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TOXICOLOGY AND CARCINOGENESIS STUDIES OF PENTAERYTHRITOL TETRANITRATE (CAS NO. 78-11-5) WITH 80% D-LACTOSE MONOHYDRATE (PETN, NF) 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 PENTAERYTHRITOL TETRANITRATE (CAS NO. 78-11-5) WITH 80% D-LACTOSE MONOHYDRATE (PETN, NF)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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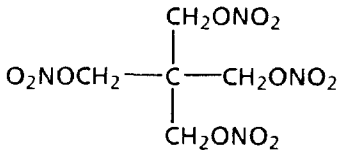

PENTAERYTHRITOL TETRANITRATE (PETN)

CAS No. 78-11-5

$C_5H_8N_4O_{12}$ Molecular weight 316 1

PETN, NF
(pentaerythritol tetranitrate:D-lactose monohydrate) (1:4)

Synonyms for PETN 2,2-bis((nitrooxy)methyl)-1,3-propanediol dinitrate (ester), 2,2-bisdihydroxy-methyl-1,3-propanediol tetranitrate, niperyt, nitropentaerythritol, pentaerythrityl tetranitrate, penthrit

Trade Names for PETN, NF Angitet, Cardiacap, Dilcoran-80, Dipentrate, Hasethrol, Lentrat, Metranil, Mycardol, Neo-Corovas, Nitropenta, Nitropenton, Pentafin, Pentanitrine, Pentitrate, Pentral 80; Pentrite, Pentritol, Pentryate, Peridex, Pergitral, Peritrate, Perityl, Prevangor, Quintrate, Subicard, Terpate, Vasodiatol

ABSTRACT

Pentaerythritol tetranitrate (PETN, NF) is a drug used to prevent angina pectoris PETN without a lactose stabilizer is used as an explosive Toxicology and carcinogenesis studies were conducted by administering PETN, NF, to groups of F344/N rats and B6C3F$_1$ mice of each sex once by gavage or in feed for 14 days, 13 or 14 weeks, or 2 years The PETN component was greater than 99% pure . Genetic toxicology studies were conducted with *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen- or Fourteen-Week Studies: All rats and mice lived to the end of the 14-day studies (dietary concentrations up to 50,000 ppm) Final mean body weights of dosed and control rats were comparable The final mean body weight of female mice that received 50,000 ppm was 13% lower than that of controls No clinical signs or toxic lesions were attributed to PETN, NF, administration

All rats and mice lived to the end of the 13-week (mice) and 14-week (rats) studies (dietary concentrations up to 50,000 ppm) Final mean body weights of dosed and control rats and mice were similar, although weight gains of female rats at 25,000 and 50,000 ppm were less than that of controls The nitrite level in urine of rats and methemoglobin levels in whole blood of rats and mice were not affected by administration of PETN, NF. An adenoma of the Zymbal gland was seen in a female rat that received 50,000 ppm A hepatocellular adenoma was seen in a female mouse that received 50,000 ppm

Based on these results and the NTP convention of limiting concentrations in 2-year feed studies to 5% of the diet, the 2-year studies were conducted by administering 0, 25,000, or 50,000 ppm PETN, NF, in feed for 104 weeks to groups of 50 male rats and for 103 weeks to groups of 49 or 50 mice of each sex

Groups of 50 female rats were given feed containing 0, 6,200, or 12,500 ppm PETN, NF, for 104 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 2%-9% lower than those of controls throughout the study; body weights of all groups of female rats were similar. No significant differences in survival were observed between any groups of rats of either sex (male: control, 23/50; low dose, 29/50; high dose, 29/50; female: 33/50; 33/50; 31/50). Mean body weights of dosed and control mice were similar. The survival of both groups of dosed male mice was significantly greater than that of the controls (26/49; 38/50; 38/50). No significant differences in survival were observed between any groups of female mice (38/50; 30/50; 38/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No nonneoplastic lesions were attributed to PETN, NF, administration in rats or mice. Neoplasms of the Zymbal gland occurred in dosed male (control, 0/49; low dose, 3/45; high dose, 2/41) and dosed female (0/36; 1/37; 3/35) rats. The historical incidence of these neoplasms is 1% ± 2% in untreated males and 0.6% ± 1% in females.

