)
Hemorrhage				1 (2%)
Ulcer, NOS	2 (4%)	2 (4%)		2 (4%)
Inflammation, acute focal	1 (2%)			
Inflammation, chronic focal			1 (2%)	2 (4%)
Necrosis, focal	1 (2%)	1 (2%)		
Hyperplasia, focal		1 (2%)		
#Forestomach	(50)	(48)	(50)	(50)
Mineralization		1 (2%)	2 (4%)	2 (4%)
Edema, NOS	4 (8%)	2 (4%)	2 (4%)	1 (2%)
Hemorrhage	1 (2%)		1 (2%)	
Ulcer, NOS	5 (10%)			2 (4%)
Inflammation, acute focal	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Inflammation, chronic focal	2 (4%)		2 (4%)	1 (2%)
Hyperplasia, epithelial	6 (12%)	4 (8%)	9 (18%)	3 (6%)
#Small intestine	(50)	(48)	(50)	(50)
Inflammation, acute focal		2 (4%)	1 (2%)	
#Jejunum	(50)	(48)	(50)	(50)
Congestion, NOS				1 (2%)
#Ileum	(50)	(48)	(50)	(50)
Adhesion, NOS			1 (2%)	

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

	Untreated Control	Low Dose	Mid Dose	High Dose
DIGESTIVE SYSTEM (Continued)				
#Large intestine	(50)	(48)	(47)	(50)
Inflammation, acute focal			1 (2%)	
#Colon	(50)	(48)	(47)	(50)
Mineralization			1 (2%)	
Edema, NOS				1 (2%)
Parasitism	3 (6%)	1 (2%)		4 (8%)
#Cecum	(50)	(48)	(47)	(50)
Congestion, NOS			1 (2%)	1 (2%)
Edema, NOS		2 (4%)	2 (4%)	1 (2%)
Inflammation, acute focal	1 (2%)	1 (2%)	1 (2%)	
Inflammation, chronic focal	1 (2%)			
Parasitism	1 (2%)			
URINARY SYSTEM				
#Urinary bladder/cavity	(50)	(49)	(50)	(50)
Dilatation, NOS	1 (2%)			
#Kidney	(50)	(49)	(50)	(50)
Mineralization			1 (2%)	7 (14%)
Cyst, NOS	2 (4%)	19 (39%)	21 (42%)	18 (36%)
Congestion, NOS			2 (4%)	2 (4%)
Hemorrhage		1 (2%)		
Inflammation, acute focal		1 (2%)		
Nephropathy	50 (100%)	49 (100%)	50 (100%)	50 (100%)
Infarct, NOS	1 (2%)			
Pigmentation, NOS	20 (40%)	10 (20%)	9 (18%)	18 (36%)
Hyperplasia, tubular cell			1 (2%)	
#Kidney/tubule	(50)	(49)	(50)	(50)
Degeneration, NOS				1 (2%)
Necrosis, focal	2 (4%)	3 (6%)		
#Kidney/pelvis	(50)	(49)	(50)	(50)
Dilatation, NOS	1 (2%)			1 (2%)
Hemorrhage			1 (2%)	
Inflammation, suppurative	1 (2%)	1 (2%)		
Hyperplasia, epithelial	6 (12%)	21 (43%)	26 (52%)	23 (46%)
#Urinary bladder/mucosa	(50)	(49)	(50)	(50)
Hyperplasia, epithelial			1 (2%)	
#Urinary bladder/submucosa	(50)	(49)	(50)	(50)
Edema, NOS	1 (2%)			
Hemorrhage	1 (2%)			
Inflammation, focal	1 (2%)			
Inflammation, acute focal			1 (2%)	
Inflammation, chronic focal		1 (2%)		
*Urethra	(50)	(49)	(50)	(50)
Hyperplasia, epithelial				1 (2%)
ENDOCRINE SYSTEM				
#Pituitary intermedia	(50)	(49)	(50)	(50)
Cyst, NOS	5 (10%)	2 (4%)	4 (8%)	1 (2%)
Pigmentation, NOS			1 (2%)	
Atrophy, focal				1 (2%)
Hyperplasia, focal			3 (6%)	
#Anterior pituitary	(50)	(49)	(50)	(50)
Cyst, NOS	5 (10%)	6 (12%)	6 (12%)	3 (6%)
Congestion, NOS		2 (4%)	4 (8%)	1 (2%)
Hemorrhage	1 (2%)			
Necrosis, focal	1 (2%)			
Hyperplasia, focal	12 (24%)	11 (22%)	11 (22%)	12 (24%)
#Pituitary posterior	(50)	(49)	(50)	(50)
Gliosis		1 (2%)		1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
ENDOCRINE SYSTEM (Continued)				
#Adrenal	(50)	(49)	(50)	(50)
Congenital hypoplasia		1 (2%)		
#Adrenal/capsule	(50)	(49)	(50)	(50)
Hyperplasia, focal				1 (2%)
#Adrenal cortex	(50)	(49)	(50)	(50)
Cyst, NOS				4 (8%)
Congestion, NOS	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Hemorrhage	1 (2%)			2 (4%)
Inflammation, acute focal			1 (2%)	
Degeneration, NOS	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Necrosis, focal	3 (6%)	1 (2%)	2 (4%)	
Cytoplasmic vacuolization	6 (12%)	2 (4%)	3 (6%)	5 (10%)
Hyperplasia, focal	22 (44%)	12 (24%)	9 (18%)	7 (14%)
Angiectasis	2 (4%)		2 (4%)	5 (10%)
#Adrenal medulla	(50)	(49)	(50)	(50)
Congestion, NOS			1 (2%)	
Inflammation, chronic focal			1 (2%)	
Hyperplasia, NOS	9 (18%)	9 (18%)	14 (28%)	14 (28%)
#Thyroid	(50)	(49)	(50)	(50)
Cyst, NOS		2 (4%)		1 (2%)
Follicular cyst, NOS	1 (2%)		1 (2%)	
Pigmentation, NOS		1 (2%)	1 (2%)	1 (2%)
Atrophy, focal				1 (2%)
Hyperplasia, C-cell	10 (20%)	6 (12%)	10 (20%)	5 (10%)
#Parathyroid	(50)	(49)	(50)	(49)
Atrophy, NOS		1 (2%)		
Hyperplasia, NOS	7 (14%)	20 (41%)	30 (60%)	28 (57%)
#Pancreatic islets	(50)	(49)	(49)	(50)
Hyperplasia, focal	3 (6%)		1 (2%)	
REPRODUCTIVE SYSTEM				
*Mammary gland	(50)	(49)	(50)	(50)
Galactocele	1 (2%)			1 (2%)
Hyperplasia, NOS	1 (2%)	2 (4%)		2 (4%)
Hyperplasia, cystic	11 (22%)	11 (22%)	10 (20%)	10 (20%)
Lactation	1 (2%)			1 (2%)
*Preputial gland	(50)	(49)	(50)	(50)
Cyst, NOS	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Inflammation, suppurative	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Inflammation, chronic focal				1 (2%)
Necrosis, focal		1 (2%)		
Atrophy, NOS	45 (90%)	44 (90%)	44 (88%)	45 (90%)
Hyperplasia, NOS	3 (6%)	6 (12%)	1 (2%)	7 (14%)
#Prostate	(50)	(49)	(50)	(50)
Inflammation, suppurative	2 (4%)	6 (12%)	1 (2%)	7 (14%)
Inflammation, chronic focal	3 (6%)	3 (6%)	4 (8%)	2 (4%)
Hyperplasia, epithelial	4 (8%)	9 (18%)	5 (10%)	1 (2%)
*Seminal vesicle	(50)	(49)	(50)	(50)
Dilatation, NOS				1 (2%)
Atrophy, NOS	23 (46%)	43 (88%)	42 (84%)	22 (44%)
#Testis	(50)	(49)	(50)	(50)
Mineralization	3 (6%)	4 (8%)	1 (2%)	2 (4%)
Congestion, NOS			1 (2%)	
Necrosis, diffuse	1 (2%)			
Atrophy, focal	42 (84%)	43 (88%)	39 (78%)	42 (84%)
Atrophy, diffuse	11 (22%)	13 (27%)	12 (24%)	17 (34%)
Hyperplasia, interstitial cell	11 (22%)	8 (16%)	10 (20%)	13 (26%)
*Epididymis	(50)	(49)	(50)	(50)
Degeneration, NOS	14 (28%)	15 (31%)	8 (16%)	8 (16%)
Hyperplasia, epithelial	1 (2%)			

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

	Untreated Control	Low Dose	Mid Dose	High Dose
NERVOUS SYSTEM				
#Brain/meninges	(50)	(49)	(50)	(50)
Hyperplasia, focal	1 (2%)			
#Brain	(50)	(49)	(50)	(50)
Mineralization	1 (2%)	1 (2%)	1 (2%)	
Hydrocephalus, NOS	1 (2%)	2 (4%)		
Congestion, NOS		1 (2%)		
Hemorrhage	7 (14%)	2 (4%)		3 (6%)
Hemorrhagic cyst				1 (2%)
Gliosis	1 (2%)			
Necrosis, focal	1 (2%)	2 (4%)		
Pigmentation, NOS	1 (2%)			
SPECIAL SENSE ORGANS				
*Eye	(50)	(49)	(50)	(50)
Acquired absence				1 (2%)
*Eye/anterior chamber	(50)	(49)	(50)	(50)
Hemorrhage		1 (2%)		
Inflammation, acute focal			1 (2%)	
*Eye/sclera	(50)	(49)	(50)	(50)
Mineralization	1 (2%)	1 (2%)		2 (4%)
*Eye/cornea	(50)	(49)	(50)	(50)
Mineralization		1 (2%)		1 (2%)
Inflammation, acute focal			1 (2%)	2 (4%)
Inflammation, chronic focal		2 (4%)		2 (4%)
Vascularization			1 (2%)	
*Eye/retina	(50)	(49)	(50)	(50)
Atrophy, NOS				1 (2%)
Atrophy, focal	3 (6%)		1 (2%)	3 (6%)
Atrophy, diffuse	2 (4%)	3 (6%)	1 (2%)	
*Eye/crystalline lens	(50)	(49)	(50)	(50)
Degeneration, NOS	2 (4%)	3 (6%)	1 (2%)	
*Nasolacrimal duct	(50)	(49)	(50)	(50)
Hemorrhage		1 (2%)	1 (2%)	
Inflammation, acute focal	2 (4%)	5 (10%)	2 (4%)	7 (14%)
*Harderian gland	(50)	(49)	(50)	(50)
Inflammation, chronic focal			1 (2%)	2 (4%)
Pigmentation, NOS			1 (2%)	
Hyperplasia, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
*Bone	(50)	(49)	(50)	(50)
Necrosis, focal	1 (2%)			1 (2%)
Fibrous osteodystrophy	2 (4%)	18 (37%)	23 (46%)	22 (44%)
*Skull	(50)	(49)	(50)	(50)
Abnormal curvature				1 (2%)
*Vertebra	(50)	(49)	(50)	(50)
Abnormal curvature		1 (2%)		
*Skeletal muscle	(50)	(49)	(50)	(50)
Hemorrhage	1 (2%)			1 (2%)
BODY CAVITIES				
*Mediastinum	(50)	(49)	(50)	(50)
Hemorrhage		1 (2%)		
*Abdominal cavity	(50)	(49)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation, chronic focal		1 (2%)	1 (2%)	
Necrosis, fat	6 (12%)	5 (10%)	5 (10%)	8 (16%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
BODY CAVITIES (Continued)				
*Mesentery	(50)	(49)	(50)	(50)
Angiectasis			1 (2%)	
ALL OTHER SYSTEMS				
*Multiple organs	(50)	(49)	(50)	(50)
Mineralization	1 (2%)	19 (39%)	26 (52%)	20 (40%)
Diaphragm				
Mineralization			1	
Foot				
Inflammation, chronic focal		1		
SPECIAL MORPHOLOGY SUMMARY				
Autolysis/no necropsy		1		

* 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 HYDROCHLOROTHIAZIDE

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

	Untreated Control	Low Dose	Mid Dose	High Dose
Animals initially in study	50	50	50	50
Animals necropsied	50	50	49	50
Animals examined histopathologically	50	50	49	50
INTEGUMENTARY SYSTEM				
*Multiple organs	(50)	(50)	(49)	(50)
Fibrous histiocytoma, malignant		1 (2%)		
*Skin	(50)	(50)	(49)	(50)
Papilloma, NOS				1 (2%)
Squamous cell papilloma			1 (2%)	1 (2%)
Squamous cell carcinoma		1 (2%)		
Basal cell tumor			1 (2%)	
Trichoepithelioma			1 (2%)	
*Subcutaneous tissue	(50)	(50)	(49)	(50)
Basal cell tumor	1 (2%)			
Sarcoma, NOS		1 (2%)		1 (2%)
RESPIRATORY SYSTEM				
#Lung	(50)	(30)	(26)	(50)
Carcinoma, NOS, metastatic	2 (4%)			1 (2%)
Alveolar/bronchiolar adenoma		1 (3%)		
Alveolar/bronchiolar carcinoma	1 (2%)			
Follicular cell carcinoma, metastatic	1 (2%)			
C-cell carcinoma, metastatic		1 (3%)		
Sarcoma, NOS, metastatic				1 (2%)
Chordoma, metastatic	1 (2%)			
HEMATOPOIETIC SYSTEM				
*Multiple organs	(50)	(50)	(49)	(50)
Leukemia, mononuclear cell	6 (12%)	5 (10%)	13 (27%)	10 (20%)
#Mandibular lymph node	(50)	(26)	(27)	(50)
Sarcoma, NOS, metastatic				1 (2%)
#Mesenteric lymph node	(50)	(26)	(27)	(50)
Endometrial stromal sarcoma, metastatic		1 (4%)		
#Liver	(50)	(50)	(49)	(50)
Leukemia, mononuclear cell			1 (2%)	
CIRCULATORY SYSTEM				
#Uterus	(50)	(50)	(49)	(50)
Hemangiosarcoma		1 (2%)		
DIGESTIVE SYSTEM				
*Palate	(50)	(50)	(49)	(50)
Squamous cell papilloma		1 (2%)		1 (2%)
Squamous cell carcinoma		1 (2%)		
*Tongue	(50)	(50)	(49)	(50)
Squamous cell papilloma			1 (2%)	
Squamous cell carcinoma	1 (2%)			
URINARY SYSTEM				
#Kidney	(50)	(50)	(49)	(50)
Tubular cell adenoma			1 (2%)	1 (2%)
#Urinary bladder/mucosa	(50)	(14)	(11)	(49)
Transitional cell carcinoma	1 (2%)			

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

	Untreated Control	Low Dose	Mid Dose	High Dose
ENDOCRINE SYSTEM				
#Anterior pituitary	(50)	(50)	(48)	(49)
Carcinoma, NOS	1 (2%)			
Adenoma, NOS	23 (46%)	13 (26%)	21 (44%)	16 (33%)
#Adrenal cortex	(50)	(49)	(49)	(50)
Adenoma, NOS				1 (2%)
#Adrenal medulla	(50)	(49)	(49)	(50)
Pheochromocytoma	2 (4%)	1 (2%)	6 (12%)	4 (8%)
#Thyroid	(50)	(48)	(49)	(50)
Follicular cell carcinoma	1 (2%)	1 (2%)		
C-cell adenoma	6 (12%)	3 (6%)	5 (10%)	5 (10%)
C-cell carcinoma		2 (4%)	2 (4%)	2 (4%)
#Pancreatic islets	(50)	(50)	(49)	(49)
Islet cell adenoma	2 (4%)		1 (2%)	
Islet cell carcinoma	1 (2%)			
REPRODUCTIVE SYSTEM				
*Mammary gland	(50)	(50)	(49)	(50)
Carcinoma, NOS		1 (2%)		
Adenoma, NOS			1 (2%)	1 (2%)
Adenocarcinoma, NOS	2 (4%)		1 (2%)	2 (4%)
Fibroadenoma	30 (60%)	12 (24%)	11 (22%)	5 (10%)
*Clitoral gland	(50)	(50)	(49)	(50)
Carcinoma, NOS	4 (8%)	1 (2%)		2 (4%)
Adenoma, NOS	1 (2%)	2 (4%)	2 (4%)	1 (2%)
#Uterus	(50)	(50)	(49)	(50)
Leiomyoma	2 (4%)	1 (2%)		
Leiomyosarcoma			1 (2%)	
Endometrial stromal polyp	18 (36%)	6 (12%)	10 (20%)	11 (22%)
Endometrial stromal sarcoma	1 (2%)	1 (2%)		
#Cervix uteri	(50)	(50)	(49)	(50)
Squamous cell carcinoma		1 (2%)		
#Uterus/endometrium	(50)	(50)	(49)	(50)
Adenoma, NOS			1 (2%)	
#Ovary	(50)	(18)	(15)	(50)
Thecoma			1 (7%)	
NERVOUS SYSTEM				
#Brain	(50)	(14)	(11)	(50)
Astrocytoma	1 (2%)			
Meningioma	1 (2%)			
SPECIAL SENSE ORGANS				
*Zymbal gland	(50)	(50)	(49)	(50)
Carcinoma, NOS	1 (2%)		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM				
*Skeletal muscle	(50)	(50)	(49)	(50)
C-cell carcinoma, metastatic		1 (2%)		
BODY CAVITIES				
*Abdominal cavity	(50)	(50)	(49)	(50)
Mesothelioma, NOS				1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
ALL OTHER SYSTEMS				
*Multiple organs	(50)	(50)	(49)	(50)
Histiocytic sarcoma				1 (2%)
ANIMAL DISPOSITION SUMMARY				
Animals initially in study	50	50	50	50
Natural death	9	16	7	10
Moribund sacrifice	11	10	16	13
Terminal sacrifice	30	24	27	27
TUMOR SUMMARY				
Total animals with primary tumors**	49	39	39	37
Total primary tumors	107	57	83	68
Total animals with benign tumors	43	32	36	31
Total benign tumors	85	40	64	48
Total animals with malignant tumors	20	16	19	18
Total malignant tumors	22	17	19	19
Total animals with secondary tumors##	4	2		2
Total secondary tumors	4	3		3
Total animals with tumors-- uncertain benign or malignant				1
Total uncertain tumors				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 HYDROCHLOROTHIAZIDE: UNTREATED CONTROL

ANIMAL NUMBER	047	044	029	005	003	027	019	026	035	043	050	011	018	009	031	012	015	014	032	010	001	002	004	006	007
WEEKS ON STUDY	073	086	087	088	092	092	093	093	093	093	093	097	097	101	101	102	102	104	105	106	107	107	107	107	107
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell tumor																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic						X												X							
Alveolar/bronchiolar carcinoma																									
Follicular cell carcinoma, metastatic																									
Chordoma, metastatic																			X						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional cell carcinoma				X																					
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS			X		X						X	X	X		X	X		X	X	X	X			X	X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
C cell adenoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS													X												
Fibroadenoma			X		X	X			X		X	X		X	X	X			X			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												X							
Adenoma, NOS							X																		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma												X													
Endometrial stromal polyp				X		X	X		X				X	X								X			
Endometrial stromal sarcoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma									X																
Meningioma										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		
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								

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
* Animals necropsied

: 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	0008	0013	0016	0017	0020	0021	0022	0023	0024	0025	0028	0030	0033	0034	0036	0037	0038	0039	0040	0041	0042	0045	0046	0048	0049	TOTAL TISSUES TUMORS
WEEKS ON STUDY	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Basal cell tumor														X												1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																										2
Alveolar/bronchiolar carcinoma															X											1
Follicular cell carcinoma, metastatic																X										1
Chordoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
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																					1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional cell carcinoma																										1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS																								X		1
Adenoma, NOS	X							X			X	X	X	X			X	X					X		X	23
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma			X								X															2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell carcinoma																X										1
C cell adenoma		X					X					X	X	X			X									6
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma													X										X			2
Islet cell carcinoma																									X	1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenocarcinoma, NOS											X															2
Fibroadenoma		X	X	X		X	X	X	X			X	X				X	X	X	X	X		X		X	30
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																			4
Adenoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma											X															2
Endometrial stromal polyp			X			X				X	X		X	X		X			X	X			X	X		18
Endometrial stromal sarcoma		X																								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma																										1
Meningioma																										1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
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													6