At no site was a significantly increased incidence of neoplasms observed in dosed male or female mice.

Genetic Toxicology: PETN, NF, was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation (S9). When tested for cytogenetic effects in cultured CHO cells, PETN, NF, induced sister chromatid exchanges (SCEs) in the presence and absence of metabolic activation; no induction of chromosomal aberrations was observed in CHO cells with or without activation.

Audit: The data, documents, and pathology materials from the 2-year studies of PETN, NF, 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 *equivocal evidence of carcinogenic activity** of PETN, NF, for male and female F344/N rats, based on a marginal increase in neoplasms of the Zymbal gland. Female rats might have tolerated a higher dose. There was *no evidence of carcinogenic activity* of PETN, NF, for male or female $B6C3F_1$ mice fed diets containing 25,000 or 50,000 ppm for 2 years. No nonneoplastic lesions were attributed to PETN, NF, administration.

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

SUMMARY OF THE TWO-YEAR FEED AND GENETIC TOXICOLOGY STUDIES OF PETN, NF

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Dietary concentration			
0, 25,000, or 50,000 ppm PETN, NF	0, 6,200, or 12,500 ppm PETN, NF	0, 25,000, or 50,000 ppm PETN, NF	0, 25,000, or 50,000 ppm PETN, NF
Body weights in the 2-year study			
Dosed and control groups similar	Dosed and control groups similar	Dosed and control groups similar	Dosed and control groups similar
Survival rates in the 2-year study			
23/50; 29/50; 29/50	33/50; 33/50; 31/50	26/49; 38/50; 38/50	38/50; 30/50; 38/50
Nonneoplastic effects			
None	None	None	None
Neoplastic effects			
Zymbal gland adenomas (0/49; 1/45; 0/41); Zymbal gland carcinomas (0/49; 2/45; 2/41)	Zymbal gland adenomas (0/36; 0/37; 2/35); Zymbal gland carcinomas (0/36; 1/37; 1/35)	None	None
Level of evidence of carcinogenic activity			
Equivocal evidence	Equivocal evidence	No evidence	No evidence
Genetic toxicology			
Salmonella (gene mutation)	**CHO Cells in Vitro**		
	SCE	**Aberration**	
Negative with and without S9	Positive with and without S9	Negative with and without S9	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

- **Inadequate Study of Carcinogenic Activity** is demonstrated by studies that because of major qualitative or quanti tative 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 ex tend 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 or gan 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 PETN, NF, is based on 13- and 14-week studies that began in January 1981 and ended in April 1981 and on 2-year studies that began in January 1982 and ended in February 1984 at EG&G Mason Research Institute (Worcester, MA)

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on PETN, NF, on October 4, 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 PETN, NF

On October 4, 1988, the draft Technical Report on the toxicology and carcinogenesis studies of PETN, NF, received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

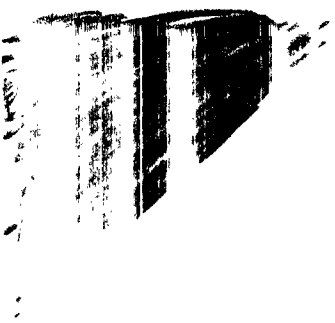
Dr. J.R. Bucher, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (equivocal evidence of carcinogenic activity for male and female rats, no evidence of carcinogenic activity for male and female mice).

Dr. Newberne, a principal reviewer, agreed with the conclusions. He asked for an explanation of why the doses for female rats in the 2-year study were only one-fourth those for the other study groups. Dr. Bucher said that at the time the 2-year study was designed, the convention for setting doses included a reduction in body weight gain of 10% or more in 13-week studies, and that was the determinant for the markedly lower doses used.