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

ANIMAL NUMBER	0034	0040	0009	0002	0113	0112	0339	0433	0530	0444	0828	0835	0838	0916	0445	0911	0937	0920	0919	0941	0006	0047	0005	0949	0014
WEEKS ON STUDY	0027	0332	0336	0442	0424	0444	0449	0550	0556	0583	0584	0587	0589	0594	0594	0595	0595	0596	0597	0598	1000	1003	1004	1005	1006
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+
Alveolar/bronchiolar adenoma																X									
C cell carcinoma, metastatic																				X					
Trachea	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	−	−	−	−	−	−
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	+	−	+	−	−	−	−
Endometrial stromal sarcoma, metastatic								X																	
Thymus	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	−	−	−	+	−	−	+	−	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma																									
Squamous cell carcinoma																									
Salivary gland	+	−	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	−	−	−	−	+	+	+	+	+	+
Small intestine	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+
Large intestine	+	+	+	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	+	−	−	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS													X	X	X		X	X		X		X			
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									X
Thyroid	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
C cell adenoma																							X		
C cell carcinoma																				X					
Parathyroid	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Fibroadenoma									X							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																									
Adenoma, NOS																					X				
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Leiomyoma																									
Endometrial stromal polyp								X										X							
Endometrial stromal sarcoma								X																	
Hemangiosarcoma										X															
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−	+	−	−	−
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−
MUSCULOSKELETAL SYSTEM																									
Muscle	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
C cell carcinoma, metastatic																				X					
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant												X													
Leukemia, mononuclear cell											X					X			X						

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

ANIMAL NUMBER	022	001	003	004	007	008	010	015	017	018	021	023	024	025	026	027	029	031	032	033	036	042	046	048	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	106	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	107	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma																			X							1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	–	+	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	–	–	+	–	–	30
Alveolar/bronchiolar adenoma																										1
C-cell carcinoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	–	–	–	–	–	–	–	+	–	–	+	+	–	–	+	–	–	–	–	–	–	+	–	–	–	21
Lymph nodes	–	+	–	+	–	–	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	+	+	+	–	26
Endometrial stromal sarcoma, meta																										1
Thymus	–	+	–	–	–	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	18
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	21
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																			X							1
Squamous cell carcinoma	X																									1
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	20
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	15
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–		14
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS			X	X		X											X	X				X				13
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell carcinoma											X															1
C cell adenoma		X					X																			3
C cell carcinoma				X																						2
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Carcinoma, NOS																							X			1
Fibroadenoma					X				X					X		X		X				X	X			12
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														1
Adenoma, NOS																			X							2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma			X																							1
Leiomyoma																								X		1
Endometrial stromal polyp						X		X		X		X														6
Endometrial stromal sarcoma																										1
Hemangiosarcoma																										1
Ovary	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	18
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
MUSCULOSKELETAL SYSTEM																										
Muscle	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
C-cell carcinoma, metastatic																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Fibrous histiocytoma, malignant																										1
Leukemia, mononuclear cell								X									X									5

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE: MID DOSE

ANIMAL NUMBER	0034	0003	0330	0332	0044	0045	0177	0220	0007	0006	0448	0095	0002	0113	0449	0224	0335	0110	0112	0001	0008	0009	0111	0114
WEEKS ON STUDY	020	022	038	066	072	073	078	087	089	091	092	096	097	098	099	102	102	103	103	106	106	106	106	106
INTEGUMENTARY SYSTEM																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+
Squamous cell papilloma										X														
Basal cell tumor																								
Trichoepithelioma																								
RESPIRATORY SYSTEM																								
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–	+	–	–	+	+	+	–
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	–	+	+	+	–	+	+	–	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	+	–	–	–	+	+	–	–
Thymus	+	+	+	+	–	+	+	+	–	+	+	–	+	+	–	–	–	–	–	–	+	+	–	–
CIRCULATORY SYSTEM																								
Heart	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	+	–	+	–	–	–	–	–
DIGESTIVE SYSTEM																								
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma																								
Salivary gland	+	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia, mononuclear cell																				X				
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–
Small intestine	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–
Large intestine	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–
URINARY SYSTEM																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																								
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS				X			X	X	X				X	X			X	X	X	X	X	X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma											X	X												X
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C cell adenoma									X			X												X
C cell carcinoma																								
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																								
REPRODUCTIVE SYSTEM																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																								
Adenocarcinoma, NOS																								
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
Adenoma, NOS													X											X
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																								
Leiomyosarcoma																								
Endometrial stromal polyp								X			X				X	X		X				X		
Ovary	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	–	–	+	–
Thecoma																								
NERVOUS SYSTEM																								
Brain	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
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
Carcinoma, 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
Leukemia, mononuclear cell					X	X	X			X		X					X	X			X	X		X

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

ANIMAL NUMBER	0 1 5	0 1 6	0 1 8	0 1 9	0 2 1	0 2 2	0 2 3	0 2 5	0 2 6	0 2 7	0 2 8	0 2 9	0 3 1	0 3 6	0 4 0	0 4 7	0 3 7	0 3 8	0 3 9	0 4 1	0 4 2	0 4 3	0 4 4	0 4 6	0 5 0	TOTAL:
WEEKS ON STUDY	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	TISSUES / TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	*49
Squamous cell papilloma																										1
Basal cell tumor	X																									1
Trichoepithelioma								X																		1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	–	–	+	–	–	–	+	+	–	–	–	–	–	A	+	–	–	–	–	–	–	–	+	+	26
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	–	–	–	–	–	+	–	+	–	–	–	+	+	A	–	–	–	–	–	–	–	–	+	–	25
Lymph nodes	+	+	–	+	–	–	+	+	+	–	–	–	–	+	A	+	–	–	–	–	+	–	+	–	–	27
Thymus	–	+	–	+	–	–	–	–	+	–	–	–	–	+	A	–	–	–	+	–	+	–	–	–	–	19
CIRCULATORY SYSTEM																										
Heart	–	+	–	–	+	–	–	+	–	–	–	–	–	+	A	–	–	–	–	–	–	–	–	+	–	–	18
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	*49
Squamous cell papilloma				X																						1
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	9
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Leukemia, *mononuclear cell*																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Stomach	–	–	–	–	–	+	–	–	–	–	–	–	–	–	A	+	–	–	–	–	–	–	–	–	–	14
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	12
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	12
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Tubular cell adenoma										X																1
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	11
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	–	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS	X		X				X	X		X	X					X				X					X	21
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma	X								X							X										6
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
C-cell adenoma																				X				X		5
C cell carcinoma				X								X														2
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Islet cell adenoma				X																						1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	*49
Adenoma, NOS												X														1
Adenocarcinoma, NOS																						X				1
Fibroadenoma	X					X		X		X							X			X				X		11
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	*49
Adenoma, NOS																										2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS																		X								1
Leiomyosarcoma																				X						1
Endometrial stromal polyp	X			X																	X	X				10
Ovary	–	+	–	–	–	–	–	–	–	–	–	–	–	+	A	–	–	–	–	–	–	–	–	–	–	15
Thecoma		X																								1
NERVOUS SYSTEM																										
Brain	–	+	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	11
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	*49
Carcinoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	*49
Leukemia, mononuclear cell	X						X		X																	13

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

ANIMAL NUMBER	0047	0014	0032	0113	0046	0027	0004	0044	0023	0037	0030	0034	0050	0036	0009	0117	0031	0038	0049	0112	0022	0029	0040	0001	0002
WEEKS ON STUDY	027	028	040	050	050	066	079	081	088	090	094	094	094	095	097	099	099	102	102	104	104	105	105	106	106
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																			X						
Squamous cell papilloma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																					X				
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																		X							
Sarcoma, NOS, metastatic																					X				
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic																					X				
Thymus	+	+	+	+	+	+	+	+	+	−	−	−	+	+	−	+	+	−	+	+	−	−	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma																			X						
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																								X	
Urinary bladder	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS								X			X	X					X		X	X		X	X	X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Pheochromocytoma							X				X						X						X		
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma																X		X							
C cell carcinoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adenocarcinoma, NOS										X												X			
Fibroadenoma																					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
Adenoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp						X	X		X				X		X	X			X						
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS						X																			
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Histiocytic sarcoma							X																		
Leukemia, mononuclear cell									X	X		X	X	X											

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

ANIMAL NUMBER	0003	0005	0006	0007	0008	0010	0011	0015	0016	0018	0019	0020	0021	0024	0025	0026	0028	0033	0035	0039	0041	0042	0043	0045	0048	TOTAL TISSUES TUMORS
WEEKS ON STUDY	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Papilloma, NOS																										1
Squamous cell papilloma										X																1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																										1
Sarcoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, NOS, metastatic																										1
Thymus	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	40
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																										1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS	X	X				X			X	X		X													X	16
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																								X		1
Pheochromocytoma																										4
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma						X							X		X											5
C cell carcinoma	X																								X	2
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	49
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenoma, NOS					X																					1
Adenocarcinoma, NOS																										2
Fibroadenoma						X												X				X	X			5
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										2
Adenoma, NOS																		X								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp		X			X					X		X														11
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Histiocytic sarcoma																										1
Leukemia, mononuclear cell							X				X						X		X				X			10

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

	Control	250 ppm	500 ppm	2,000 ppm
Hematopoietic System: Mononuclear Cell Leukemia				
Overall Rates (a)	6/50 (12%)	(b) 5/50 (10%)	(b) 14/49 (29%)	10/50 (20%)
Adjusted Rates (c)	16.0%	15.3%	36.7%	28.3%
Terminal Rates (d)	3/31 (10%)	2/26 (8%)	7/29 (24%)	5/27 (19%)
Week of First Observation	86	84	72	88
Life Table Tests (e)	P=0.185	P=0.619N	P=0.036	P=0.161
Incidental Tumor Tests (e)	P=0.170	P=0.625N	P=0.041	P=0.144
Cochran-Armitage Trend Test (e)	P=0.208			
Fisher Exact Test (e)		P=0.500N	P=0.035	P=0.207
Anterior Pituitary Gland: Adenoma				
Overall Rates (a)	23/50 (46%)	13/50 (26%)	21/48 (44%)	16/49 (33%)
Adjusted Rates (c)	56.8%	37.6%	55.2%	44.6%
Terminal Rates (d)	14/31 (45%)	6/26 (23%)	12/28 (43%)	8/27 (30%)
Week of First Observation	87	89	72	81
Life Table Tests (e)	P=0.330N	P=0.135N	P=0.569	P=0.246N
Incidental Tumor Tests (e)	P=0.300N	P=0.104N	P=0.452	P=0.224N
Cochran-Armitage Trend Test (e)	P=0.263N			
Fisher Exact Test (e)		P=0.030N	P=0.492N	P=0.124N
Anterior Pituitary Gland: Adenoma or Carcinoma				
Overall Rates (a)	24/50 (48%)	13/50 (26%)	21/48 (44%)	16/49 (33%)
Adjusted Rates (c)	59.3%	37.6%	55.2%	44.6%
Terminal Rates (d)	15/31 (48%)	6/26 (23%)	12/28 (43%)	8/27 (30%)
Week of First Observation	87	89	72	81
Life Table Tests (e)	P=0.291N	P=0.103N	P=0.505N	P=0.197N
Incidental Tumor Tests (e)	P=0.257N	P=0.074N	P=0.531	P=0.171N
Cochran-Armitage Trend Test (e)	P=0.223N			
Fisher Exact Test (e)		P=0.019N	P=0.413N	P=0.088N
Adrenal Gland Medulla: Pheochromocytoma				
Overall Rates (a)	2/50 (4%)	1/49 (2%)	6/49 (12%)	4/50 (8%)
Adjusted Rates (c)	6.5%	4.0%	18.2%	10.6%
Terminal Rates (d)	2/31 (6%)	1/25 (4%)	4/29 (14%)	0/27 (0%)
Week of First Observation	106	106	92	79
Life Table Tests (e)	P=0.266	P=0.575N	P=0.114	P=0.303
Incidental Tumor Tests (e)	P=0.276	P=0.575N	P=0.104	P=0.300
Cochran-Armitage Trend Test (e)	P=0.288			
Fisher Exact Test (e)		P=0.508N	P=0.128	P=0.339
Thyroid Gland: C-Cell Adenoma				
Overall Rates (a)	6/50 (12%)	3/48 (6%)	5/49 (10%)	5/50 (10%)
Adjusted Rates (c)	19.4%	11.0%	14.8%	16.3%
Terminal Rates (d)	6/31 (19%)	2/26 (8%)	3/29 (10%)	3/27 (11%)
Week of First Observation	106	104	89	99
Life Table Tests (e)	P=0.527	P=0.333N	P=0.547N	P=0.586N
Incidental Tumor Tests (e)	P=0.542	P=0.333N	P=0.569N	P=0.579N
Cochran-Armitage Trend Test (e)	P=0.581			
Fisher Exact Test (e)		P=0.264N	P=0.514N	P=0.500N
Thyroid Gland: C-Cell Adenoma or Carcinoma				
Overall Rates (a)	6/50 (12%)	5/48 (10%)	7/49 (14%)	7/50 (14%)
Adjusted Rates (c)	19.4%	17.4%	21.4%	23.2%
Terminal Rates (d)	6/31 (19%)	3/26 (12%)	5/29 (17%)	5/27 (19%)
Week of First Observation	106	98	89	99
Life Table Tests (e)	P=0.363	P=0.621N	P=0.448	P=0.403
Incidental Tumor Tests (e)	P=0.380	P=0.621N	P=0.428	P=0.409
Cochran-Armitage Trend Test (e)	P=0.426			
Fisher Exact Test (e)		P=0.529N	P=0.484	P=0.500

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

	Control	250 ppm	500 ppm	2,000 ppm
Pancreatic Islets: Islet Cell Adenoma or Carcinoma				
Overall Rates (a)	3/50 (6%)	0/50 (0%)	1/49 (2%)	0/49 (0%)
Adjusted Rates (c)	9.7%	0.0%	3.4%	0.0%
Terminal Rates (d)	3/31 (10%)	0/26 (0%)	1/29 (3%)	0/27 (0%)
Week of First Observation	106		106	
Life Table Tests (e)	P=0.178N	P=0.153N	P=0.328N	P=0.145N
Incidental Tumor Tests (e)	P=0.178N	P=0.153N	P=0.328N	P=0.145N
Cochran-Armitage Trend Test (e)	P=0.168N			
Fisher Exact Test (e)		P=0.121N	P=0.316N	P=0.125N
Mammary Gland: Fibroadenoma				
Overall Rates (a)	30/50 (60%)	12/50 (24%)	11/49 (22%)	5/50 (10%)
Adjusted Rates (c)	72.5%	37.7%	34.8%	17.6%
Terminal Rates (d)	20/31 (65%)	7/26 (27%)	9/29 (31%)	4/27 (15%)
Week of First Observation	87	56	92	104
Life Table Tests (e)	P<0.001N	P=0.007N	P<0.001N	P<0.001N
Incidental Tumor Tests (e)	P<0.001N	P=0.003N	P<0.001N	P<0.001N
Cochran-Armitage Trend Test (e)	P<0.001N			
Fisher Exact Test (e)		P<0.001N	P<0.001N	P<0.001N
Mammary Gland: Adenoma or Fibroadenoma				
Overall Rates (a)	30/50 (60%)	12/50 (24%)	12/49 (24%)	6/50 (12%)
Adjusted Rates (c)	72.5%	37.7%	38.0%	21.1%
Terminal Rates (d)	20/31 (65%)	7/26 (27%)	10/29 (34%)	5/27 (19%)
Week of First Observation	87	56	92	104
Life Table Tests (e)	P<0.001N	P=0.007N	P=0.002N	P<0.001N
Incidental Tumor Tests (e)	P<0.001N	P=0.003N	P=0.002N	P<0.001N
Cochran-Armitage Trend Test (e)	P<0.001N			
Fisher Exact Test (e)		P<0.001N	P<0.001N	P<0.001N
Mammary Gland: Adenoma, Adenocarcinoma, or Carcinoma				
Overall Rates (a)	2/50 (4%)	1/50 (2%)	2/49 (4%)	3/50 (6%)
Adjusted Rates (c)	5.7%	3.8%	6.9%	9.3%
Terminal Rates (d)	1/31 (3%)	1/26 (4%)	2/29 (7%)	1/27 (4%)
Week of First Observation	97	106	106	90
Life Table Tests (e)	P=0.299	P=0.563N	P=0.671	P=0.449
Incidental Tumor Tests (e)	P=0.309	P=0.560N	P=0.632	P=0.455
Cochran-Armitage Trend Test (e)	P=0.323			
Fisher Exact Test (e)		P=0.500N	P=0.684	P=0.500
Mammary Gland: Adenoma, Fibroadenoma, Adenocarcinoma, or Carcinoma				
Overall Rates (a)	32/50 (64%)	12/50 (24%)	13/49 (27%)	8/50 (16%)
Adjusted Rates (c)	75.6%	37.7%	41.3%	25.7%
Terminal Rates (d)	21/31 (68%)	7/26 (27%)	11/29 (38%)	5/27 (19%)
Week of First Observation	87	56	92	90
Life Table Tests (e)	P<0.001N	P=0.003N	P=0.001N	P<0.001N
Incidental Tumor Tests (e)	P<0.001N	P<0.001N	P=0.001N	P<0.001N
Cochran-Armitage Trend Test (e)	P<0.001N			
Fisher Exact Test (e)		P<0.001N	P<0.001N	P<0.001N
Clitoral Gland: Carcinoma				
Overall Rates (a)	4/50 (8%)	1/50 (2%)	0/49 (0%)	2/50 (4%)
Adjusted Rates (c)	11.3%	3.8%	0.0%	7.4%
Terminal Rates (d)	2/31 (6%)	1/26 (4%)	0/29 (0%)	2/27 (7%)
Week of First Observation	92	106		106
Life Table Tests (e)	P=0.563N	P=0.241N	P=0.079N	P=0.398N
Incidental Tumor Tests (e)	P=0.562N	P=0.242N	P=0.086N	P=0.399N
Cochran-Armitage Trend Test (e)	P=0.536N			
Fisher Exact Test (e)		P=0.181N	P=0.061N	P=0.339N

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

	Control	250 ppm	500 ppm	2,000 ppm
Clitoral Gland: Adenoma or Carcinoma				
Overall Rates (a)	5/50 (10%)	3/50 (6%)	2/49 (4%)	3/50 (6%)
Adjusted Rates (c)	13.3%	10.8%	6.0%	11.1%
Terminal Rates (d)	2/31 (6%)	2/26 (8%)	1/29 (3%)	3/27 (11%)
Week of First Observation	92	100	96	106
Life Table Tests (e)	P=0.470N	P=0.462N	P=0.260N	P=0.427N
Incidental Tumor Tests (e)	P=0.448N	P=0.458N	P=0.320N	P=0.421N
Cochran-Armitage Trend Test (e)	P=0.434N			
Fisher Exact Test (e)		P=0.357N	P=0.226N	P=0.357N
Uterus: Endometrial Stromal Polyp				
Overall Rates (a)	18/50 (36%)	6/50 (12%)	10/49 (20%)	11/50 (22%)
Adjusted Rates (c)	46.9%	19.9%	27.8%	29.0%
Terminal Rates (d)	12/31 (39%)	4/26 (15%)	5/29 (17%)	4/27 (15%)
Week of First Observation	88	50	87	66
Life Table Tests (e)	P=0.419N	P=0.023N	P=0.111N	P=0.191N
Incidental Tumor Tests (e)	P=0.384N	P=0.009N	P=0.141N	P=0.165N
Cochran-Armitage Trend Test (e)	P=0.354N			
Fisher Exact Test (e)		P=0.005N	P=0.067N	P=0.093N
All Sites: Benign Tumors				
Overall Rates (a)	43/50 (86%)	32/50 (64%)	36/49 (73%)	31/50 (62%)
Adjusted Rates (c)	95.5%	79.9%	85.4%	71.9%
Terminal Rates (d)	29/31 (94%)	18/26 (69%)	23/29 (79%)	15/27 (56%)
Week of First Observation	87	50	72	66
Life Table Tests (e)	P=0.169N	P=0.281N	P=0.300N	P=0.143N
Incidental Tumor Tests (e)	P=0.039N	P=0.115N	P=0.406N	P=0.041N
Cochran-Armitage Trend Test (e)	P=0.035N			
Fisher Exact Test (e)		P=0.010N	P=0.096N	P=0.006N
All Sites: Malignant Tumors				
Overall Rates (a)	20/50 (40%)	16/50 (32%)	19/49 (39%)	18/50 (36%)
Adjusted Rates (c)	48.2%	46.1%	49.3%	48.0%
Terminal Rates (d)	11/31 (35%)	9/26 (35%)	11/29 (38%)	9/27 (33%)
Week of First Observation	86	50	66	79
Life Table Tests (e)	P=0.516	P=0.517N	P=0.532	P=0.564
Incidental Tumor Tests (e)	P=0.543	P=0.429N	P=0.540N	P=0.580
Cochran-Armitage Trend Test (e)	P=0.500N			
Fisher Exact Test (e)		P=0.266N	P=0.532N	P=0.419N
All Sites: All Tumors				
Overall Rates (a)	49/50 (98%)	39/50 (78%)	39/49 (80%)	37/50 (74%)
Adjusted Rates (c)	100.0%	90.7%	86.4%	82.2%
Terminal Rates (d)	31/31 (100%)	22/26 (85%)	23/29 (79%)	19/27 (70%)
Week of First Observation	86	50	66	66
Life Table Tests (e)	P=0.197N	P=0.418N	P=0.184N	P=0.175N
Incidental Tumor Tests (e)	P=0.016N	P=0.141N	P=0.034N	P=0.010N
Cochran-Armitage Trend Test (e)	P=0.017N			
Fisher Exact Test (e)		P=0.002N	P=0.004N	P=0.001N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Twenty-one spleens were examined in the 250-ppm group and 25 in the 500-ppm group. Trend and pairwise statistical comparisons assume that all animals were examined equally for leukemia.
(c) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(d) Observed tumor incidence at terminal kill
(e) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE B4a. HISTORICAL INCIDENCE OF RENAL TUBULAR CELL TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

	Incidence of Adenomas or Adenocarcinomas in Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	(b) 4/1,928 (0.2%)
SD (c)	1.17%
Range (d)	
High	1/49
Low	0/50

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

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

	Incidence in Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	383/1,983 (19.3%)
SD (b)	6.66%
Range (c)	
High	15/49
Low	3/50

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

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

	Incidence in Controls		
	Fibroadenoma	Adenocarcinoma	Fibroadenoma or Adenocarcinoma
No 2-year studies by SRI International are included in the historical data base.			
Overall Historical Incidence			
TOTAL	(b) 589/1,983 (29.7%)	(c) 52/1,983 (2.6%)	(b,c) 622/1,983 (31.4%)
SD (d)	10.19%	2.09%	10.00%
Range (e)			
High	24/49	4/50	25/50
Low	5/50	0/50	6/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes 14 adenomas, NOS, 2 cystadenomas, NOS, 2 papillary cystadenomas, NOS, and 4 cystfibroadenomas
(c) Includes three papillary adenocarcinomas and two papillary cystadenocarcinomas, NOS
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.

TABLE B4d. HISTORICAL INCIDENCE OF ENDOMETRIAL STROMAL POLYPS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

	Incidence in Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	420/1,966 (21.4%)
SD (b)	7.55%
Range (c)	
High	18/49
Low	4/50

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

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

	Untreated Control	Low Dose	Mid Dose	High Dose
Animals initially in study	50	50	50	50
Animals necropsied	50	50	49	50
Animals examined histopathologically	50	50	49	50
INTEGUMENTARY SYSTEM				
*Skin	(50)	(50)	(49)	(50)
Epidermal inclusion cyst		1 (2%)	1 (2%)	
Hemorrhage	1 (2%)			
Hyperplasia, epithelial	1 (2%)			
Hyperkeratosis		1 (2%)		
*Subcutaneous tissue	(50)	(50)	(49)	(50)
Hemorrhage				1 (2%)
Inflammation, suppurative	3 (6%)			
Inflammation, chronic focal	1 (2%)			1 (2%)
RESPIRATORY SYSTEM				
#Nasal cavity	(49)	(50)	(48)	(49)
Foreign body, NOS	1 (2%)	6 (12%)	2 (4%)	2 (4%)
Congestion, NOS			1 (2%)	1 (2%)
Hemorrhage		4 (8%)	2 (4%)	2 (4%)
Inflammation, acute focal	1 (2%)	5 (10%)	3 (6%)	5 (10%)
Inflammation, chronic focal	1 (2%)			
#Tracheal submucosa	(50)	(48)	(49)	(50)
Dilatation/ducts			1 (2%)	
#Lung/bronchiole	(50)	(30)	(26)	(50)
Foreign body, NOS		1 (3%)		
Hyperplasia, epithelial		1 (3%)		
#Lung	(50)	(30)	(26)	(50)
Foreign body, NOS		3 (10%)		1 (2%)
Mineralization		1 (3%)		
Congestion, NOS	6 (12%)	11 (37%)	4 (15%)	10 (20%)
Edema, NOS		2 (7%)	3 (12%)	
Hemorrhage	1 (2%)	9 (30%)	4 (15%)	7 (14%)
Inflammation, interstitial		1 (3%)		
Inflammation, acute focal		2 (7%)	2 (8%)	2 (4%)
Inflammation, chronic focal	4 (8%)	5 (17%)	2 (8%)	9 (18%)
Infarct, focal			1 (4%)	
Pigmentation, NOS	25 (50%)	4 (13%)	4 (15%)	30 (60%)
Alveolar macrophages	6 (12%)	4 (13%)	4 (15%)	4 (8%)
Hyperplasia, alveolar epithelium	2 (4%)			2 (4%)
HEMATOPOIETIC SYSTEM				
*Abdominal cavity	(50)	(50)	(49)	(50)
Hyperplasia, lymphoid			1 (2%)	
#Bone marrow	(50)	(50)	(49)	(50)
Hyperplasia, granulocytic	1 (2%)	1 (2%)	2 (4%)	
Hyperplasia, reticulum cell	1 (2%)	2 (4%)	1 (2%)	1 (2%)
#Spleen	(50)	(21)	(25)	(49)
Abnormal curvature			1 (4%)	1 (2%)
Congestion, NOS				1 (2%)
Fibrosis, focal				1 (2%)
Infarct, NOS			1 (4%)	
Pigmentation, NOS	1 (2%)	1 (5%)		
Atrophy, NOS	1 (2%)			
Atrophy, focal	1 (2%)		3 (12%)	2 (4%)
Atrophy, diffuse	1 (2%)	1 (5%)		1 (2%)
Hematopoiesis	10 (20%)	8 (38%)	5 (20%)	7 (14%)
#Splenic capsule	(50)	(21)	(25)	(49)
Fibrosis, focal				1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)				
#Lymph node	(50)	(26)	(27)	(50)
Cyst, NOS	1 (2%)			
Inflammation, acute			1 (4%)	
Hyperplasia, plasma cell			1 (4%)	
Hyperplasia, lymphoid		1 (4%)		
#Mandibular lymph node	(50)	(26)	(27)	(50)
Cyst, NOS	3 (6%)			4 (8%)
Congestion, NOS	2 (4%)	1 (4%)		2 (4%)
Necrosis, focal			1 (4%)	
Hyperplasia, plasma cell	1 (2%)		2 (7%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
#Thoracic lymph node	(50)	(26)	(27)	(50)
Angiectasis	1 (2%)			
Hyperplasia, plasma cell		1 (4%)	1 (4%)	
Hyperplasia, reticulum cell	1 (2%)			
#Hepatic lymph node	(50)	(26)	(27)	(50)
Pigmentation, NOS				1 (2%)
Hyperplasia, plasma cell		1 (4%)		
#Pancreatic lymph node	(50)	(26)	(27)	(50)
Hematopoiesis	1 (2%)			
#Mesenteric lymph node	(50)	(26)	(27)	(50)
Multiple cysts		1 (4%)		
Congestion, NOS	2 (4%)		1 (4%)	
Edema, NOS			1 (4%)	3 (6%)
Hemorrhage	1 (2%)	2 (8%)	2 (7%)	1 (2%)
Necrosis, focal			1 (4%)	
Angiectasis	1 (2%)			
Hyperplasia, plasma cell				1 (2%)
Hyperplasia, reticulum cell				1 (2%)
Hyperplasia, lymphoid		4 (15%)		3 (6%)
#Renal lymph node	(50)	(26)	(27)	(50)
Edema, NOS			1 (4%)	
#Inguinal lymph node	(50)	(26)	(27)	(50)
Cyst, NOS	1 (2%)			
#Liver	(50)	(50)	(49)	(50)
Hematopoiesis	2 (4%)	3 (6%)	3 (6%)	2 (4%)
#Thymus	(48)	(18)	(19)	(40)
Cyst, NOS	1 (2%)			
Congestion, NOS	1 (2%)	1 (6%)		3 (8%)
Hemorrhage	1 (2%)	2 (11%)	1 (5%)	1 (3%)
Necrosis, focal		1 (6%)		
Involution, NOS	45 (94%)	9 (50%)	18 (95%)	33 (83%)
CIRCULATORY SYSTEM				
#Hepatic lymph node	(50)	(26)	(27)	(50)
Lymphangiectasis				1 (2%)
#Mesenteric lymph node	(50)	(26)	(27)	(50)
Lymphangiectasis				1 (2%)
#Heart	(50)	(21)	(18)	(50)
Mineralization				1 (2%)
Dilatation, NOS			1 (6%)	
Cyst, NOS			1 (6%)	
Inflammation, chronic focal	47 (94%)	18 (86%)	14 (78%)	45 (90%)
Fibrosis, focal				1 (2%)
Periarteritis	1 (2%)			
*Aorta	(50)	(50)	(49)	(50)
Mineralization			1 (2%)	
Hemorrhage		1 (2%)		
*Pulmonary artery	(50)	(50)	(49)	(50)
Mineralization	37 (74%)	6 (12%)	4 (8%)	25 (50%)
Thrombosis, NOS	1 (2%)			

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

	Untreated Control	Low Dose	Mid Dose	High Dose
CIRCULATORY SYSTEM (Continued)				
#Pancreas	(50)	(50)	(49)	(49)
Periarteritis	1 (2%)			
DIGESTIVE SYSTEM				
*Tooth	(50)	(50)	(49)	(50)
Inflammation, chronic focal		2 (4%)		
#Salivary gland	(50)	(11)	(9)	(50)
Inflammation, acute focal	1 (2%)			
Inflammation, chronic focal	1 (2%)			1 (2%)
Basophilic cyto change				2 (4%)
Atrophy, focal	2 (4%)			6 (12%)
Hyperplasia, focal	1 (2%)			1 (2%)
#Liver	(50)	(50)	(49)	(50)
Abnormal curvature	4 (8%)	6 (12%)	2 (4%)	1 (2%)
Congestion, NOS	3 (6%)	4 (8%)	4 (8%)	2 (4%)
Hemorrhage	1 (2%)			1 (2%)
Hemorrhagic cyst	1 (2%)		1 (2%)	1 (2%)
Inflammation, acute focal		1 (2%)		
Inflammation, chronic focal	27 (54%)	15 (30%)	14 (29%)	20 (40%)
Fibrosis		1 (2%)		
Peliosis hepatis	2 (4%)		1 (2%)	1 (2%)
Necrosis, focal	7 (14%)	3 (6%)	2 (4%)	2 (4%)
Infarct, NOS		1 (2%)		
Pigmentation, NOS	1 (2%)		1 (2%)	1 (2%)
#Liver/hepatocytes	(50)	(50)	(49)	(50)
Degeneration, NOS	1 (2%)			2 (4%)
Cytoplasmic vacuolization	15 (30%)	9 (18%)	10 (20%)	10 (20%)
Basophilic cyto change	36 (72%)	34 (68%)	36 (73%)	32 (64%)
Eosinophilic cyto change	1 (2%)		1 (2%)	
Clear cell change	6 (12%)	4 (8%)	1 (2%)	1 (2%)
#Bile duct	(50)	(50)	(49)	(50)
Hyperplasia, focal	25 (50%)	8 (16%)	15 (31%)	20 (40%)
#Pancreas	(50)	(50)	(49)	(49)
Hemorrhage		2 (4%)		1 (2%)
Inflammation, chronic focal	8 (16%)	3 (6%)	1 (2%)	6 (12%)
Pigmentation, NOS			1 (2%)	
#Pancreatic acinus	(50)	(50)	(49)	(49)
Basophilic cyto change				3 (6%)
Eosinophilic cyto change		1 (2%)		
Atrophy, focal	19 (38%)	21 (42%)	19 (39%)	24 (49%)
Atrophy, diffuse			1 (2%)	
Hyperplasia, focal			1 (2%)	
#Esophagus	(50)	(49)	(49)	(50)
Hemorrhage		1 (2%)		
Inflammation, suppurative		1 (2%)		
Inflammation, chronic				1 (2%)
#Glandular stomach	(50)	(20)	(14)	(49)
Ulcer, NOS	1 (2%)		1 (7%)	1 (2%)
Inflammation, acute focal		1 (5%)		
Inflammation, chronic focal			1 (7%)	
Necrosis, focal		1 (5%)		2 (4%)
Atrophy, focal	3 (6%)		1 (7%)	2 (4%)
#Forestomach	(50)	(20)	(14)	(49)
Edema, NOS		3 (15%)		2 (4%)
Hemorrhage		1 (5%)		1 (2%)
Ulcer, NOS	1 (2%)	2 (10%)	2 (14%)	3 (6%)
Inflammation, acute focal		5 (25%)		1 (2%)
Inflammation, chronic focal	2 (4%)	1 (5%)	5 (36%)	2 (4%)
Erosion				1 (2%)
Hyperplasia, epithelial	4 (8%)	3 (15%)	6 (43%)	7 (14%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
DIGESTIVE SYSTEM (Continued)				
#Small intestine	(50)	(13)	(12)	(49)
Congestion, NOS	1 (2%)			
#Duodenal mucosa	(50)	(13)	(12)	(49)
Hemorrhage				1 (2%)
#Colon	(50)	(15)	(12)	(48)
Edema, NOS	1 (2%)			
Hemorrhage	1 (2%)			
Inflammation, acute focal	1 (2%)			
Parasitism	2 (4%)			2 (4%)
#Cecum	(50)	(15)	(12)	(48)
Edema, NOS	1 (2%)			
Congestion, NOS	1 (2%)			
Inflammation, acute focal	1 (2%)			
Inflammation, chronic focal		1 (7%)		
*Rectum	(50)	(50)	(49)	(50)
Dilatation, NOS				1 (2%)
URINARY SYSTEM				
#Kidney	(50)	(50)	(49)	(50)
Mineralization	10 (20%)	34 (68%)	33 (67%)	35 (70%)
Cyst, NOS		3 (6%)	4 (8%)	3 (6%)
Congestion, NOS		1 (2%)		
Hemorrhage		1 (2%)		
Nephropathy	47 (94%)	42 (84%)	44 (90%)	47 (94%)
Infarct, NOS	2 (4%)			
Pigmentation, NOS	4 (8%)	4 (8%)	8 (16%)	6 (12%)
#Kidney/tubule	(50)	(50)	(49)	(50)
Degeneration, NOS		1 (2%)		
Necrosis, focal	2 (4%)			1 (2%)
#Kidney/pelvis	(50)	(50)	(49)	(50)
Dilatation, NOS		1 (2%)	1 (2%)	
Hyperplasia, epithelial		4 (8%)	2 (4%)	3 (6%)
#Urinary bladder/mucosa	(50)	(14)	(11)	(49)
Hyperplasia, epithelial	1 (2%)			
#Urinary bladder/submucosa	(50)	(14)	(11)	(49)
Inflammation, acute focal			1 (9%)	
Inflammation, chronic focal	1 (2%)			2 (4%)
#Urinary bladder/muscularis	(50)	(14)	(11)	(49)
Mineralization		1 (7%)		
ENDOCRINE SYSTEM				
#Pituitary intermedia	(50)	(50)	(48)	(49)
Cyst, NOS	4 (8%)	2 (4%)	4 (8%)	
Hyperplasia, focal				1 (2%)
Angiectasis		2 (4%)		
#Anterior pituitary	(50)	(50)	(48)	(49)
Cyst, NOS	12 (24%)	21 (42%)	18 (38%)	14 (29%)
Congestion, NOS		1 (2%)	1 (2%)	2 (4%)
Pigmentation, NOS				1 (2%)
Atrophy, focal				1 (2%)
Hyperplasia, focal	20 (40%)	21 (42%)	18 (38%)	20 (41%)
Angiectasis	1 (2%)		1 (2%)	1 (2%)
#Pituitary posterior	(50)	(50)	(48)	(49)
Cyst, NOS			1 (2%)	
Gliosis			1 (2%)	
#Adrenal/capsule	(50)	(49)	(49)	(50)
Hyperplasia, focal				1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
ENDOCRINE SYSTEM (Continued)				
#Adrenal cortex	(50)	(49)	(49)	(50)
Cyst, NOS			1 (2%)	
Congestion, NOS	3 (6%)	7 (14%)	4 (8%)	4 (8%)
Hemorrhage	1 (2%)		2 (4%)	
Fibrosis, focal	1 (2%)			
Degeneration, NOS	1 (2%)			
Necrosis, focal				1 (2%)
Cytoplasmic vacuolization	7 (14%)	4 (8%)	4 (8%)	1 (2%)
Hypertrophy, focal	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Hyperplasia, focal	22 (44%)	12 (24%)	16 (33%)	13 (26%)
Angiectasis	13 (26%)	1 (2%)	4 (8%)	4 (8%)
#Adrenal medulla	(50)	(49)	(49)	(50)
Cyst, NOS			1 (2%)	
Inflammation, chronic focal				1 (2%)
Atrophy, NOS				1 (2%)
Hyperplasia, NOS	2 (4%)	3 (6%)	12 (24%)	5 (10%)
#Thyroid	(50)	(48)	(49)	(50)
Cyst, NOS	2 (4%)			1 (2%)
Follicular cyst, NOS		1 (2%)		
Inflammation, chronic focal				1 (2%)
Hyperplasia, C-cell	22 (44%)	22 (46%)	21 (43%)	16 (32%)
#Parathyroid	(50)	(47)	(49)	(49)
Hyperplasia, NOS		12 (26%)	11 (22%)	10 (20%)
#Pancreatic islets	(50)	(50)	(49)	(49)
Hyperplasia, focal	1 (2%)			
REPRODUCTIVE SYSTEM				
*Mammary gland	(50)	(50)	(49)	(50)
Galactocele	1 (2%)		1 (2%)	
Inflammation, suppurative			1 (2%)	
Hyperplasia, cystic	37 (74%)	32 (64%)	34 (69%)	33 (66%)
Lactation	5 (10%)			4 (8%)
*Clitoral gland	(50)	(50)	(49)	(50)
Cyst, NOS	3 (6%)			
Inflammation, suppurative	1 (2%)	1 (2%)		2 (4%)
Inflammation, chronic focal				3 (6%)
Atrophy, NOS	41 (82%)	5 (10%)	4 (8%)	43 (86%)
Hyperplasia, NOS	4 (8%)	1 (2%)	1 (2%)	6 (12%)
#Uterus	(50)	(50)	(49)	(50)
Hydrometra	11 (22%)	11 (22%)	11 (22%)	6 (12%)
Cyst, NOS			1 (2%)	2 (4%)
Inflammation, acute focal			1 (2%)	
#Cervix uteri	(50)	(50)	(49)	(50)
Epidermal inclusion cyst	3 (6%)	2 (4%)		
Inflammation, suppurative	1 (2%)			1 (2%)
#Uterus/endometrium	(50)	(50)	(49)	(50)
Cyst, NOS				1 (2%)
Hyperplasia, epithelial		1 (2%)		
#Ovary	(50)	(18)	(15)	(50)
Cyst, NOS	2 (4%)	5 (28%)	4 (27%)	6 (12%)
Congestion, NOS		1 (6%)		2 (4%)
Hemorrhage		2 (11%)		1 (2%)
Inflammation, chronic focal	1 (2%)		1 (7%)	
Necrosis, focal		1 (6%)		1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
NERVOUS SYSTEM				
#Brain	(50)	(14)	(11)	(50)
Hydrocephalus, NOS	4 (8%)		2 (18%)	2 (4%)
Congestion, NOS				2 (4%)
Hemorrhage		1 (7%)	1 (9%)	5 (10%)
Inflammation, chronic focal				1 (2%)
SPECIAL SENSE ORGANS				
*Eye/anterior chamber	(50)	(50)	(49)	(50)
Inflammation, acute focal		2 (4%)		1 (2%)
*Eye/sclera	(50)	(50)	(49)	(50)
Inflammation, chronic focal				1 (2%)
*Eye/cornea	(50)	(50)	(49)	(50)
Mineralization		1 (2%)	1 (2%)	
Inflammation, acute focal	1 (2%)		2 (4%)	
Inflammation, chronic focal		1 (2%)		1 (2%)
Vascularization				1 (2%)
*Eye/retina	(50)	(50)	(49)	(50)
Atrophy, focal	2 (4%)			3 (6%)
Atrophy, diffuse	2 (4%)	1 (2%)	1 (2%)	1 (2%)
*Eye/crystalline lens	(50)	(50)	(49)	(50)
Degeneration, NOS	2 (4%)	2 (4%)	1 (2%)	
*Eye/conjunctiva	(50)	(50)	(49)	(50)
Inflammation, acute focal				1 (2%)
*Eye/lacrimal gland	(50)	(50)	(49)	(50)
Pigmentation, NOS				1 (2%)
*Nasolacrimal duct	(50)	(50)	(49)	(50)
Hemorrhage	1 (2%)			1 (2%)
Inflammation, acute focal	8 (16%)	10 (20%)	9 (18%)	11 (22%)
*Harderian gland	(50)	(50)	(49)	(50)
Inflammation, chronic focal	6 (12%)	1 (2%)		4 (8%)
Fibrosis	1 (2%)			
Pigmentation, NOS	1 (2%)	1 (2%)	2 (4%)	
*Zymbal gland	(50)	(50)	(49)	(50)
Hyperplasia, epithelial				1 (2%)
MUSCULOSKELETAL SYSTEM				
*Bone	(50)	(50)	(49)	(50)
Fibrous osteodystrophy		9 (18%)	9 (18%)	5 (10%)
Osteosclerosis	5 (10%)			
*Skeletal muscle	(50)	(50)	(49)	(50)
Inflammation, chronic focal				1 (2%)
*Muscle of trunk	(50)	(50)	(49)	(50)
Hemorrhage	1 (2%)			
BODY CAVITIES				
*Mediastinum	(50)	(50)	(49)	(50)
Hemorrhage		2 (4%)	3 (6%)	1 (2%)
Inflammation, acute focal		1 (2%)		
*Abdominal cavity	(50)	(50)	(49)	(50)
Hemorrhage	1 (2%)			
Inflammation, suppurative			1 (2%)	
Inflammation, chronic focal	1 (2%)			1 (2%)
Necrosis, fat	13 (26%)	2 (4%)	8 (16%)	10 (20%)
*Mesentery	(50)	(50)	(49)	(50)
Hemorrhage				1 (2%)