Dr. McKnight, the second principal reviewer, agreed with the conclusions. She commented on the three lots of PETN, NF, and asked how they were used. Dr. Bucher said that they were used sequentially, with all of the formulated diets being made from the lot in use at a particular time. Dr. McKnight asked why so many Zymbal glands were missing from all three groups of female rats. Dr. Bucher said that the glands are very small and hard to find unless they are enlarged with a tumor. The sections are taken through the inner ear and certain other relevant tissues as well; sometimes the Zymbal gland is missed.

Dr. Gold, the third principal reviewer, agreed with the conclusions. She requested clarification of a statement in the Discussion that all compounds in the NTP data base (except benzene and PETN, NF) that induce tumors of the Zymbal gland are also positive in the Salmonella assay. She indicated that all nine non-NTP chemicals that induced Zymbal gland tumors were also genotoxic. Dr. Bucher responded that this represented one of the first complete assessments of tumor incidence vs. genotoxicity that NTP has put together and was included as a discussion point. He noted that the level of evidence chosen was based on the tumor incidence and not on whether PETN, NF, was genotoxic.

Dr. Gold moved that the Technical Report on PETN, NF, be accepted with the revisions discussed and with the conclusions as written for male and female rats, equivocal evidence of carcinogenic activity, and for male and female mice, no evidence of carcinogenic activity. Dr. Newberne seconded the motion, which was approved unanimously by seven members.



I. INTRODUCTION

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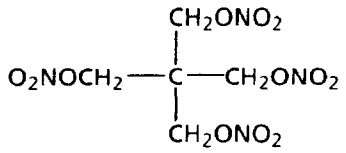

PENTAERYTHRITOL TETRANITRATE (PETN)

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PETN, NF
(pentaerythritol tetranitrate:D-lactose monohydrate) (1:4)

Synonyms for PETN 2,2-bis((nitrooxy)methyl)-1,3-propanediol dinitrate (ester), 2,2-bisdihydroxy-methyl-1,3-propanediol tetranitrate, niperyt, nitropentaerythritol, pentaerythrityl tetranitrate, penthrit

Trade Names for PETN, NF Angitet, Cardiacap, Dilcoran-80, Dipentrate, Hasethrol, Lentrat, Metranil; Mycardol; Neo-Corovas; Nitropenta, Nitropenton, Pentafin, Pentanitrine, Pentitrate, Pentral 80, Pentrite, Pentritol, Pentryate; Peridex, Pergitral, Peritrate, Perityl, Prevangor, Quintrate, Subicard, Terpate, Vasodiatol

Physical Properties, Use, Production, and Exposure

Pentaerythritol tetranitrate (PETN) is a nitric acid ester of a tetrahydric alcohol, pentaerythritol It is a white crystalline material first prepared in 1901 by Vignon and Gerin (Dept of the Army, 1967) Crystalline PETN has a melting point of 140° C and a specific gravity of 1 77 at 20° C (Merck, 1983) It is insoluble in water, slightly soluble in alcohol, and soluble in acetone (von Oettingen et al , 1944).

PETN is an explosive that came into general use after World War I. It is used as an admixture with TNT for loading small-caliber projectiles and grenades and has limited use in detonating fuses, boosters, and detonators. PETN and nitroglycerin are approximately equivalent in explosive power and are among the most potent of the standard military explosives (Dept. of the Army, 1967) PETN is also one of a number of organic nitrates used in the treatment of angina pectoris (Gilman et al , 1985) For this purpose, PETN is formulated with an inert ingredient, usually lactose, to decrease the hazard of explosion (Merck, 1983)