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

	Untreated Control	Low Dose	Mid Dose	High Dose
ALL OTHER SYSTEMS				
*Multiple organs	(50)	(50)	(49)	(50)
Mineralization		6 (12%)	6 (12%)	5 (10%)
Tail				
Abnormal curvature	1			
SPECIAL MORPHOLOGY SUMMARY				
Autolysis/no necropsy			1	

* Number of animals receiving complete necropsy examinations; 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 HYDROCHLOROTHIAZIDE

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

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing		1	
Animals necropsied	50	49	50
Animals examined histopathologically	49	49	50
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(50)	(49)	(50)
Sarcoma, NOS	1 (2%)	3 (6%)	1 (2%)
Fibroma			2 (4%)
Fibrosarcoma	2 (4%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#Nasal cavity	(48)	(2)	(50)
Carcinoma, NOS, metastatic			1 (2%)
#Lung	(49)	(20)	(50)
Carcinoma, NOS, metastatic		1 (5%)	1 (2%)
Hepatocellular carcinoma, metastatic	2 (4%)		2 (4%)
Alveolar/bronchiolar adenoma	7 (14%)	2 (10%)	8 (16%)
Alveolar/bronchiolar carcinoma	2 (4%)	2 (10%)	2 (4%)
Sarcoma, NOS, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(49)	(50)
Malignant lymphoma, lymphocytic type	1 (2%)	1 (2%)	
Malignant lymphoma, histiocytic type	1 (2%)		3 (6%)
Malignant lymphoma, mixed type	4 (8%)	1 (2%)	6 (12%)
Mast cell tumor	1 (2%)		
#Bone marrow	(48)	(48)	(50)
Sarcoma, NOS, metastatic	1 (2%)		
#Spleen	(48)	(49)	(50)
Malignant lymphoma, mixed type	1 (2%)		
#Mesenteric lymph node	(47)	(19)	(49)
Malignant lymphoma, mixed type			1 (2%)
CIRCULATORY SYSTEM			
#Spleen	(48)	(49)	(50)
Hemangiosarcoma	1 (2%)		
#Heart	(49)	(6)	(50)
Sarcoma, NOS, metastatic	1 (2%)		
#Liver	(48)	(49)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)
DIGESTIVE SYSTEM			
#Liver	(48)	(49)	(50)
Hepatocellular adenoma	3 (6%)	8 (16%)	14 (28%)
Hepatocellular carcinoma	4 (8%)	4 (8%)	9 (18%)
Mixed hepato/cholangio carcinoma			1 (2%)
#Glandular stomach	(48)	(6)	(50)
Adenomatous polyp, NOS	1 (2%)		
#Forestomach	(48)	(6)	(50)
Squamous cell papilloma	1 (2%)		1 (2%)
#Duodenum	(48)	(7)	(50)
Adenomatous polyp, NOS	2 (4%)		
URINARY SYSTEM			
None			

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

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Anterior pituitary	(48)	(6)	(49)
Adenoma, NOS	1 (2%)		1 (2%)
#Adrenal medulla	(48)	(49)	(50)
Pheochromocytoma	3 (6%)	1 (2%)	2 (4%)
#Thyroid	(48)	(6)	(50)
Follicular cell adenoma	3 (6%)		
REPRODUCTIVE SYSTEM			
*Seminal vesicle	(50)	(49)	(50)
Sarcoma, NOS	1 (2%)		
#Testis	(48)	(49)	(50)
Interstitial cell tumor	1 (2%)		
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(49)	(50)
Carcinoma, NOS		1 (2%)	1 (2%)
Adenoma, NOS	1 (2%)	1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
*Rib	(50)	(49)	(50)
Sarcoma, NOS, metastatic	1 (2%)		
*Muscle of upper extremity	(50)	(49)	(50)
Sarcoma, NOS	1 (2%)		
BODY CAVITIES			
*Mediastinum	(50)	(49)	(50)
Sarcoma, NOS, metastatic	1 (2%)		
*Abdominal cavity	(50)	(49)	(50)
Sarcoma, NOS, metastatic			1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(49)	(50)
Mixed hepato/cholangiocarcinoma, metastatic			1 (2%)
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	5	4	2
Moribund sacrifice	2	3	5
Terminal sacrifice	43	42	43
Animal missing		1	

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

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	30	20	34
Total primary tumors	44	25	55
Total animals with benign tumors	18	12	22
Total benign tumors	23	12	29
Total animals with malignant tumors	19	12	19
Total malignant tumors	20	13	26
Total animals with secondary tumors##	3	1	5
Total secondary tumors	7	1	6
Total animals with tumors-- uncertain benign or malignant	1		
Total uncertain tumors	1		

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

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

ANIMAL NUMBER	0003	0031	0042	0045	0004	0038	0002	0001	0005	0006	0007	0008	0009	0010	0011	0012	0013	0014	0015	0016	0017	0018	0019	0020	0021
WEEKS ON STUDY	041	046	048	083	100	102	104	106	106	106	106	106	108	106	106	106	106	106	106	106	106	106	106	106	106
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS					X																				
Fibrosarcoma														X											
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic							X						X												
Alveolar/bronchiolar adenoma													X			X			X						X
Alveolar/bronchiolar carcinoma																	X		X						
Sarcoma, NOS, metastatic					X																				
Trachea	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic					X																				
Spleen	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Malignant lymphoma, mixed type								X																	
Lymph nodes	+	A	−	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	A	+	A	−	+	−	+	−	−	+	−	+	+	+	−	−	+	−	−	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic					X																				
DIGESTIVE SYSTEM																									
Salivary gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																X	X								
Hepatocellular carcinoma							X						X												X
Hemangiosarcoma											X														
Bile duct	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma									X																
Adenomatous polyp, NOS																									
Small intestine	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenomatous polyp, NOS																X									
Large intestine	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adrenal	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																				X					
Parathyroid	+	A	+	A	−	+	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−
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	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor																									
Prostate	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	+	N	N	N	+	N	N	N
Sarcoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
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
Sarcoma, NOS, metastatic					X																				
Muscle	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															
BODY CAVITIES																									
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS, metastatic					X																				
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type																				X					
Malignant lymphoma, histiocytic type						X																			
Malignant lymphoma, mixed type												X									X	X		
Mast cell tumor																									

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	022	023	024	025	026	027	028	029	030	032	033	034	035	036	037	039	040	041	043	044	046	047	048	049	050	TOTAL:
WEEKS ON STUDY	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
Fibrosarcoma														X												2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic																										2
Alveolar/bronchiolar adenoma												X		X				X								7
Alveolar/bronchiolar carcinoma																										2
Sarcoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, NOS, metastatic																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																										1
Malignant lymphoma, mixed type																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thymus	+	+	+	−	+	−	+	+	+	+	−	+	−	+	−	+	+	+	+	+	+	+	+	+	−	33
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma, NOS, metastatic																										1
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma												X														3
Hepatocellular carcinoma				X																						4
Hemangiosarcoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell papilloma																										1
Adenomatous polyp, NOS										X																1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenomatous polyp, NOS																					X					2
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS																				X						1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma			X	X																X						3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell adenoma		X																			X					3
Parathyroid	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	+	−	−	−	+	+	30
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	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Interstitial cell tumor								X																		1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Seminal vesicle	N	N	N	N	N	N	N	N	N	N	+	+	+	+	+	+	+	+	N	+	N	N	N	N	N	*50
Sarcoma, NOS											X															1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS													X													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
Sarcoma, NOS, metastatic																										1
Muscle	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
BODY CAVITIES																										
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS, metastatic																										1
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																										1
Malignant lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type																X										4
Mast cell tumor										X																1

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

ANIMAL NUMBER	032	004	044	030	028	005	048	047	001	002	003	006	007	008	009	110	111	112	113	114	115	116	117	118	119
WEEKS ON STUDY	063	067	070	076	079	086	087	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	M	+	+	+	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS								X			X														
Fibrosarcoma																					X				
RESPIRATORY SYSTEM																									
Lungs and bronchi	M	+	+	+	+	+	+	+	−	+	+	−	−	−	−	+	+	+	−	−	−	−	−	−	+
Carcinoma, NOS, metastatic			X																						
Alveolar/bronchiolar adenoma																	X								X
Alveolar/bronchiolar carcinoma										X															
Trachea	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Nasal cavity	M	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HEMATOPOIETIC SYSTEM																									
Bone marrow	M	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	M	+	+	+	+	+	+	+	+	−	+	+	−	+	−	+	−	−	−	−	−	−	+	−	
Thymus	M	+	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
CIRCULATORY SYSTEM																									
Heart	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
DIGESTIVE SYSTEM																									
Salivary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma					X						X		X	X											
Hepatocellular carcinoma														X		X									
Bile duct	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	M	+	+	+	+	+	N	N	N	N	N	+	N	+	N	N	N	N	N	N	N	N	N	N	N
Pancreas	M	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Esophagus	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Stomach	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Small intestine	M	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Large intestine	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
URINARY SYSTEM																									
Kidney	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ENDOCRINE SYSTEM																									
Pituitary	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Adrenal	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Parathyroid	M	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
REPRODUCTIVE SYSTEM																									
Mammary gland	M	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
NERVOUS SYSTEM																									
Brain	M	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
SPECIAL SENSE ORGANS																									
Harderian gland	M	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																						
Adenoma, NOS															X										
ALL OTHER SYSTEMS																									
Multiple organs, NOS	M	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											

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE: LOW DOSE (Continued)

ANIMAL NUMBER	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5	0 2 6	0 2 7	0 2 9	0 3 1	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 5	0 4 6	0 4 9	0 5 0	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	
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	*49
Sarcoma, NOS																							X			3
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	−	−	−	−	−	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−	+	−	−	+	20
Carcinoma, NOS, metastatic																										1
Alveolar/bronchiolar adenoma																										2
Alveolar/bronchiolar carcinoma									X																	2
Trachea	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
Nasal cavity	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	2
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	−	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	+	−	19
Thymus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	4
CIRCULATORY SYSTEM																										
Heart	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma				X	X											X							X		8
Hepatocellular carcinoma				X													X									4
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
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	*49
Pancreas	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	7
Esophagus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
Stomach	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
Small intestine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	7
Large intestine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	8
ENDOCRINE SYSTEM																										
Pituitary	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma							X																			1
Thyroid	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
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	*49
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prostate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
NERVOUS SYSTEM																										
Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	6
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	*49
Carcinoma, NOS																										1
Adenoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Malignant lymphoma, lymphocytic type																										1
Malignant lymphoma, mixed type																										1

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

ANIMAL NUMBER	0161	0111	0241	0291	0331	0155	0099	0101	0102	0103	0104	0105	0106	0107	0108	0110	0112	0113	0114	0117	0118	0119	0200	0221	0222
WEEKS ON STUDY	057	079	081	084	091	092	093	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																			X						
Fibroma															X										
Fibrosarcoma																							X		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic					X																				
Hepatocellular carcinoma, metastatic																									
Alveolar/bronchiolar adenoma											X	X	X												
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic					X																				
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																					X				
Thymus	−	−	+	+	−	+	−	−	+	−	−	−	+	+	−	−	+	−	+	+	−	+	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma		X		X				X			X	X							X				X		
Hepatocellular carcinoma					X	X	X														X		X		
Mixed hepato/cholangiocarcinoma				X																					
Hemangiosarcoma																							X		
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma														X											
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
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
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS					X																				
Adenoma, NOS																									
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS, metastatic																			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
Mixed hepato/cholangiocarcinoma, metastatic				X																					
Malignant lymphoma, histiocytic type			X				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
* Animals necropsied

: 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 IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE: HIGH DOSE (Continued)

ANIMAL NUMBER	0 2 3	0 2 5	0 2 6	0 2 7	0 2 8	0 3 0	0 3 1	0 3 2	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 4	0 4 5	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	TOTAL:
WEEKS ON STUDY	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	TISSUES / TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50	
Sarcoma, NOS																									1	
Fibroma					X																				2	
Fibrosarcoma																									1	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Carcinoma, NOS, metastatic																									1	
Hepatocellular carcinoma, metastatic	X					X																			2	
Alveolar/bronchiolar adenoma			X							X	X			X		X									8	
Alveolar/bronchiolar carcinoma																X						X			2	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Carcinoma, NOS, metastatic																									1	
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Lymph nodes	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Malignant lymphoma, mixed type																									1	
Thymus	+	+	−	−	+	−	+	+	+	−	+	+	−	+	+	−	−	−	+	+	−	−	+	+	27	
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Hepatocellular adenoma					X	X			X		X									X	X			X	14	
Hepatocellular carcinoma	X					X					X					X									9	
Mixed hepato/cholangiocarcinoma																									1	
Hemangiosarcoma																									1	
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Squamous cell papilloma																									1	
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	49	
Adenoma, NOS			X																						1	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Pheochromocytoma		X																							2	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Parathyroid	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	−	+	−	+	36	
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50	
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
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	*50	
Carcinoma, NOS																									1	
Adenoma, NOS																				X					1	
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50	
Sarcoma, NOS, metastatic																									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	*50	
Mixed hepato/cholangiocarcinoma meta.																									1	
Malignant lymphoma, histiocytic type							X																		3	
Malignant lymphoma, mixed type									X				X			X			X					X		6	

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

	Control	2,500 ppm	5,000 ppm
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	2/50 (4%)	1/49 (2%)	3/50 (6%)
Adjusted Rates (b)	4.7%	2.4%	7.0%
Terminal Rates (c)	2/43 (5%)	1/42 (2%)	3/43 (7%)
Week of First Observation	105	105	105
Life Table Tests (d)	P=0.400	P=0.508N	P=0.500
Incidental Tumor Tests (d)	P=0.400	P=0.508N	P=0.500
Cochran-Armitage Trend Test (d)	P=0.400		
Fisher Exact Test (d)		P=0.508N	P=0.500
Subcutaneous Tissue: Sarcoma or Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	4/49 (8%)	2/50 (4%)
Adjusted Rates (b)	6.7%	9.3%	4.7%
Terminal Rates (c)	2/43 (5%)	3/42 (7%)	2/43 (5%)
Week of First Observation	100	104	105
Life Table Tests (d)	P=0.423N	P=0.484	P=0.506N
Incidental Tumor Tests (d)	P=0.551N	P=0.330	P=0.592N
Cochran-Armitage Trend Test (d)	P=0.417N		
Fisher Exact Test (d)		P=0.489	P=0.500N
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	4/49 (8%)	4/50 (8%)
Adjusted Rates (b)	6.7%	9.3%	9.3%
Terminal Rates (c)	2/43 (5%)	3/42 (7%)	4/43 (9%)
Week of First Observation	100	104	105
Life Table Tests (d)	P=0.421	P=0.484	P=0.495
Incidental Tumor Tests (d)	P=0.307	P=0.330	P=0.422
Cochran-Armitage Trend Test (d)	P=0.424		
Fisher Exact Test (d)		P=0.489	P=0.500
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	7/49 (14%)	(e) 2/20 (10%)	8/50 (16%)
Adjusted Rates (b)	16.3%		18.6%
Terminal Rates (c)	7/43 (16%)		8/43 (19%)
Week of First Observation	105		105
Life Table Test (d)			P=0.500
Incidental Tumor Test (d)			P=0.500
Fisher Exact Test (d)			P=0.517
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	8/49 (16%)	(e) 4/20 (20%)	9/50 (18%)
Adjusted Rates (b)	18.6%		20.9%
Terminal Rates (c)	8/43 (19%)		9/43 (21%)
Week of First Observation	105		105
Life Table Test (d)			P=0.500
Incidental Tumor Test (d)			P=0.500
Fisher Exact Test (d)			P=0.518
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	1/50 (2%)	0/49 (0%)	3/50 (6%)
Adjusted Rates (b)	2.2%	0.0%	6.5%
Terminal Rates (c)	0/43 (0%)	0/42 (0%)	1/43 (2%)
Week of First Observation	102		81
Life Table Tests (d)	P=0.177	P=0.509N	P=0.302
Incidental Tumor Tests (d)	P=0.100	P=0.718N	P=0.202
Cochran-Armitage Trend Test (d)	P=0.177		
Fisher Exact Test (d)		P=0.505N	P=0.309

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

	Control	2,500 ppm	5,000 ppm
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	5/50 (10%)	1/49 (2%)	7/50 (14%)
Adjusted Rates (b)	11.6%	2.4%	16.3%
Terminal Rates (c)	5/43 (12%)	1/42 (2%)	7/43 (16%)
Week of First Observation	105	105	105
Life Table Tests (d)	P=0.297	P=0.109N	P=0.379
Incidental Tumor Tests (d)	P=0.297	P=0.109N	P=0.379
Cochran-Armitage Trend Test (d)	P=0.298		
Fisher Exact Test (d)		P=0.107N	P=0.380
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	7/50 (14%)	2/49 (4%)	10/50 (20%)
Adjusted Rates (b)	15.9%	4.6%	22.1%
Terminal Rates (c)	6/43 (14%)	1/42 (2%)	8/43 (19%)
Week of First Observation	102	86	81
Life Table Tests (d)	P=0.230	P=0.091N	P=0.299
Incidental Tumor Tests (d)	P=0.231	P=0.083N	P=0.262
Cochran-Armitage Trend Test (d)	P=0.227		
Fisher Exact Test (d)		P=0.085N	P=0.298
Liver: Hepatocellular Adenoma			
Overall Rates (a)	3/48 (6%)	8/49 (16%)	14/50 (28%)
Adjusted Rates (b)	7.0%	18.5%	30.9%
Terminal Rates (c)	3/43 (7%)	7/42 (17%)	12/43 (28%)
Week of First Observation	105	79	79
Life Table Tests (d)	P=0.003	P=0.095	P=0.004
Incidental Tumor Tests (d)	P=0.008	P=0.148	P=0.012
Cochran-Armitage Trend Test (d)	P=0.003		
Fisher Exact Test (d)		P=0.106	P=0.004
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	4/48 (8%)	4/49 (8%)	9/50 (18%)
Adjusted Rates (b)	9.1%	9.5%	19.6%
Terminal Rates (c)	3/43 (7%)	4/42 (10%)	6/43 (14%)
Week of First Observation	104	105	91
Life Table Tests (d)	P=0.082	P=0.630	P=0.123
Incidental Tumor Tests (d)	P=0.092	P=0.562	P=0.161
Cochran-Armitage Trend Test (d)	P=0.089		
Fisher Exact Test (d)		P=0.631N	P=0.133
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	7/48 (15%)	10/49 (20%)	21/50 (42%)
Adjusted Rates (b)	15.9%	23.1%	43.7%
Terminal Rates (c)	6/43 (14%)	9/42 (21%)	16/43 (37%)
Week of First Observation	104	79	79
Life Table Tests (d)	P=0.001	P=0.282	P=0.003
Incidental Tumor Tests (d)	P=0.004	P=0.323	P=0.009
Cochran-Armitage Trend Test (d)	P=0.001		
Fisher Exact Test (d)		P=0.314	P=0.002
Adrenal Gland Medulla: Pheochromocytoma			
Overall Rates (a)	3/48 (6%)	1/49 (2%)	2/50 (4%)
Adjusted Rates (b)	7.0%	2.4%	4.7%
Terminal Rates (c)	3/43 (7%)	1/42 (2%)	2/43 (5%)
Week of First Observation	105	105	105
Life Table Tests (d)	P=0.400N	P=0.314N	P=0.500N
Incidental Tumor Tests (d)	P=0.400N	P=0.314N	P=0.500N
Cochran-Armitage Trend Test (d)	P=0.383N		
Fisher Exact Test (d)		P=0.301N	P=0.480N

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

	Control	2,500 ppm	5,000 ppm
Thyroid Gland: Follicular Cell Adenoma			
Overall Rates (a)	3/48 (6%)	(e) 0/6 (0%)	0/50 (0%)
Adjusted Rates (b)	7.0%		0.0%
Terminal Rates (c)	3/43 (7%)		0/43 (0%)
Week of First Observation	105		
Life Table Test (d)			P=0.121N
Incidental Tumor Test (d)			P=0.121N
Fisher Exact Test (d)			P=0.114N
All Sites: Benign Tumors			
Overall Rates (a)	18/50 (36%)	12/49 (24%)	22/50 (44%)
Adjusted Rates (b)	41.9%	27.8%	48.7%
Terminal Rates (c)	18/43 (42%)	11/42 (26%)	20/43 (47%)
Week of First Observation	105	79	79
Life Table Tests (d)	P=0.233	P=0.151N	P=0.270
Incidental Tumor Tests (d)	P=0.320	P=0.125N	P=0.362
Cochran-Armitage Trend Test (d)	P=0.231		
Fisher Exact Test (d)		P=0.152N	P=0.270
All Sites: Malignant Tumors			
Overall Rates (a)	19/50 (38%)	12/49 (24%)	19/50 (38%)
Adjusted Rates (b)	41.3%	26.5%	39.6%
Terminal Rates (c)	16/43 (37%)	9/42 (21%)	14/43 (33%)
Week of First Observation	100	70	81
Life Table Tests (d)	P=0.536	P=0.125N	P=0.569
Incidental Tumor Tests (d)	P=0.502N	P=0.114N	P=0.529N
Cochran-Armitage Trend Test (d)	P=0.542		
Fisher Exact Test (d)		P=0.109N	P=0.581
All Sites: All Tumors			
Overall Rates (a)	30/50 (60%)	20/49 (41%)	34/50 (68%)
Adjusted Rates (b)	65.2%	43.4%	69.4%
Terminal Rates (c)	27/43 (63%)	16/42 (38%)	28/43 (65%)
Week of First Observation	100	70	79
Life Table Tests (d)	P=0.261	P=0.058N	P=0.285
Incidental Tumor Tests (d)	P=0.326	P=0.037N	P=0.357
Cochran-Armitage Trend Test (d)	P=0.240		
Fisher Exact Test (d)		P=0.044N	P=0.266

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

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

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

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Second highest: 12/50
(e) Second highest: 20/50

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

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing		1	
Animals necropsied	50	49	50
Animals examined histopathologically	49	49	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(49)	(50)
Impaction, fecal			1 (2%)
Ulcer, NOS			2 (4%)
Inflammation, acute focal	1 (2%)	1 (2%)	
Inflammation, chronic focal			1 (2%)
Fibrosis, focal		1 (2%)	1 (2%)
Pigmentation, NOS			1 (2%)
Hyperplasia, epithelial			1 (2%)
Hyperkeratosis	1 (2%)		
*Subcutaneous tissue	(50)	(49)	(50)
Cyst, NOS			1 (2%)
Inflammation, suppurative	1 (2%)	5 (10%)	2 (4%)
Inflammation, chronic focal	3 (6%)	4 (8%)	2 (4%)
RESPIRATORY SYSTEM			
#Nasal cavity	(48)	(2)	(50)
Foreign body, NOS			1 (2%)
Hemorrhage	6 (13%)		1 (2%)
Inflammation, acute focal	1 (2%)		1 (2%)
Inflammation, chronic focal			1 (2%)
#Lung	(49)	(20)	(50)
Mineralization			1 (2%)
Congestion, NOS	4 (8%)	4 (20%)	2 (4%)
Hemorrhage	3 (6%)	2 (10%)	2 (4%)
Inflammation, interstitial	1 (2%)	1 (5%)	2 (4%)
Inflammation, acute focal		1 (5%)	
Inflammation, chronic focal	22 (45%)	10 (50%)	19 (38%)
Pigmentation, NOS			1 (2%)
Alveolar macrophages	4 (8%)	1 (5%)	
Hyperplasia, alveolar epithelium	6 (12%)	2 (10%)	7 (14%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(49)	(50)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)
Hematopoiesis			1 (2%)
#Bone marrow	(48)	(48)	(50)
Pigmentation, NOS		1 (2%)	
Hyperplasia, granulocytic	2 (4%)	7 (15%)	9 (18%)
#Spleen	(48)	(49)	(50)
Abnormal curvature	1 (2%)	2 (4%)	
Fibrosis, focal	1 (2%)		
Necrosis, focal	2 (4%)	1 (2%)	
Amyloidosis		1 (2%)	
Depletion, lymphoid	1 (2%)		
Angiectasis		1 (2%)	
Hyperplasia, lymphoid	2 (4%)	6 (12%)	6 (12%)
Hematopoiesis	3 (6%)	11 (22%)	10 (20%)
#Lymph node	(47)	(19)	(49)
Pigmentation, NOS			2 (4%)
Hyperplasia, plasma cell	4 (9%)	2 (11%)	
Hyperplasia, lymphoid	3 (6%)		4 (8%)
#Mandibular lymph node	(47)	(19)	(49)
Pigmentation, NOS		1 (5%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)		

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

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Thoracic lymph node	(47)	(19)	(49)
Hemorrhage			1 (2%)
Hyperplasia, plasma cell		1 (5%)	
Hyperplasia, lymphoid			1 (2%)
#Mesenteric lymph node	(47)	(19)	(49)
Congestion, NOS	21 (45%)	5 (26%)	12 (24%)
Edema, NOS			1 (2%)
Hemorrhage		1 (5%)	2 (4%)
Inflammation, acute focal		2 (11%)	
Inflammation, chronic			1 (2%)
Necrosis, focal			1 (2%)
Angiectasis	2 (4%)	1 (5%)	2 (4%)
Hyperplasia, reticulum cell		1 (5%)	
Hyperplasia, lymphoid	7 (15%)	3 (16%)	5 (10%)
Hematopoiesis	1 (2%)	3 (16%)	5 (10%)
#Renal lymph node	(47)	(19)	(49)
Hyperplasia, plasma cell		1 (5%)	
Hyperplasia, lymphoid			2 (4%)
#Inguinal lymph node	(47)	(19)	(49)
Hyperplasia, plasma cell		2 (11%)	1 (2%)
#Liver	(48)	(49)	(50)
Hematopoiesis	1 (2%)	3 (6%)	5 (10%)
#Kidney	(49)	(49)	(50)
Hyperplasia, lymphoid		3 (6%)	1 (2%)
#Thymus	(33)	(4)	(27)
Cyst, NOS			2 (7%)
Necrosis, NOS	1 (3%)		
Involution, NOS	2 (6%)	3 (75%)	6 (22%)
CIRCULATORY SYSTEM			
*Mediastinum	(50)	(49)	(50)
Periarteritis			1 (2%)
*Lymphatics of abdome	(50)	(49)	(50)
Dilatation, NOS			1 (2%)
#Lung	(49)	(20)	(50)
Thrombosis, NOS		1 (5%)	
#Heart	(49)	(6)	(50)
Mineralization	1 (2%)		
Inflammation, chronic focal	5 (10%)	3 (50%)	7 (14%)
Periarteritis			1 (2%)
#Heart/atrium	(49)	(6)	(50)
Pigmentation, NOS	1 (2%)		1 (2%)
#Cardiac valve	(49)	(6)	(50)
Pigmentation, NOS	3 (6%)		5 (10%)
DIGESTIVE SYSTEM			
*Tooth	(50)	(49)	(50)
Abnormal curvature	1 (2%)		1 (2%)
Dysplasia, NOS	1 (2%)		
#Salivary gland	(49)	(49)	(49)
Inflammation, chronic focal	17 (35%)	22 (45%)	23 (47%)
Hyperplasia, intraductal	1 (2%)		
#Liver	(48)	(49)	(50)
Cyst, NOS		1 (2%)	
Congestion, NOS	1 (2%)		1 (2%)
Hemorrhagic cyst	1 (2%)		
Inflammation, chronic focal	12 (25%)	7 (14%)	10 (20%)
Necrosis, focal	1 (2%)	1 (2%)	6 (12%)
Eosinophilic cyto change			1 (2%)
Atrophy, focal		1 (2%)	1 (2%)

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

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Liver/hepatocytes	(48)	(49)	(50)
Cytoplasmic vacuolization	2 (4%)	3 (6%)	
Basophilic cyto change	1 (2%)	3 (6%)	
Eosinophilic cyto change			1 (2%)
Clear cell change	3 (6%)	2 (4%)	4 (8%)
*Gallbladder	(50)	(49)	(50)
Inflammation, chronic focal	1 (2%)	2 (4%)	2 (4%)
Hyperplasia, NOS	1 (2%)		
#Pancreas	(48)	(7)	(50)
Congestion, NOS	1 (2%)		
Inflammation, acute focal		1 (14%)	
Inflammation, chronic focal	9 (19%)	2 (29%)	16 (32%)
#Pancreatic acinus	(48)	(7)	(50)
Atrophy, focal	2 (4%)		4 (8%)
#Esophagus	(49)	(6)	(50)
Hyperkeratosis			1 (2%)
#Glandular stomach	(48)	(6)	(50)
Mineralization	1 (2%)		3 (6%)
Cyst, NOS	1 (2%)		
Inflammation, acute focal		1 (17%)	
Inflammation, chronic focal	2 (4%)	1 (17%)	6 (12%)
Necrosis, focal			1 (2%)
#Forestomach	(48)	(6)	(50)
Hemorrhage	1 (2%)		
Ulcer, NOS	1 (2%)		
Inflammation, acute focal	1 (2%)		
Inflammation, chronic focal			1 (2%)
Hyperplasia, epithelial	1 (2%)	1 (17%)	2 (4%)
Hyperkeratosis	1 (2%)		1 (2%)
#Small intestine	(48)	(7)	(50)
Amyloid, NOS	1 (2%)		
#Peyer's patch	(48)	(7)	(50)
Congestion, NOS	1 (2%)		
#Duodenum	(48)	(7)	(50)
Polypoid hyperplasia			1 (2%)
URINARY SYSTEM			
#Kidney	(49)	(49)	(50)
Mineralization	18 (37%)	11 (22%)	13 (26%)
Cyst, NOS	3 (6%)	1 (2%)	5 (10%)
Congestion, NOS	1 (2%)		1 (2%)
Hemorrhage	1 (2%)		
Glomerulonephritis, NOS	1 (2%)	2 (4%)	2 (4%)
Inflammation, chronic focal	5 (10%)		1 (2%)
Inflammation, chronic diffuse		1 (2%)	
Nephropathy	17 (35%)	14 (29%)	8 (16%)
Degeneration, NOS			1 (2%)
Nephrosis, NOS			1 (2%)
Necrosis, focal		2 (4%)	
Infarct, NOS	1 (2%)		1 (2%)
Atrophy			1 (2%)
Hyperplasia, tubular cell			1 (2%)
#Kidney/pelvis	(49)	(49)	(50)
Dilatation, NOS	1 (2%)	1 (2%)	
Inflammation, suppurative	1 (2%)	2 (4%)	
Hyperplasia, epithelial	1 (2%)		
#Urinary bladder/mucosa	(48)	(8)	(50)
Necrosis, focal		1 (13%)	
#Urinary bladder/submucosa	(48)	(8)	(50)
Congestion, NOS	1 (2%)		
Inflammation, acute focal		1 (13%)	

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

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
#Urinary bladder/submucosa (Continued)	(48)	(8)	(50)
Inflammation, chronic focal	7 (15%)		11 (22%)
#Urinary bladder/muscularis	(48)	(8)	(50)
Inflammation, chronic focal		1 (13%)	
ENDOCRINE SYSTEM			
#Anterior pituitary	(48)	(6)	(49)
Cyst, NOS	5 (10%)	1 (17%)	2 (4%)
Hyperplasia, focal	1 (2%)	1 (17%)	4 (8%)
#Adrenal/capsule	(48)	(49)	(50)
Hyperplasia, focal	44 (92%)	47 (96%)	45 (90%)
#Adrenal cortex	(48)	(49)	(50)
Degeneration, NOS	1 (2%)		1 (2%)
Necrosis, focal		1 (2%)	
Hyperplasia, focal	17 (35%)	17 (35%)	10 (20%)
#Adrenal medulla	(48)	(49)	(50)
Hyperplasia, NOS	2 (4%)	2 (4%)	3 (6%)
#Thyroid	(48)	(6)	(50)
Cyst, NOS	1 (2%)		1 (2%)
Follicular cyst, NOS	6 (13%)		8 (16%)
Inflammation, chronic focal	1 (2%)		
Atrophy, focal	1 (2%)		
#Parathyroid	(30)	(5)	(36)
Cyst, NOS	1 (3%)		
#Pancreatic islets	(48)	(7)	(50)
Hyperplasia, NOS	2 (4%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(49)	(50)
Cyst, NOS			1 (2%)
Hyperplasia, intraductal		1 (2%)	
*Penis	(50)	(49)	(50)
Inflammation, suppurative		1 (2%)	
Inflammation, chronic focal	1 (2%)		
Necrosis, diffuse		1 (2%)	
Hyperkeratosis	1 (2%)		
*Prepuce	(50)	(49)	(50)
Inflammation, acute focal	2 (4%)		
Inflammation, chronic focal			1 (2%)
Hyperplasia, epithelial			1 (2%)
*Preputial gland	(50)	(49)	(50)
Cyst, NOS		2 (4%)	1 (2%)
Abscess, NOS	2 (4%)		1 (2%)
Inflammation, chronic focal		1 (2%)	1 (2%)
Atrophy, NOS	1 (2%)		
#Prostate	(48)	(6)	(49)
Inflammation, suppurative	1 (2%)	1 (17%)	1 (2%)
Inflammation, chronic focal	4 (8%)		9 (18%)
Hyperplasia, epithelial	1 (2%)		
*Seminal vesicle	(50)	(49)	(50)
Dilatation, NOS	5 (10%)	2 (4%)	5 (10%)
Hemorrhage	1 (2%)		
Inflammation, suppurative		2 (4%)	
Inflammation, chronic focal	2 (4%)		
Atrophy, NOS			2 (4%)
#Testis	(48)	(49)	(50)
Mineralization		1 (2%)	5 (10%)
Inflammation, chronic focal		1 (2%)	
Atrophy, focal	2 (4%)	2 (4%)	1 (2%)
Atrophy, diffuse		1 (2%)	1 (2%)

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

	Untreated Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM (Continued)			
*Epididymis	(50)	(49)	(50)
Inflammation, chronic focal			1 (2%)
NERVOUS SYSTEM			
#Brain/meninges	(48)	(6)	(50)
Inflammation, chronic focal			1 (2%)
Pigmentation, NOS	4 (8%)	1 (17%)	
Hyperplasia, focal			1 (2%)
*Choroid plexus	(50)	(49)	(50)
Hyperplasia, NOS	1 (2%)		
#Brain	(48)	(6)	(50)
Mineralization	22 (46%)	1 (17%)	15 (30%)
Hydrocephalus, NOS	1 (2%)		2 (4%)
#Cerebellum	(48)	(6)	(50)
Leukodystrophy, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*Nasolacrimal duct	(50)	(49)	(50)
Hemorrhage			3 (6%)
Inflammation, acute focal			1 (2%)
*Harderian gland	(50)	(49)	(50)
Inflammation, chronic focal	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Skeletal muscle	(50)	(49)	(50)
Inflammation, chronic focal			1 (2%)
*Muscle of trunk	(50)	(49)	(50)
Inflammation, chronic	1 (2%)		
*Muscle of perineum	(50)	(49)	(50)
Inflammation, acute necrotizing	1 (2%)		
*Muscle of leg	(50)	(49)	(50)
Hemorrhage		1 (2%)	
Necrosis, focal		1 (2%)	
BODY CAVITIES			
*Abdominal cavity	(50)	(49)	(50)
Cyst, NOS			1 (2%)
Hemorrhage		1 (2%)	
Necrosis, fat	4 (8%)	3 (6%)	4 (8%)
*Tunica vaginalis	(50)	(49)	(50)
Cyst, NOS			1 (2%)
Inflammation, chronic focal	1 (2%)		
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(49)	(50)
Amyloidosis			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
Animal missing/no necropsy		1	
Auto/necropsy/histo perf	1		
Auto/necropsy/no histo	1		