Current production data for PETN were not found, but estimates in the NCI/SRI data base indicate that in 1973, 1 8-2 3 $\times 10^9$ kg was used for production of explosives and approximately 2 3 $\times 10^4$ kg was used for production of ethical drugs PETN is produced by the nitration of pentaerythritol, which is accomplished by slowly adding pentaerythritol to 96% nitric acid at an initial temperature of 18° C (Dept of the Army, 1967) The exothermic reaction is kept below 23° C by regulating the addition of pentaerythritol PETN is precipitated from solution by addition of cold water and is then washed, dissolved in acetone, and reprecipitated by cold water

McConnell et al (1946) reviewed the industrial hygiene and the incidence of occupational disease in government-owned ordnance plants in the United States during World War II An apparent increase in the number of sudden deaths among explosives workers was observed, but in 915,000 man-years of exposure to the various organic nitrates, no fatalities were attributed to the aliphatic nitrates An undetermined number of episodes of mild illness or dermatitis were attributed to exposure to PETN Workers involved in the production of nitroglycerin and

other organic nitrates that are readily absorbed through the skin suffered at times from a syndrome called "dynamite head" or "powder headache," which was manifested as severe headache, dizziness, and postural weakness upon initial exposure (Gilman et al., 1985). These symptoms diminished with time but then often reappeared at the beginning of the work week. The initial symptoms reflected the vasodilator action of the nitrates, and the so-called "Monday disease" was attributed to tolerance to this action developed during the work week and to expression of an organic nitrate dependence that became apparent after a several-day break in exposure. The risk of developing this condition while working with PETN is not considered to be high because of the relatively poor dermal absorption of the chemical and because it is usually processed as a wet slurry or precipitate (Dept. of the Army, 1967).

Pharmacologic Action

The use of organic nitrites for treating angina dates from 1857, when Brunton first administered amyl nitrite by inhalation and noted relief of anginal pain within 30-60 seconds (Gilman et al., 1985). In 1879, William Murrell demonstrated that sublingual administration of nitroglycerin provided similar relief of angina as well as prophylactic action if taken before exercise. Early studies with PETN were performed by Takeshita (1937), who demonstrated the ability of the compound to lower blood pressure in rabbits. Further characterization of the pharmacologic and toxicologic action of PETN was carried out by the U.S. Government during World War II because of potential exposure during munitions manufacture (von Oettingen et al., 1944). After the war, research on PETN continued in efforts to develop antianginal agents that could be taken orally and would provide long-acting prophylaxis. This research (1943-69) has been reviewed by Dunning (1971).

Angina pectoris is associated with ischemic heart disease and is usually secondary to advanced atherosclerosis; its onset can be prompted by increases in oxygen demand by the heart or by decreases in myocardial blood flow. Organic nitrates are vasodilators, but their mode of action in the relief of angina is complicated and incompletely understood (Gilman et al., 1985). In the peripheral circulation, organic nitrates cause dilation of venous-capacitance and arteriolar-resistance vessels, which in effect decreases the preload and afterload on the heart (Kafka et al., 1985). Nitrates also dilate large coronary vessels, but in typical angina, total coronary blood flow is not increased by nitrates; rather, blood flow tends to redistribute to areas of poor perfusion, especially the subendocardial regions (Uchida et al., 1972). Despite improved regional coronary blood flow, the primary benefit of nitrate therapy appears to result from a reduction in the oxygen requirement of the heart. This observation is supported by studies showing that angina occurs in patients at the same value of the "triple product" (aortic pressure × heart rate × ejection time) with or without nitrate therapy (Gilman et al., 1985).

PETN is prescribed to reduce the number, intensity, and duration of angina attacks and to reduce the need to use nitroglycerin for relief of acute attacks. The recommended dosage for adults is one 40-mg tablet four times per day, or about 2.5 mg/kg per day (PDR, 1987). This dose is higher than the doses recommended in the 1960s and early 1970s after it was recognized that hepatic degradation was sufficient to rapidly and totally inactivate lower doses (Abrams, 1980).