* 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 HYDROCHLOROTHIAZIDE

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

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(50)	(50)	(50)
Sarcoma, NOS	4 (8%)	1 (2%)	1 (2%)
Osteosarcoma	1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(49)	(15)	(50)
Hepatocellular carcinoma, metastatic	1 (2%)		
Alveolar/bronchiolar adenoma	4 (8%)	2 (13%)	1 (2%)
Alveolar/bronchiolar carcinoma		1 (7%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, undifferentiated type			1 (2%)
Malignant lymphoma, lymphocytic type		4 (8%)	1 (2%)
Malignant lymphoma, histiocytic type	3 (6%)	3 (6%)	2 (4%)
Malignant lymphoma, mixed type	13 (26%)	12 (24%)	13 (26%)
#Spleen	(50)	(32)	(50)
Malignant lymphoma, lymphocytic type		1 (3%)	
Malignant lymphoma, histiocytic type			1 (2%)
Malignant lymphoma, mixed type		1 (3%)	
#Lymph node	(49)	(24)	(50)
Malignant lymphoma, mixed type		1 (4%)	
#Thoracic lymph node	(49)	(24)	(50)
Sarcoma, NOS, metastatic			1 (2%)
#Peyer's patch	(47)	(9)	(49)
Malignant lymphoma, mixed type	2 (4%)		
#Uterus	(49)	(45)	(50)
Malignant lymphoma, histiocytic type	1 (2%)		
#Thymus	(42)	(7)	(39)
Sarcoma, NOS, metastatic			1 (3%)
CIRCULATORY SYSTEM			
#Liver	(50)	(21)	(50)
Hemangiosarcoma		2 (10%)	
#Uterus	(49)	(45)	(50)
Hemangioma		1 (2%)	
Hemangiosarcoma	1 (2%)		
#Ovary	(48)	(20)	(50)
Hemangioma	1 (2%)		
DIGESTIVE SYSTEM			
#Liver	(50)	(21)	(50)
Hepatocellular adenoma	2 (4%)	4 (19%)	1 (2%)
Hepatocellular carcinoma	2 (4%)	1 (5%)	
#Pancreas	(48)	(9)	(50)
Sarcoma, NOS, metastatic			1 (2%)
URINARY SYSTEM			
None			

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

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(49)	(49)
Carcinoma, NOS			1 (2%)
Adenoma, NOS		1 (2%)	
#Anterior pituitary	(49)	(49)	(49)
Adenoma, NOS	5 (10%)	8 (16%)	2 (4%)
#Adrenal/capsule	(50)	(9)	(50)
Adenoma, NOS		1 (11%)	
#Thyroid	(49)	(7)	(50)
Follicular cell adenoma	2 (4%)	2 (29%)	2 (4%)
#Pancreatic islets	(48)	(9)	(50)
Islet cell adenoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Acinar cell carcinoma	1 (2%)	1 (2%)	1 (2%)
Mixed tumor, malignant	1 (2%)	2 (4%)	
#Uterus	(49)	(45)	(50)
Sarcoma, NOS			1 (2%)
Leiomyoma			1 (2%)
Endometrial stromal polyp	1 (2%)	2 (4%)	
#Uterus/endometrium	(49)	(45)	(50)
Adenocarcinoma, NOS	1 (2%)		
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(50)
Adenoma, NOS	6 (12%)	5 (10%)	3 (6%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	10	6	11
Moribund sacrifice	3	5	4
Terminal sacrifice	37	39	35

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

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	36	35	27
Total primary tumors	52	56	33
Total animals with benign tumors	20	23	8
Total benign tumors	22	26	10
Total animals with malignant tumors	28	25	22
Total malignant tumors	30	30	23
Total animals with secondary tumors##	1		1
Total secondary tumors	1		3

* 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 HYDROCHLOROTHIAZIDE: UNTREATED CONTROL

ANIMAL NUMBER	0046	0043	0037	0032	0017	0008	0016	0050	0045	0034	0049	0031	0030	0001	0002	0003	0004	0005	0006	0007	0009	0010	0011	0012	0013
WEEKS ON STUDY	054	058	084	085	086	091	100	100	101	102	103	104	105	106	106	106	106	106	106	106	106	106	106	106	106
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS						X																X			
Osteosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																									
Alveolar/bronchiolar adenoma																X							X	X	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	−	+	+	−	+	−	+	−	+	+	−	+	−	+	+	+	+	+	+	+	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																	X								
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	−	−	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																									
Large intestine	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									X
Parathyroid	+	−	+	+	+	+	-	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell carcinoma																									
Mixed tumor, malignant																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																						X			
Endometrial stromal polyp																									
Hemangiosarcoma																									
Malignant lymphoma, histiocytic type																							X		
Ovary	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma												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				
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								X		X														
Malignant lymphoma, mixed type		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
* Animals necropsied
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 IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0 1 4	0 1 5	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5	0 2 6	0 2 7	0 2 8	0 2 9	0 3 3	0 3 5	0 3 6	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 4	0 4 7	0 4 8	TOTAL TISSUES TUMORS
WEEKS ON STUDY	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS							X										X									4
Osteosarcoma																					X					1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic			X																							1
Alveolar/bronchiolar adenoma	X																									4
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	42
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma															X											2
Hepatocellular carcinoma			X												X											2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Malignant lymphoma, mixed type										X													X			2
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS								X			X						X		X				X			5
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma											X															2
Parathyroid	–	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	42
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islet cell adenoma																								X		1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	*50
Acinar cell carcinoma																			X							1
Mixed tumor, malignant		X																								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, NOS																										1
Endometrial stromal polyp		X																								1
Hemangiosarcoma					X																					1
Malignant lymphoma, histiocytic type																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangioma																										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	X															X				X		X				6
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																										3
Malignant lymphoma, mixed type									X		X	X	X		X	X				X				X		13

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

ANIMAL NUMBER	0343	0338	0008	0004	0224	0445	0330	0339	0443	0115	0001	0002	0003	0005	0006	0007	0009	0110	0111	0112	0113	0114	0116	0117	0118
WEEKS ON STUDY	0777	0883	0877	0889	0990	0992	0999	1002	1022	1044	1051	1055	1055	1055	1056	1055	1055	1055	1055	1055	1055	1055	1056	1057	1055
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS										X															
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Nasal cavity	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	-	−	−		−	−		−	−	−
Spleen	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−		−	+	−	+	+	+	+	+	+
Malignant lymphoma, lymphocytic type																									X
Malignant lymphoma, mixed type																									
Lymph nodes	+	+	+	+	+	−	+	+	+	−	−	−	+	−	−	-	−	+	+	+	−	−	+	−	−
Malignant lymphoma, mixed type																									
Thymus	−	+	−	+	−	−	−	−	+	−	−	−	−	−	−	-	−	−	−	−	−	−	−	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	−	+	−	+	+	−	−	−	−	−	-	−	−	−	−	−	−	−	−	−
Liver	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+	−	−	−	+	−	−	+	−	+
Hepatocellular adenoma																							X		
Hepatocellular carcinoma																									
Hemangiosarcoma							X																		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	N	N
Pancreas	+	+	+	+	+	+	−	−	+	−	−	−	−	−	−	-	−	−	+	−	−	−	−	−	−
Esophagus	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	-	−	−	−	−	−	−	−	−	−
Stomach	+	+	+	+	+	−	−	−	+	+	−	−	−	−	−	-	−	−	−	−	−	−	−	−	−
Small intestine	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	-	−	−	−	+	−	−	−	−	−
Large intestine	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	-	−	−	−	−	−	−	−	−	−
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	-	−	−	−	−	−	−	−	−	−
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS													X			X			X						X
Adrenal	+	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Adenoma, NOS																				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
Acinar cell carcinoma																									
Mixed tumor, malignant							X																		
Uterus	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+
Endometrial stromal polyp	X																								
Hemangioma																									
Ovary	+	+	+	+	+	+	~	−	+	−	+	−	−	−	+	−	−	+	+	+	−	+	−	−	−
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, lymphocytic type					X																X				
Malignant lymphoma, histiocytic type			X			X		X																	
Malignant lymphoma, mixed type	X								X				X							X					

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE: LOW DOSE (Continued)

ANIMAL NUMBER	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 5	0 2 6	0 2 7	0 2 8	0 2 9	0 3 1	0 3 2	0 3 5	0 3 6	0 3 7	0 3 8	0 4 0	0 4 1	0 4 2	0 4 4	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	TOTAL TISSUES TUMORS
WEEKS ON STUDY	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	
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	N	*50
Sarcoma, NOS																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	+	–	–	+	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	+	+	+	–	15
Alveolar/bronchiolar adenoma														X										X		2
Alveolar/bronchiolar carcinoma																							X			1
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Nasal cavity	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Spleen	+	–	+	+	–	+	–	–	–	+	+	–	+	+	–	+	+	+	+	–	–	–	–	+	+	32
Malignant lymphoma, lymphocytic type																										1
Malignant lymphoma, mixed type																									X	1
Lymph nodes	+	–	+	+	–	+	+	–	–	+	–	+	–	–	–	+	–	–	+	–	–	–	+	+	–	24
Malignant lymphoma, mixed type																							X			1
Thymus	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	–	–	7
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Liver	–	–	+	–	–	+	–	–	–	–	–	–	+	–	–	–	+	–	+	–	–	–	+	–	–	21
Hepatocellular adenoma			X										X				X									4
Hepatocellular carcinoma						X																				1
Hemangiosarcoma																										2
Bile duct	–	–	+	–	–	+	–	–	–	–	–	–	+	–	–	–	+	–	+	–	–	–	+	–	–	21
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	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
Small intestine	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	9
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	8
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS				X		X				X							X		X							9
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Adenoma, NOS																										1
Thyroid	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
Follicular cell adenoma							X																			2
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	*50
Acinar cell carcinoma																	X									1
Mixed tumor, malignant															X											2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	45
Endometrial stromal polyp																X										2
Hemangioma																		X								1
Ovary	–	–	–	+	–	–	–	+	–	–	+	–	–	+	–	–	–	–	–	+	–	–	–	+	+	20
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS							X		X			X									X					5
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, lymphocytic type														X					X							4
Malignant lymphoma, histiocytic type																										3
Malignant lymphoma, mixed type	X		X	X		X				X		X			X									X		12

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

ANIMAL NUMBER	0048	0036	0023	0006	0047	0025	0049	0037	0018	0016	0030	0041	0022	0032	0038	0001	0002	0003	0004	0005	0007	0008	0009	0010	0011
WEEKS ON STUDY	0211	0078	0800	0833	0833	0911	0911	0933	0966	0977	0997	1000	1022	1022	1033	1055	1055	1055	1055	1055	1055	1055	1055	1055	1055
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS													X												
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X																		
Alveolar/bronchiolar carcinoma														X											
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, histiocytic type																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic													X												
Thymus	+	−	+	+	+	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	+	−	+	+	−
Sarcoma, NOS, metastatic													X												
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic													X												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS																X									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma								X										X							
Parathyroid	+	−	−	+	+	−	+	−	+	+	−	−	+	+	+	+	−	+	+	−	−	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell carcinoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS													X												
Leiomyoma																			X						
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS							X											X							
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, undifferentiated type									X																
Malignant lymphoma, lymphocytic type																									X
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 IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE: HIGH DOSE (Continued)

ANIMAL NUMBER	0 1 2	0 1 3	0 1 4	0 1 5	0 1 7	0 1 9	0 2 0	0 2 1	0 2 4	0 2 6	0 2 7	0 2 8	0 2 9	0 3 1	0 3 3	0 3 4	0 3 5	0 3 9	0 4 0	0 4 2	0 4 3	0 4 4	0 4 5	0 4 6	0 5 0	TOTAL:
WEEKS ON STUDY	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50	
Sarcoma, NOS																									1	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Alveolar/bronchiolar adenoma																									1	
Alveolar/bronchiolar carcinoma																									1	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Malignant lymphoma, histiocytic type							X																		1	
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Sarcoma, NOS, metastatic																									1	
Thymus	+	+	+	+	+	+	+	–	+	+	+	+	+	–	–	+	–	+	–	+	+	+	+	+	39	
Sarcoma, NOS, metastatic																									1	
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Hepatocellular adenoma															X										1	
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Gallbladder & common bile duct	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Sarcoma, NOS, metastatic																									1	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Carcinoma, NOS																								X	1	
Adenoma, NOS			X																						2	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Follicular cell adenoma																									2	
Parathyroid	+	–	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	+	+	–	+	+	+	+	33	
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50	
Acinar cell carcinoma									X																1	
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Sarcoma, NOS																									1	
Leiomyoma																									1	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50	
Adenoma, NOS																								X	3	
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	*50	
Malignant lymphoma, undiffer type																									1	
Malignant lymphoma, lymphocytic type																									1	
Malignant lymphoma, histiocytic type																									2	
Malignant lymphoma, mixed type						X					X						X			X	X				13	

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

	Control	2,500 ppm	5,000 ppm
Subcutaneous Tissue: Sarcoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	9.9%	2.4%	2.6%
Terminal Rates (c)	3/38 (8%)	0/40 (0%)	0/35 (0%)
Week of First Observation	91	104	102
Life Table Tests (d)	P=0.115N	P=0.172N	P=0.203N
Incidental Tumor Tests (d)	P=0.087N	P=0.184N	P=0.167N
Cochran-Armitage Trend Test (d)	P=0.101N		
Fisher Exact Test (d)		P=0.181N	P=0.181N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	4/49 (8%)	(e) 2/15 (13%)	1/50 (2%)
Adjusted Rates (b)	10.8%		2.2%
Terminal Rates (c)	4/37 (11%)		0/35 (0%)
Week of First Observation	105		91
Life Table Test (d)			P=0.195N
Incidental Tumor Test (d)			P=0.181N
Fisher Exact Test (d)			P=0.175N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	4/49 (8%)	(e) 3/15 (20%)	2/50 (4%)
Adjusted Rates (b)	10.8%		4.8%
Terminal Rates (c)	4/37 (11%)		0/35 (0%)
Week of First Observation	105		91
Life Table Test (d)			P=0.362N
Incidental Tumor Test (d)			P=0.317N
Fisher Exact Test (d)			P=0.329N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	0/50 (0%)	(e,f) 5/50 (10%)	1/50 (2%)
Adjusted Rates (b)	0.0%		2.9%
Terminal Rates (c)	0/38 (0%)		1/35 (3%)
Week of First Observation			105
Life Table Test (d)			P=0.484
Incidental Tumor Test (d)			P=0.484
Fisher Exact Test (d)			P=0.500
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	4/50 (8%)	(e,f) 3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	9.2%		7.5%
Terminal Rates (c)	1/38 (3%)		1/35 (3%)
Week of First Observation	54		80
Life Table Test (d)			P=0.537N
Incidental Tumor Test (d)			P=0.480N
Fisher Exact Test (d)			P=0.500N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	15/50 (30%)	(e,f) 14/50 (28%)	13/50 (26%)
Adjusted Rates (b)	35.9%		30.4%
Terminal Rates (c)	12/38 (32%)		7/35 (20%)
Week of First Observation	58		78
Life Table Test (d)			P=0.498N
Incidental Tumor Test (d)			P=0.460N
Fisher Exact Test (d)			P=0.412N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	19/50 (38%)	(e,f) 22/50 (44%)	18/50 (36%)
Adjusted Rates (b)	42.5%		39.9%
Terminal Rates (c)	13/38 (34%)		9/35 (26%)
Week of First Observation	54		78
Life Table Test (d)			P=0.552
Incidental Tumor Test (d)			P=0.527N
Fisher Exact Test (d)			P=0.500N

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

	Control	2,500 ppm	5,000 ppm
Circulatory System: Hemangioma or Hemangiosarcoma			
Overall Rates (a)	2/50 (4%)	(e,f) 3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	5.1%		0.0%
Terminal Rates (c)	1/38 (3%)		0/35 (0%)
Week of First Observation	104		
Life Table Test (d)			P=0.261N
Incidental Tumor Test (d)			P=0.217N
Fisher Exact Test (d)			P=0.248N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	2/50 (4%)	(e) 4/21 (19%)	1/50 (2%)
Adjusted Rates (b)	5.3%		2.9%
Terminal Rates (c)	2/38 (5%)		1/35 (3%)
Week of First Observation	105		105
Life Table Test (d)			P=0.529N
Incidental Tumor Test (d)			P=0.529N
Fisher Exact Test (d)			P=0.500N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	3/50 (6%)	(e) 5/21 (24%)	1/50 (2%)
Adjusted Rates (b)	7.9%		2.9%
Terminal Rates (c)	3/38 (8%)		1/35 (3%)
Week of First Observation	105		105
Life Table Test (d)			P=0.335N
Incidental Tumor Test (d)			P=0.335N
Fisher Exact Test (d)			P=0.309N
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	5/49 (10%)	8/49 (16%)	2/49 (4%)
Adjusted Rates (b)	13.5%	20.0%	5.7%
Terminal Rates (c)	5/37 (14%)	8/40 (20%)	2/35 (6%)
Week of First Observation	105	105	105
Life Table Tests (d)	P=0.221N	P=0.326	P=0.238N
Incidental Tumor Tests (d)	P=0.221N	P=0.326	P=0.238N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.276	P=0.218N
Mammary Gland: Acinar Cell Carcinoma or Mixed Tumor, Malignant			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	5.3%	7.2%	2.9%
Terminal Rates (c)	2/38 (5%)	2/40 (5%)	1/35 (3%)
Week of First Observation	105	99	105
Life Table Tests (d)	P=0.433N	P=0.519	P=0.529N
Incidental Tumor Tests (d)	P=0.404N	P=0.482	P=0.529N
Cochran-Armitage Trend Test (d)	P=0.399N		
Fisher Exact Test (d)		P=0.500	P=0.500N
Harderian Gland: Adenoma			
Overall Rates (a)	6/50 (12%)	5/50 (10%)	3/50 (6%)
Adjusted Rates (b)	15.0%	12.5%	7.8%
Terminal Rates (c)	5/38 (13%)	5/40 (13%)	2/35 (6%)
Week of First Observation	85	105	91
Life Table Tests (d)	P=0.226N	P=0.466N	P=0.280N
Incidental Tumor Tests (d)	P=0.208N	P=0.453N	P=0.249N
Cochran-Armitage Trend Test (d)	P=0.195N		
Fisher Exact Test (d)		P=0.500N	P=0.243N

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

	Control	2,500 ppm	5,000 ppm
All Sites: Benign Tumors			
Overall Rates (a)	20/50 (40%)	23/50 (46%)	8/50 (16%)
Adjusted Rates (b)	49.8%	55.9%	20.9%
Terminal Rates (c)	18/38 (47%)	22/40 (55%)	6/35 (17%)
Week of First Observation	85	77	91
Life Table Tests (d)	P=0.013N	P=0.424	P=0.014N
Incidental Tumor Tests (d)	P=0.012N	P=0.384	P=0.009N
Cochran-Armitage Trend Test (d)	P=0.008N		
Fisher Exact Test (d)		P=0.343	P=0.007N
All Sites: Malignant Tumors			
Overall Rates (a)	28/50 (56%)	25/50 (50%)	22/50 (44%)
Adjusted Rates (b)	61.8%	52.0%	47.5%
Terminal Rates (c)	21/38 (55%)	17/40 (43%)	11/35 (31%)
Week of First Observation	54	77	78
Life Table Tests (d)	P=0.262N	P=0.296N	P=0.295N
Incidental Tumor Tests (d)	P=0.133N	P=0.410N	P=0.169N
Cochran-Armitage Trend Test (d)	P=0.136N		
Fisher Exact Test (d)		P=0.345N	P=0.159N
All Sites: All Tumors			
Overall Rates (a)	36/50 (72%)	35/50 (70%)	27/50 (54%)
Adjusted Rates (b)	78.1%	72.9%	57.2%
Terminal Rates (c)	28/38 (74%)	27/40 (68%)	15/35 (43%)
Week of First Observation	54	77	78
Life Table Tests (d)	P=0.150N	P=0.388N	P=0.176N
Incidental Tumor Tests (d)	P=0.043N	P=0.556N	P=0.058N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.500N	P=0.049N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) Incomplete sampling of tissues
(f) Thirty-two spleens, 21 livers, and 24 lymph nodes were examined microscopically.

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE

	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)
Epidermal inclusion cyst			1 (2%)
Inflammation, chronic focal			1 (2%)
*Subcutaneous tissue	(50)	(50)	(50)
Mineralization	1 (2%)		
Cyst, NOS			1 (2%)
Inflammation, chronic focal			1 (2%)
RESPIRATORY SYSTEM			
#Nasal cavity	(50)	(4)	(50)
Foreign body, NOS	1 (2%)		1 (2%)
Hemorrhage			2 (4%)
Inflammation, acute focal	2 (4%)		3 (6%)
#Tracheal submucosa	(50)	(5)	(50)
Inflammation, chronic focal			1 (2%)
#Lung	(49)	(15)	(50)
Mineralization	1 (2%)		
Congestion, NOS	3 (6%)	1 (7%)	1 (2%)
Edema, NOS			1 (2%)
Hemorrhage	2 (4%)	2 (13%)	5 (10%)
Inflammation, chronic focal	22 (45%)	6 (40%)	14 (28%)
Pigmentation, NOS	2 (4%)		1 (2%)
Alveolar macrophages	1 (2%)	1 (7%)	2 (4%)
Hyperplasia, alveolar epithelium	2 (4%)		1 (2%)
HEMATOPOIETIC SYSTEM			
#Brain/meninges	(50)	(5)	(50)
Hyperplasia, lymphoid			1 (2%)
*Multiple organs	(50)	(50)	(50)
Hyperplasia, lymphoid	3 (6%)	2 (4%)	7 (14%)
#Bone marrow	(50)	(5)	(50)
Pigmentation, NOS		3 (60%)	2 (4%)
Hyperplasia, granulocytic	6 (12%)	2 (40%)	2 (4%)
#Spleen	(50)	(32)	(50)
Necrosis, focal		1 (3%)	
Russell body	1 (2%)		
Angiectasis		1 (3%)	
Hyperplasia, lymphoid	4 (8%)	3 (9%)	5 (10%)
Hematopoiesis	8 (16%)	11 (34%)	5 (10%)
#Lymph node	(49)	(24)	(50)
Hyperplasia, lymphoid	1 (2%)	1 (4%)	
#Mandibular lymph node	(49)	(24)	(50)
Hemorrhage			1 (2%)
Pigmentation, NOS			1 (2%)
Angiectasis		1 (4%)	
Hyperplasia, lymphoid		1 (4%)	
#Thoracic lymph node	(49)	(24)	(50)
Hyperplasia, plasma cell	3 (6%)	1 (4%)	
Hyperplasia, reticulum cell			1 (2%)
Hyperplasia, lymphoid	3 (6%)		
#Hepatic lymph node	(49)	(24)	(50)
Hyperplasia, plasma cell	1 (2%)		
#Lumbar lymph node	(49)	(24)	(50)
Hyperplasia, NOS		1 (4%)	

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE (Continued)

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Mesenteric lymph node	(49)	(24)	(50)
Congestion, NOS	3 (6%)	1 (4%)	3 (6%)
Edema, NOS			1 (2%)
Hemorrhage		1 (4%)	1 (2%)
Abscess, NOS	1 (2%)		
Angiectasis	2 (4%)		2 (4%)
Hyperplasia, plasma cell	1 (2%)		1 (2%)
Hyperplasia, reticulum cell			1 (2%)
Hyperplasia, lymphoid	4 (8%)		2 (4%)
Hematopoiesis	3 (6%)	2 (8%)	1 (2%)
#Inguinal lymph node	(49)	(24)	(50)
Hyperplasia, plasma cell	1 (2%)		
#Lung	(49)	(15)	(50)
Hyperplasia, lymphoid	1 (2%)		4 (8%)
#Liver	(50)	(21)	(50)
Hyperplasia, lymphoid			1 (2%)
Hematopoiesis	4 (8%)	2 (10%)	2 (4%)
#Pancreas	(48)	(9)	(50)
Hyperplasia, lymphoid			1 (2%)
#Peyer's patch	(47)	(9)	(49)
Hyperplasia, lymphoid	1 (2%)		1 (2%)
#Kidney	(50)	(50)	(50)
Hyperplasia, lymphoid	2 (4%)	2 (4%)	4 (8%)
#Urinary bladder/submucosa	(48)	(8)	(50)
Hyperplasia, lymphoid			1 (2%)
#Adrenal cortex	(50)	(9)	(50)
Hematopoiesis		1 (11%)	1 (2%)
#Adrenal medulla	(50)	(9)	(50)
Hematopoiesis	1 (2%)		
#Thymus	(42)	(7)	(39)
Involution, NOS	5 (12%)	2 (29%)	3 (8%)
Hyperplasia, epithelial	1 (2%)		
Angiectasis	1 (2%)		
Hyperplasia, lymphoid	1 (2%)	2 (29%)	
CIRCULATORY SYSTEM			
#Heart	(50)	(50)	(50)
Hemorrhage	1 (2%)		
Inflammation, acute focal			1 (2%)
Inflammation, chronic focal	5 (10%)	8 (16%)	2 (4%)
Periarteritis			1 (2%)
Degeneration, NOS			1 (2%)
Pigmentation, NOS		1 (2%)	
#Cardiac valve	(50)	(50)	(50)
Inflammation, chronic focal	1 (2%)		
Pigmentation, NOS	6 (12%)		5 (10%)
#Uterus/endometrium	(49)	(45)	(50)
Thrombosis, NOS			1 (2%)
DIGESTIVE SYSTEM			
#Salivary gland	(49)	(8)	(50)
Inflammation, chronic focal	12 (24%)	3 (38%)	10 (20%)
#Liver	(50)	(21)	(50)
Cyst, NOS		1 (5%)	
Hemorrhage	2 (4%)		1 (2%)
Inflammation, chronic focal	23 (46%)	4 (19%)	21 (42%)
Fibrosis, focal		1 (5%)	

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE (Continued)

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM			
#Liver (Continued)	(50)	(21)	(50)
Peliosis hepatis			2 (4%)
Necrosis, focal	6 (12%)	4 (19%)	4 (8%)
Pigmentation, NOS	1 (2%)		
Atrophy, focal	1 (2%)		
Angiectasis		1 (5%)	
#Liver/hepatocytes	(50)	(21)	(50)
Cytoplasmic vacuolization	4 (8%)	1 (5%)	3 (6%)
Basophilic cyto change	2 (4%)	3 (14%)	1 (2%)
Eosinophilic cyto change			1 (2%)
Clear cell change	1 (2%)	1 (5%)	2 (4%)
*Gallbladder	(50)	(50)	(50)
Inflammation, chronic focal	6 (12%)		
#Pancreas	(48)	(9)	(50)
Cyst, NOS	1 (2%)	1 (11%)	
Inflammation, acute focal	1 (2%)		
Inflammation, chronic focal	16 (33%)	1 (11%)	14 (28%)
#Pancreatic acinus	(48)	(9)	(50)
Atrophy, focal			3 (6%)
Atrophy, diffuse	2 (4%)	1 (11%)	2 (4%)
Hyperplasia, focal	1 (2%)		
#Glandular stomach	(46)	(7)	(49)
Mineralization			1 (2%)
Cyst, NOS	1 (2%)		1 (2%)
Inflammation, chronic focal	4 (9%)		4 (8%)
Degeneration, cystic	2 (4%)		
Hyperplasia, epithelial	1 (2%)		
#Forestomach	(46)	(7)	(49)
Mineralization			1 (2%)
Inflammation, chronic focal	3 (7%)	1 (14%)	
Hyperplasia, epithelial	1 (2%)	1 (14%)	2 (4%)
Hyperkeratosis			1 (2%)
#Small intestine	(47)	(9)	(49)
Inflammation, acute focal		1 (11%)	
Amyloidosis	1 (2%)		5 (10%)
#Duodenum	(47)	(9)	(49)
Impaction, fecal	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
URINARY SYSTEM			
#Urinary bladder/cavity	(48)	(8)	(50)
Calculus, microscopic examination			1 (2%)
Dilatation, NOS	1 (2%)		
Inflammation, suppurative			1 (2%)
#Kidney	(50)	(50)	(50)
Hydronephrosis		1 (2%)	
Hemorrhage		1 (2%)	
Glomerulonephritis, NOS	1 (2%)	1 (2%)	1 (2%)
Inflammation, suppurative			1 (2%)
Inflammation, chronic focal	28 (56%)	20 (40%)	19 (38%)
Nephrosis, NOS	6 (12%)	7 (14%)	6 (12%)
Glomerulosclerosis, NOS	2 (4%)	1 (2%)	3 (6%)
Necrosis, focal		2 (4%)	
Infarct, NOS			2 (4%)
Metaplasia, osseous			2 (4%)
#Kidney/tubule	(50)	(50)	(50)
Degeneration, NOS	2 (4%)	2 (4%)	6 (12%)
#Kidney/pelvis	(50)	(50)	(50)
Dilatation, NOS	1 (2%)		

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM (Continued)			
#Urinary bladder/submucosa	(48)	(8)	(50)
Congestion, NOS		1 (13%)	
Inflammation, focal			1 (2%)
Inflammation, suppurative			1 (2%)
Inflammation, chronic focal	20 (42%)	2 (25%)	14 (28%)
Inflammation, chronic diffuse			1 (2%)
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(49)	(49)
Hyperplasia, focal		1 (2%)	
#Anterior pituitary	(49)	(49)	(49)
Cyst, NOS		1 (2%)	
Congestion, NOS	1 (2%)	1 (2%)	3 (6%)
Hyperplasia, focal	28 (57%)	21 (43%)	22 (45%)
Angiectasis		2 (4%)	
#Adrenal/capsule	(50)	(9)	(50)
Hyperplasia, focal	48 (96%)	7 (78%)	44 (88%)
#Adrenal cortex	(50)	(9)	(50)
Congestion, NOS	1 (2%)		1 (2%)
Inflammation, acute focal	1 (2%)		
Degeneration, NOS	2 (4%)	1 (11%)	2 (4%)
Hyperplasia, focal	4 (8%)	1 (11%)	1 (2%)
#Adrenal medulla	(50)	(9)	(50)
Hemorrhagic cyst			1 (2%)
Hyperplasia, NOS	1 (2%)		
#Thyroid	(49)	(7)	(50)
Cyst, NOS			1 (2%)
Follicular cyst, NOS	12 (24%)	1 (14%)	14 (28%)
Inflammation, acute focal	1 (2%)		
Inflammation, chronic focal	4 (8%)		
Necrosis, focal		1 (14%)	
Hyperplasia, follicular cell	4 (8%)		4 (8%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Hyperplasia, NOS			1 (2%)
Hyperplasia, cystic	5 (10%)	2 (4%)	2 (4%)
#Uterus	(49)	(45)	(50)
Hydrometra	1 (2%)	2 (4%)	1 (2%)
Hemorrhagic cyst		1 (2%)	
#Uterus/endometrium	(49)	(45)	(50)
Inflammation, suppurative	1 (2%)		1 (2%)
Hyperplasia, NOS	2 (4%)		1 (2%)
Hyperplasia, cystic	44 (90%)	43 (96%)	43 (86%)
#Ovary	(48)	(20)	(50)
Mineralization	1 (2%)		
Cyst, NOS	19 (40%)	12 (60%)	20 (40%)
Hemorrhage			1 (2%)
Hemorrhagic cyst	4 (8%)		2 (4%)
Inflammation, suppurative	1 (2%)	1 (5%)	
Inflammation, chronic focal	1 (2%)		
Pigmentation, NOS	1 (2%)		
Atrophy, NOS			1 (2%)
Angiectasis		1 (5%)	

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HYDROCHLOROTHIAZIDE (Continued)

	Untreated Control	Low Dose	High Dose
NERVOUS SYSTEM			
#Brain/meninges	(50)	(5)	(50)
Inflammation, chronic focal	4 (8%)		1 (2%)
Pigmentation, NOS	1 (2%)		1 (2%)
*Choroid plexus	(50)	(50)	(50)
Inflammation, chronic focal	1 (2%)		
#Brain	(50)	(5)	(50)
Mineralization	15 (30%)	1 (20%)	17 (34%)
Hydrocephalus, NOS		1 (20%)	1 (2%)
Hemorrhage			1 (2%)
Inflammation, chronic focal	1 (2%)		1 (2%)
Gliosis	1 (2%)		
SPECIAL SENSE ORGANS			
*Nasolacrimal duct	(50)	(50)	(50)
Hemorrhage	12 (24%)		6 (12%)
Inflammation, acute focal	1 (2%)		1 (2%)
*Harderian gland	(50)	(50)	(50)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, focal		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
*Bone	(50)	(50)	(50)
Fibrous osteodystrophy	36 (72%)		37 (74%)
BODY CAVITIES			
*Abdominal cavity	(50)	(50)	(50)
Cyst, NOS		1 (2%)	
Inflammation, suppurative	3 (6%)	1 (2%)	
Inflammation, chronic focal			1 (2%)
Necrosis, fat	3 (6%)	6 (12%)	1 (2%)
*Pleural cavity	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)		
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mineralization			1 (2%)
Tail			
Necrosis, focal			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 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 viral 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 viral 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 (6,18,24 mo)	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) Sendai (12 mo)	MHV (mouse hepatitis virus)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai (6,18,24 mo)	RCV (rat coronavirus) (6,12,18 mo) Sendai (12 mo)	RCV/SDA(sialodacryoadenitis virus) (24 mo)

Results

Results are presented in Table E1.

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

Interval (months)	Number of Animals	Positive Serologic Reaction for
RATS		
6	--	None positive
12	1/10	KRV
18	--	None positive
24	--	None positive
MICE		
6	--	None positive
12	10/10	Sendai
18	8/10	Sendai
24	6/9	Sendai

(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.

APPENDIX F

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

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

	Control		Low Dose			Mid 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)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
1	19	215	16	196	20	16	194	41	16	190	168
5	18	305	17	273	16	17	271	31	17	269	126
9	18	354	17	315	13	17	313	27	17	315	108
15	18	398	17	350	12	17	342	25	17	347	98
19	17	419	16	367	11	17	362	23	16	363	88
23	18	437	16	377	11	17	371	23	17	374	91
27	18	452	16	394	10	16	388	21	16	390	82
31	17	467	15	405	9	15	401	19	16	404	79
36	18	476	16	414	10	16	408	20	16	413	77
39	17	483	15	419	9	16	414	19	16	417	77
44	17	490	15	426	9	15	421	18	15	424	71
49	18	500	16	434	9	16	431	19	16	433	74
53	17	507	15	447	8	16	443	18	16	444	72
57	18	513	15	450	8	15	445	17	15	442	68
61	18	516	16	453	9	16	448	18	16	453	71
67	18	516	16	451	9	16	446	18	16	454	70
71	17	518	13	435	7	15	442	17	15	452	66
75	17	514	14	448	8	15	440	17	15	451	67
79	17	508	13	440	7	15	432	17	14	440	64
83	16	503	14	429	8	15	423	18	15	437	69
87	17	496	15	421	9	15	416	18	14	423	66
91	17	482	14	414	8	16	418	19	13	398	65
96	12	467	12	393	8	11	390	14	11	388	57
99	21	461	21	379	14	21	383	27	20	378	106
103	18	458	15	360	10	18	381	24	15	370	81
Mean	17.4	458	15.4	396	10	16.0	393	21	15.6	395	82
SD (c)	1.5		1.7		3	1.7		6	1.6		24
CV (d)	8.6		11.0		30.0	10.6		28.6	10.3		29.3

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Estimated milligrams of hydrochlorothiazide 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 HYDROCHLOROTHIAZIDE

	Control		Low Dose			Mid 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)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
1	13	150	11	140	20	12	138	43	11	138	159
5	12	189	11	173	16	12	174	34	11	172	128
9	11	209	11	190	14	11	192	29	11	191	115
15	12	226	11	199	14	12	200	30	11	199	111
19	11	232	10	207	12	10	207	24	10	205	98
23	12	238	11	208	13	11	208	26	11	206	107
27	11	247	10	216	12	11	219	25	11	215	102
31	11	256	10	221	11	11	224	25	10	223	90
36	11	264	10	225	11	10	227	22	10	224	89
39	11	270	10	228	11	10	229	22	10	227	88
44	11	277	10	231	11	10	235	21	10	233	86
49	12	290	11	240	11	11	242	23	11	240	92
53	12	299	10	249	10	11	251	22	11	250	88
57	13	314	11	252	11	12	258	23	11	253	87
61	13	326	12	265	11	12	261	23	12	262	92
67	12	338	13	277	12	13	282	23	13	275	95
71	13	347	12	288	10	12	290	21	13	286	91
75	14	356	13	300	11	13	300	22	13	296	88
79	14	364	13	303	11	13	310	21	13	300	87
83	13	366	12	307	10	12	307	20	12	306	78
87	12	365	12	304	10	12	305	20	12	303	79
91	13	365	12	295	10	12	303	20	12	299	80
96	11	366	10	297	8	9	282	16	9	289	62
99	19	365	16	290	14	17	283	30	17	287	118
103	14	366	12	277	11	13	275	24	12	275	87
Mean	12.4	295	11.4	247	12	11.7	248	24	11.5	246	96
SD (c)	1.7		1.4		2	1.5		5	1.6		19
CV (d)	13.7		12.3		16.7	12.8		20.8	13.9		19.8

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Estimated milligrams of hydrochlorothiazide 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 HYDROCHLOROTHIAZIDE

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)
1	3.0	23.3	2.9	22.9	317	3.0	22.8	658
5	3.7	27.0	3.7	26.7	346	3.9	26.5	736
10	3.6	30.9	3.8	30.0	317	3.9	29.8	654
16	3.7	32.8	3.8	31.5	302	3.7	31.6	585
20	3.8	34.2	3.9	33.3	293	4.0	33.1	604
24	4.2	35.5	4.2	34.8	302	4.4	35.0	629
28	3.8	36.6	3.9	35.8	272	4.1	36.3	565
32	3.8	37.3	4.0	36.8	272	4.2	37.8	556
37	3.5	37.8	3.5	36.7	238	3.7	37.4	495
40	3.6	37.7	3.9	37.5	260	3.9	37.7	517
45	3.7	38.0	3.6	38.1	236	4.0	38.5	519
49	3.8	38.1	3.8	38.6	246	4.2	39.2	536
54	3.8	38.7	3.7	39.1	237	4.0	40.3	496
58	3.9	39.0	4.0	38.8	258	4.0	40.2	498
62	3.7	38.7	4.1	38.6	266	4.3	40.3	533
68	3.9	39.6	3.7	39.4	235	3.9	40.1	486
72	3.8	39.7	3.8	39.6	240	3.8	40.0	475
76	3.8	39.9	3.9	39.8	245	3.9	39.8	490
80	3.8	39.6	4.0	40.3	248	4.1	40.0	513
84	3.7	39.1	3.8	40.1	237	3.8	38.5	494
88	3.7	38.7	3.9	39.4	247	3.9	37.1	526
92	4.1	39.0	4.3	39.7	271	4.3	37.2	578
96	3.8	38.5	3.8	39.0	244	3.8	36.4	522
100	3.5	37.2	3.7	38.6	240	3.8	36.6	519
104	3.6	37.9	3.8	38.5	247	3.5	35.1	499
Mean	3.7	36.6	3.8	36.5	265	3.9	36.3	547
SD (c)	0.2		0.3		31	0.3		65
CV (d)	5.4		7.9		11.7	7.7		11.9