Absorption, Distribution, Metabolism, and Excretion

Von Oettingen et al. (1944) administered PETN by gavage with a tenfold excess of starch in a 10% gum arabic solution (PETN concentration, 20 mg/ml) to young female albino rats. Six hours later, the entire gastrointestinal tract was isolated, and only 13% of the PETN had been absorbed. PETN was also mixed with acetone and rubbed onto the palm of a human hand; after 1 hour, essentially all of the PETN could be recovered by washing. In contrast, PETN was absorbed after insufflation of 100 mg into the lower trachea of dogs. The resulting decrease in blood pressure peaked at about 90 minutes.

DiCarlo et al. (1967a) studied the absorption of [^{14}C]PETN from four ligated sections of the gastrointestinal tract in female Wistar rats.

I. INTRODUCTION

Absorption from the stomach was slow, and PETN was stable in stomach acid. Absorption was rapid from the small intestine and somewhat slower from the large intestine. Although the drug remaining in the small intestine was unchanged, bacterial action appeared to cause denitration in the large intestine, resulting in the uptake of the denitrated metabolites.

PETN binds to both plasma proteins and erythrocytes, and denitration reactions (the major metabolic pathway) occur in vitro with both blood components, primarily with erythrocytes. Denitration reactions appear to be most rapid with the more highly nitrated metabolites, resulting in accumulation of the mono- and dinitrated forms (DiCarlo et al., 1965). Denitration reactions can be catalyzed by subcellular fractions of heart (DiCarlo et al., 1967b) and by liver parenchymal and reticuloendothelial cells (DiCarlo et al., 1967c; Melgar et al., 1974). The reaction requires reduced glutathione and a rather nonspecific enzyme termed glutathione-organic nitrate reductase (Needleman and Hunter, 1965). Removal of one or more nitro groups allows the resulting alcohol to form glucuronide conjugates. The conjugates of pentaerythritol mono-, di-, and trinitrate were isolated from the bile of Wistar rats given [^{14}C]pentaerythritol trinitrate by intravenous injection (Crew et al., 1971).

DiCarlo et al. (1967d) administered [^{14}C]PETN (10 mg/kg) by gavage to female Wistar rats. Approximately 8% of the radiolabel was absorbed during the first hour, 14% after 2 hours, 24% after 4 hours, and 60% after 18 hours. Radioactivity was first found in feces after 2 hours, and 10% of the dose was eliminated by this route after 18 hours. Most of the radioactivity absorbed during the first hour was cleared from blood and found in tissues, primarily fat and carcass. Pentaerythritol was determined to be the major final metabolite in rats.

Little or no carbon dioxide results from PETN metabolism (Crew et al., 1966). Most of the absorbed PETN is excreted in urine. Pentaerythritol di- and mononitrate and pentaerythritol were found in the urine in different proportions, depending on the time after administration. Crew et al. (1971) found that urinary excretion of the radiolabel was reduced by 60% in biliary cannulated Wistar rats compared with noncannulated rats that had received [^{14}C]pentaerythritol trinitrate. This suggests that glucuronidated metabolites normally undergo enterohepatic circulation through reabsorption from the intestine after removal of glucuronic acid. Studies of metabolism patterns in mice have indicated a basic similarity to those of rats (Litchfield, 1971).

A quantitative study of the pharmacokinetics of PETN after oral or intra-arterial dosing in Sprague Dawley rats was performed by King and Fung (1986). PETN appeared to be rapidly converted to the denitrated metabolites after oral or intra-arterial administration, and only the di- or mononitrated metabolites were detected after oral dosing. The half-life of PETN in blood was 5.8 minutes, and that of the trinitrate and dinitrate was about 62 minutes each. The clearance of total label was 620 ml/minute per kilogram, which exceeds the cardiac output by about one-third and exceeds the denitrating capacity of blood plasma and erythrocytes. To account for this, King and Fung proposed that PETN and its metabolites are extracted from blood by the blood vessels.

Studies in humans have indicated absorption of at least 60% of an oral dose of [^{14}C]PETN. Label appeared in the blood within 15 minutes, but only the mono- and dinitrated forms were found (Davidson et al., 1970). Predominant forms in the urine were the mononitrate and the completely denitrated pentaerythritol. These results are similar to those observed for rats. In vitro studies with human blood have indicated a capability to degrade PETN primarily to the trinitrate but no further (King and Fung, 1985). The half-life for denitration of PETN in human blood was three to four times slower than that in rat blood. Studies of the in vivo pharmacodynamics of pentaerythritol trinitrate indicated that it was metabolized to pentaerythritol dinitrate and pentaerythritol mononitrate within a few minutes; the elimination half-life of pentaerythritol dinitrate from human blood was 10.5 hours, and that of pentaerythritol mononitrate was 7.3 hours (Davidson et al., 1971). Taken together, these results suggest a major role in humans for the absorption of the trinitrate following bacterial denitration of PETN in the

intestine. However, the studies of Carter and Goldman (1976) have shown no evidence for the involvement of intestinal microflora in the absorption of PETN in the rat.

Proposed Mechanism of Vascular Smooth-Muscle Relaxation by Organic Nitrates

Recently, considerable evidence has pointed to an activation of cGMP formation in the relaxation of smooth muscle by organic nitrates (Kreye et al., 1986). Guanylate cyclase has been shown to be activated by some nitrates directly or by derivatives such as the *S*-nitrosothiols. In brief, this theory holds that organic nitrates enter smooth-muscle cells, where they undergo denitration. The nitrite formed is metabolically activated by thiols such as cysteine to form an unstable *S*-nitrosothiol capable of activating guanylate cyclase (Ignarro et al., 1981). Activation of guanylate cyclase and stimulation of cGMP production may result in phosphorylation of a protein kinase, which in turn activates a sarcolemmal ATPase responsible for the extrusion of calcium (Kukovetz and Holzmann, 1986).

Toxicity

Toxicity associated with organic nitrate exposure is generally secondary to cardiovascular effects. Symptoms of headache in munitions workers were described earlier, and weakness, dizziness, and other manifestations of cerebral ischemia associated with postural hypotension may develop. Even in the most severe cases of overdose, simple changes in position to restore venous flow to the heart is sufficient therapy (Gilman et al., 1985). Cutaneous sensitivity to PETN has been reported in humans and appears to be a common effect of exposure to all organic nitrates (Ryan, 1972).

Von Oettingen et al. (1944) gave volunteers 64-mg capsules orally and measured various physiologic functions for several hours. No changes occurred in respiration or blood pressure, and no increase in blood nitrite was found. When dogs were given doses of 5 mg/kg orally, a gradual, transient decrease (about 28%) in blood pressure was noted with a corresponding increase in respiratory rate and minute volume. No reports of LD_{50} determinations in animals were found in the literature (NIOSH, 1987).

Von Oettingen et al. (1944) also studied the effects of 1-year administration of PETN in feed to an unspecified strain of rats. Groups of 45 rats were given either a control diet or a diet containing sufficient PETN to provide doses of 2 mg/kg body weight. No effects on body weight were noted, and deaths in both groups were attributed to parasitic infestations. Monthly blood collections tended to show slightly higher values for hemoglobin and erythrocytes in the dosed animals. Microscopic examination of the brain, heart, lungs, liver, spleen, kidney, adrenal glands, testis, and femur revealed no clear compound-related changes.

Reproductive Toxicity

No studies of reproductive or developmental toxicity or teratology in animals or humans were found in the literature.

Genetic Toxicity

Little information is available in the literature regarding the mutagenic potential of PETN, but available data suggest that the chemical is not mutagenic. PETN did not induce mutations in *Salmonella typhimurium* when tested with or without S9 metabolic activation in a variety of strains by the spot test, the plate incorporation test, and a preincubation protocol (Simmon et al., 1977; Whong et al., 1980; Mortelmans et al., 1986). PETN was reported to be negative for induction of mitotic recombination when tested in *Saccharomyces cerevisiae* D3 (Simmon et al., 1977).