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Estimated milligrams of hydrochlorothiazide 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 HYDROCHLOROTHIAZIDE

	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)
1	3.0	19.2	2.8	19.1	366	2.7	18.7	722
5	3.5	21.9	3.6	21.8	413	3.7	21.7	853
10	3.5	24.7	3.5	24.7	354	3.4	24.0	708
16	3.8	25.8	3.4	25.1	339	3.7	25.2	734
20	3.8	27.4	3.8	26.9	353	3.7	26.7	693
24	4.1	28.5	4.1	28.4	361	4.1	28.0	732
28	3.8	29.9	3.9	29.0	336	3.7	28.9	640
32	3.8	30.4	4.1	30.4	337	4.1	30.5	672
37	3.6	31.0	3.6	30.3	297	3.5	30.4	576
40	3.7	31.3	4.0	31.2	321	3.9	31.3	623
45	3.6	32.4	3.6	31.9	282	3.6	31.6	570
49	3.9	33.8	4.0	33.3	300	4.0	32.9	608
54	3.7	34.2	3.8	33.9	280	3.8	33.5	567
58	4.0	35.1	4.2	34.9	301	4.2	34.1	616
62	4.2	36.5	4.4	36.3	303	4.4	36.2	608
68	3.6	38.9	4.0	38.5	260	3.7	38.7	478
72	3.9	39.6	3.8	39.7	239	3.9	40.0	488
76	3.9	41.4	4.0	40.6	246	3.9	40.2	485
80	3.8	41.5	4.0	41.6	240	3.9	41.1	474
84	3.8	41.5	3.9	41.0	238	3.9	41.3	472
88	4.0	41.8	4.1	40.6	252	4.0	40.8	490
92	4.3	41.3	4.3	39.1	275	4.2	39.4	533
96	4.1	40.1	4.2	40.3	261	3.9	37.2	524
100	3.9	39.5	4.1	38.7	265	4.0	36.4	549
104	4.3	40.3	4.3	39.0	276	4.1	37.1	553
Mean	3.8	33.9	3.9	33.5	300	3.8	33.0	599
SD (c)	0.3		0.3		47	0.3		101
CV (d)	7.9		7.7		15.7	7.9		16.9

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Estimated milligrams of hydrochlorothiazide 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

Pelleted Diet: July 1981 to July 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

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Crude protein (percent by weight)	23.53 ± 0.81	22.2-25.1	24
Crude fat (percent by weight)	4.92 ± 0.55	3.3-5.7	24
Crude fiber (percent by weight)	3.29 ± 0.26	2.9-3.8	24
Ash (percent by weight)	6.43 ± 0.40	5.7-7.24	24
Amino Acids (percent of total diet) (a)			
Arginine	1.260	1.21-1.31	2
Cystine	0.395	0.39-0.40	2
Glycine	1.175	1.15-1.20	2
Histidine	0.553	0.530-0.576	2
Isoleucine	0.908	0.881-0.934	2
Leucine	1.905	1.85-1.96	2
Lysine	1.250	1.20-1.30	2
Methionine	0.310	0.306-0.314	2
Phenylalanine	0.967	0.960-0.974	2
Threonine	0.834	0.827-0.840	2
Tryptophan	0.175	0.171-0.178	2
Tyrosine	0.587	0.566-0.607	2
Valine	1.085	1.05-1.12	
Essential Fatty Acids (percent of total diet) (a)			
Linoleic	2.37		1
Linolenic	0.308		1
Arachidonic	0.008		1
Vitamins (a)			
Vitamin A (IU/kg)	12,167 ± 4,192	7,500-24,000	24
Vitamin D (IU/kg)	6,300		1
α-Tocopherol (ppm)	37.6	31.1-44.0	2
Thiamine (ppm)	16.2 ± 2.3	12.0-21.0	24
Riboflavin (ppm)	6.9	6.1-7.4	2
Niacin (ppm)	75	65-85	2
Pantothenic acid (ppm)	30.2	29.8-30.5	2
Pyridoxine (ppm)	7.2	5.6-8.8	2
Folic acid (ppm)	2.1	1.8-2.4	2
Biotin (ppm)	0.24	0.21-0.27	2
Vitamin B_{12} (ppb)	12.8	10.6-15.0	2
Choline (ppm)	3,315	3,200-3,430	2
Minerals (a)			
Calcium (percent)	1.23 ± 0.11	1.08-1.44	24
Phosphorus (percent)	0.97 ± 0.03	0.88-1.00	24
Potassium (percent)	0.809	0.772-0.846	2
Chloride (percent)	0.557	0.479-0.635	2
Sodium (percent)	0.304	0.258-0.349	2
Magnesium (percent)	0.172	0.166-0.177	2
Sulfur (percent)	0.278	0.270-0.285	2
Iron (ppm)	418	409-426	2
Manganese (ppm)	90.8	86.0-95.5	2
Zinc (ppm)	55.1	54.2-56.0	2
Copper (ppm)	12.68	9.65-15.70	2
Iodine (ppm)	2.58	1.52-3.64	2
Chromium (ppm)	1.86	1.79-1.93	2
Cobalt (ppm)	0.57	0.49-0.65	2

(a) One or two batches of feed analyzed for nutrients reported in this table were manufactured in January and/or April 1983.

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

Contaminants	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.51 ± 0.13	0.29-0.77	24
Cadmium (ppm) (a)	<0.10		24
Lead (ppm)	0.84 ± 0.81	0.33-3.37	24
Mercury (ppm) (a)	<0.05		24
Selenium (ppm)	0.29 ± 0.06	0.13-0.40	24
Aflatoxins (ppb) (a,b)	<5		24
Nitrate nitrogen (ppm) (c)	9.80 ± 4.44	1.9-22.0	24
Nitrite nitrogen (ppm) (c)	2.21 ± 1.58	2.0-17.0	24
BHA (ppm) (d)	5.80 ± 4.96	2.0-17.0	24
BHT (ppm) (d)	3.07 ± 2.71	0.9-12.0	24
Aerobic plate count (CFU/g) (e,f)	39,761 ± 32,066	4,900-110,000	23
Aerobic plate count (CFU/g) (g)	76,854 ± 184,406	4,900-930,000	24
Coliform (MPN/g) (h,i)	14.2 ± 22.4	<3-93	23
Coliform (MPN/g) (j)	51.4 ± 127.7	<3-460	24
E. coli (MPN/g) (a)	<3		24
Total nitrosamines (ppb) (k)	3.87 ± 2.56	0.8-9.3	24
N-Nitrosodimethylamine (ppb) (k)	2.74 ± 2.40	0.8-9.3	24
N-Nitrosopyrrolidine (ppb)	1.05 ± 0.60	<0.3-2.9	24
Pesticides (ppm) (c)			
α-BHC (a,l)	<0.01		24
β-BHC (a)	<0.02		24
γ-BHC-Lindane (a)	<0.01		24
δ-BHC (a)	<0.01		24
Heptachlor (a)	<0.01		24
Aldrin (a)	<0.01		24
Heptachlor epoxide (a)	<0.01		24
DDE (a)	<0.01		24
DDD (a)	<0.01		24
DDT (a)	<0.01		24
HCB (a)	<0.01		24
Mirex (a)	<0.01		24
Methoxychlor (m)	<0.05	0.09 (8/26/81)	24
Dieldrin (a)	<0.01		24
Endrin (a)	<0.01		24
Telodrin (a)	<0.01		24
Chlordane (a)	<0.05		24
Toxaphene (a)	<0.1		24
Estimated PCBs (a)	<0.2		24
Ronnel (a)	<0.01		24
Ethion (a)	<0.02		24
Trithion (a)	<0.05		24
Diazinon (a)	<0.1		24
Methyl parathion (a)	<0.02		24
Ethyl parathion (a)	<0.02		24
Malathion (n)	0.09 ± 0.06	<0.05-0.27	24
Endosulfan I (a)	<0.01		24
Endosulfan II (a)	<0.01		24
Endosulfan sulfate (a)	<0.03		24

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 reduced from 10 ppb to 5 ppb after 7/81
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) CFU = colony-forming units
(f) Mean, standard deviation, and range exclude one very high value of 930,000 obtained for the batch produced on 12/22/82.
(g) Mean, standard deviation, and range include the high value given in (f).
(h) MPN = most probable number
(i) Excludes one very high value of 460 obtained for the batch produced on 9/23/82.
(j) Includes the high value listed in (i).
(k) All values were corrected for percentage recovery.
(l) BHC = hexachlorocyclohexane or benzene hexachloride
(m) One observation was above the detection limit; the value and the date it was obtained are given under the range.
(n) Eleven batches contained more than 0.05 ppm.

APPENDIX H

TERATOLOGIC EVALUATION OF HYDROCHLOROTHIAZIDE IN CD® RATS AND MICE

APPENDIX H. TERATOLOGIC EVALUATION

A study of teratologic effects of hydrochlorothiazide in rats and mice was conducted at the Research Triangle Institute under the sponsorship of the National Toxicology Program (NCTR contract number 222-80-2031[c]). The laboratory report is on file at NTP, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Materials and Methods

Hydrochlorothiazide (lot no. 44-779-CA from Abbott Laboratories, North Chicago, Illinois) was administered in food-grade corn oil by gavage (dose volume: 10 ml/kg) to timed-pregnant CD® rats (0, 100, 300, or 1,000 mg/kg per day) and CD®-1 mice (0, 300, 1,000, or 3,000 mg/kg per day). Dose selection was based on the results of preliminary maternal and embryo toxicity studies conducted on gestational days 6 through 15. Females were weighed and observed during compound administration and 4 hours after dosing for clinical signs of toxicity.

Male rats were housed individually and female rats were housed four per cage on Ab-Sorb-Dri® cage bedding (Laboratory Products, Garfield, New Jersey) in solid-bottom polypropylene or polycarbonate cages (Laboratory Products, Rochelle Park, New Jersey). Male mice were housed individually in solid-bottom polycarbonate cages, and females were housed 10 per cage in solid-bottom polypropylene or polycarbonate cages (Laboratory Products, Rochelle Park, New Jersey).

Purina Certified Rodent Chow® (5002) and deionized/filtered water were available ad libitum throughout the study. Animal holding rooms were equipped with individual temperature controls and automatic adjustable light cycles (lights on 7:00 a.m. to 7:00 p.m.).

Thirty-six rat dams per dose group were killed and evaluated on gestational day 20; 20-27 mouse dams per dose group were evaluated on gestational day 17. The gravid uterus of each dam was weighed, and the number of implantation sites and of live, dead, or resorbed fetuses was recorded. All live fetuses were weighed and examined for external, visceral, and skeletal malformations.

Several statistical procedures were employed to aid in analysis of data from these studies, and these are fully described in the laboratory report. Nonparametric statistical procedures were applied to data from preliminary toxicity studies, with the litter used as the experimental unit for measures of embryotoxicity and fetotoxicity. One-way ANOVA was used to test for differences among dose groups, with followup comparisons using the Mann-Whitney U test, Jonckheere's test, and the Fisher exact test. For data collected in the teratology study, parametric evaluation of the dose effect and replicate effect and tests of interactions were conducted by using appropriate ANOVA designs.

Results

Compound-related effects during exposure included piloerection, weight loss, diarrhea, chromodacryorrhea, light-colored feces, and bloody urogenital area for the rat dams and weight loss, piloerection, lethargy, and rough coats for the mouse dams. None of the dams died during the exposure period. The maternal body weight of dosed rats was significantly lower than that of vehicle controls on gestational days 11 and 15. The maternal liver and relative kidney weights of dosed rats were significantly lower than those of vehicle controls. The maternal body weight of dosed mice was not significantly lower than that of vehicle controls.

Rats: Hydrochlorothiazide had no effect on gravid uterine weight, maternal water consumption, number of corpora lutea per dam, implantation sites per litter, percentage preimplantation loss, or the number or proportion of resorbed, dead, nonlive (i.e., dead plus resorbed) or affected (i.e., nonlive plus malformed) fetuses. Likewise, neither the number nor the proportion of litters with resorbed, dead, nonlive, or affected fetuses in any dose group was significantly different from that in vehicle

controls. In those litters containing live fetuses, there were no differences among dose groups in the number of live fetuses per live litter, the proportion of males per live litter, or the average fetal body weight per litter. In addition, hydrochlorothiazide had no effect on the number or proportion of litters with malformed fetuses or on the number or proportion of malformed male or female pups per litter.

Mice: The number of corpora lutea per dam differed among dose groups and was inconsistent across replicates and thus was not considered to be biologically significant. There were no dose-related differences in the number or proportion per litter of resorbed, dead, nonlive, or affected fetuses. Likewise, the number or proportion of litters with resorbed, dead, nonlive, or affected fetuses was not significantly different from that of vehicle controls in any dosed group.

In those litters containing live fetuses, there were no differences among dose groups in the number of live fetuses per live litter, the proportion of males per live litter, or the average fetal body weight per litter. There was a significant dose-response trend for the absolute number of litters with malformed fetuses, the proportion of malformed fetuses per litter, and the proportion of malformed females per litter. These trends were based solely on the occurrence of two malformed female fetuses (out of a total of 1,041 male and female fetuses examined) in the 3,000 mg/kg group and a malformation incidence of 0% for female fetuses from the vehicle control, 300 mg/kg, and 1,000 mg/kg groups and for male fetuses from the vehicle control and all dosed groups.

Conclusions

Hydrochlorothiazide (0, 100, 300, or 1,000 mg/kg per day) administered to rats on gestational days 6 through 15 produced no dose-related fetal toxicity and did not increase significantly the incidence of malformations in CD® rats, even in the presence of significant maternal toxicity. Hydrochlorothiazide (0, 300, 1,000, or 3,000 mg/kg per day) administered to mice on gestational days 6 through 15 produced no dose-related maternal or fetal toxicity and did not increase significantly the incidence of malformations in CD®-1 mice.

APPENDIX I

METHODS FOR MEASURING BLOOD-CLOTTING ACTIVITY

APPENDIX I. MEASURING BLOOD-CLOTTING ACTIVITY

I. Samples

Nine parts of freshly collected blood were mixed with one part of 3.8% sodium citrate solution. As soon as possible, blood was centrifuged for 5 minutes at 3,000 rpm. Plasma was removed immediately from cells, and the plasma container was stoppered. The plasma was then frozen in a dry ice/methanol bath, and mailed frozen to the NTP. At the NTP, the samples were thawed.

II. Fibrometer

The overall activity of the blood factors was measured by a fibrometer. The fibrometer uses a moving electrode that is located in front of a stationary electrode on the probe arm. When a coagulation time is being measured, the moving electrode cycles through the plasma-thromboplastin mixture every half second until a clot is formed. This clot formation triggers an electronic circuit that stops the timer.

III. Prothrombin Time

The calcium in whole blood is bound by added sodium citrate; thus, coagulation is prevented. When thromboplastin-C is added to plasma, factor VII in the plasma reacts with a tissue factor to form a reaction product that converts factor X to its activated form, factor Xa; this in turn reacts with factor V, calcium, and the phospholipid in the tissue extract to form extrinsic prothrombinase, which converts prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin. The rate of fibrin formation depends on the concentration of factors V, VII, and X and of prothrombin and fibrinogen; the test measures the overall activity of these factors.

Reconstituted Dade thromboplastin-C (rabbit brain) was used for the test, which was conducted at 37° C, and four plasma controls were used:

Dade Level I normal plasma control
Dade Level II abnormal plasma control
Dade Level III abnormal plasma control
Pooled normal plasma control

IV. Activated Partial Thromboplastin Time Test

The activated partial thromboplastin time test was used to screen for deficiencies of the plasma coagulation factors except for factors VII and XIII. This test is generally more sensitive than the partial thromboplastin time test.

One of the chief variables in the partial thromboplastin time test is the surface area of glass with which the blood has contact before the test is performed. The activated partial thromboplastin time test eliminates this contact variable by assuring complete activation through addition of a controlled plasma activator, actin, to the partial thromboplastin reagent.

The calcium in whole blood is bound by added sodium citrate; thus, coagulation is prevented. After centrifugation, the plasma contains all intrinsic coagulation factors except calcium and platelets. Calcium chloride, a phospholipid substitute for platelets (partial thromboplastin), and actin, which ensures maximal activation, are added to the plasma.

The time required for the plasma to clot--the activated partial thromboplastin time--was measured with a fibrometer.

The sample was thawed at the NTP. Actin (activated cephaloplastin reagent) was mixed by inversion immediately before use. Four plasma controls were used for the test, which was conducted at 37° C:

Dade Level I normal plasma control
Dade Level II abnormal plasma control
Dade Level III abnormal plasma control
Pooled normal plasma control

V. Fibrinogen Determinations

Fibrinogen is cleaved by the enzyme thrombin to form fibrin monomers, which then aggregate and form the fibrin clot. Thrombin is added in excess so that fibrinogen is the only rate-limiting factor in the reaction. The time required to form the clot is then a function only of the fibrinogen level.

Data-Fi Thrombin Reagent, Data-Fi Fibrinogen Calibration Reference, two plasma controls (Dade Level I normal plasma control and Dade Level II abnormal plasma control), and Owren's Veronal Buffer (adjusted to pH 7.35 with hydrochloric acid) were used. The test was conducted at 37.5° C. The clotting time was determined with a fibrometer, and the fibrinogen concentration was determined from a standard curve.

APPENDIX J

AUDIT SUMMARY

APPENDIX J. AUDIT SUMMARY

The experimental data, documents, the draft Technical Report, and pathology specimens for the 1- and 2-year studies of hydrochlorothiazide in rats and mice were audited for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations of the Food and Drug Administration (fully implemented by the National Toxicology Program (NTP) beginning on October 1, 1981). The laboratory studies were conducted for the NTP by SRI International, Menlo Park, California. Animal exposures to the chemical in feed began in October 1981. The retrospective audit was conducted for the National Institute of Environmental Health Sciences (NIEHS) at the NTP Archives during November and December 1987 by Dynamac Corporation. The audit of pathology specimens was conducted in June 1986 by Dynamac Corporation. The full audit report is on file at the NIEHS. The audit included a review of the following:

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

The audit showed that inlife procedures and events were documented by archival records, with minor exceptions that were explained in the laboratory's final reports. The archival records indicated that doses were prepared and administered to animals according to protocols and that feed consumption measurements were computed accurately. Of the external masses noted inlife, 178/183 in rats and 38/40 in mice were correlated with necropsy observations. The inlife mode and date of death records for all early-death animals were correlated with necropsy records. The analytical chemistry records from the study laboratory were present and accurate, and they documented procedures adequately.

Inspection of residual wet tissues for individual animal identifiers (punched ears) showed that 29/42 rats and 59/62 mice were identified correctly. Although ears for the other animals were documented as not saved, other toxicology and pathology audit findings corroborated the animal identity throughout the studies. The audit found untrimmed potential lesions in one rat and three mice. It also revealed that the residual segments of the intestinal tract were not completely opened and that a few hollow organs in the low dose mouse group were not incised or opened for full examination. No potential lesions were visible by external examination during the audit. All gross observations made at necropsy were found to have a corresponding microscopic diagnosis. Full details about these and other audit findings are presented in the audit report, which was reviewed by NTP staff when the study interpretations were prepared.

In conclusion, the data and results presented in the preliminary draft of the Technical Report for the 2-year studies of hydrochlorothiazide are supported by the records at the NTP Archives.

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