Pentaerythritol mononitrate was reported to induce gene mutations in the *Escherichia coli* bacteriophage T4B (Kononova et al., 1972), but the completely denitrated metabolite pentaerythritol was negative when tested for gene reversion in *E. coli* and *S. typhimurium* at doses up to 5 mg/plate (Shimizu et al., 1985).

I. INTRODUCTION

Study Rationale

PETN was nominated for study by the National Cancer Institute from a review of vasodilator drugs and was selected because of its potential widespread use in angina therapy and because of the lack of adequate toxicologic and carcinogenic characterization in animals. The oral route of exposure was chosen to mimic the principal mode of human exposure.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF PETN, NF

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES AND FORMULATED DIETS

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK AND FOURTEEN-WEEK 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 PETN, NF

Pentaerythritol tetranitrate D-lactose monohydrate (1 4) (PETN, NF) was obtained in three lots lot no G23-H2 from ICI America, Inc (Wilmington, DE) and lot nos 80124 and 81130 from R W Greeff and Company (Old Greenwich, CT) (Table 1) The PETN component was National Formulary grade, and the lactose was USP grade Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, MO) MRI reports on the analyses performed in support of the PETN, NF, studies are on file at the National Institute of Environmental Health Sciences

Analysis of cumulative data on all lots of the study material indicated that the PETN was greater than 99% pure and was incorporated in a 20 80 mixture of PETN lactose monohydrate All lots of the study chemical were identified as a mixture of PETN and lactose by infrared and nuclear magnetic resonance spectroscopy The infrared and nuclear magnetic resonance spectra of the study material were consistent with a mixture of PETN and lactose Isolation of PETN from the study material by acetone extraction and subsequent infrared and nuclear magnetic resonance spectral analysis gave spectra that were consistent with that expected for the structure of PETN and the literature spectra (Sadtler Standard Spectra, infrared only, no nuclear magnetic resonance spectrum found in the literature) (Representative spectra of the study material are presented in Figures 1 to 5)

The purity of all lots was determined by elemental analysis, Karl Fischer water analysis, specific rotation measurements to determine lactose content, and thin-layer chromatography Thin-layer chromatographic analysis was performed on water (lot no G23-H2 only), water methanol (1 1), and acetone extracts of the study chemical by using aluminum oxide plates with two solvent systems toluene (system 1) and petroleum ether acetone (85 10, for acetone extracts only) (system 2) Visualization for nitrate esters was performed under ultraviolet light with 5% diphenylamine in 95% ethanol and with 50% aqueous sulfuric acid spray reagents High-performance liquid chromatography was per formed concurrently for lot nos G23-H2 and 81130 with a Waters μBondapak C_{18} column and a water methanol (45 55) solvent system, acetanilide was the internal standard, and ultraviolet detection was at 210 nm The USP assay to determine the concentration of PETN was performed by extraction of the study material with acetone, reaction of the extracted PETN with phenoldisulfonic acid, measurement of the absorbance maximum near 409 nm, and comparison with a potassium nitrate standard Acetone-insoluble material in lot no 81130 was determined by weighing the dried residue from a Soxhlet extraction of the study material with acetone

TABLE 1. IDENTITY AND SOURCE OF LOTS USED IN THE STUDIES OF PETN, NF

Fourteen-Day Studies	Thirteen- and Fourteen-Week Studies	Two-Year Studies
Lot Numbers		
G23-H2	G23 H2, 80124	80124, 81130
Date of Initial Use		
10/15/80	Lot no 80124 2/26/81	Lot no 81130 8/17/83
Supplier		
ICI America, Inc (Wilmington, DE)	G23 H2 same as 14 d studies, 80124- R W Greeff and Company (Old Greenwich, CT)	R W Greeff and Company (Old Greenwich, CT)

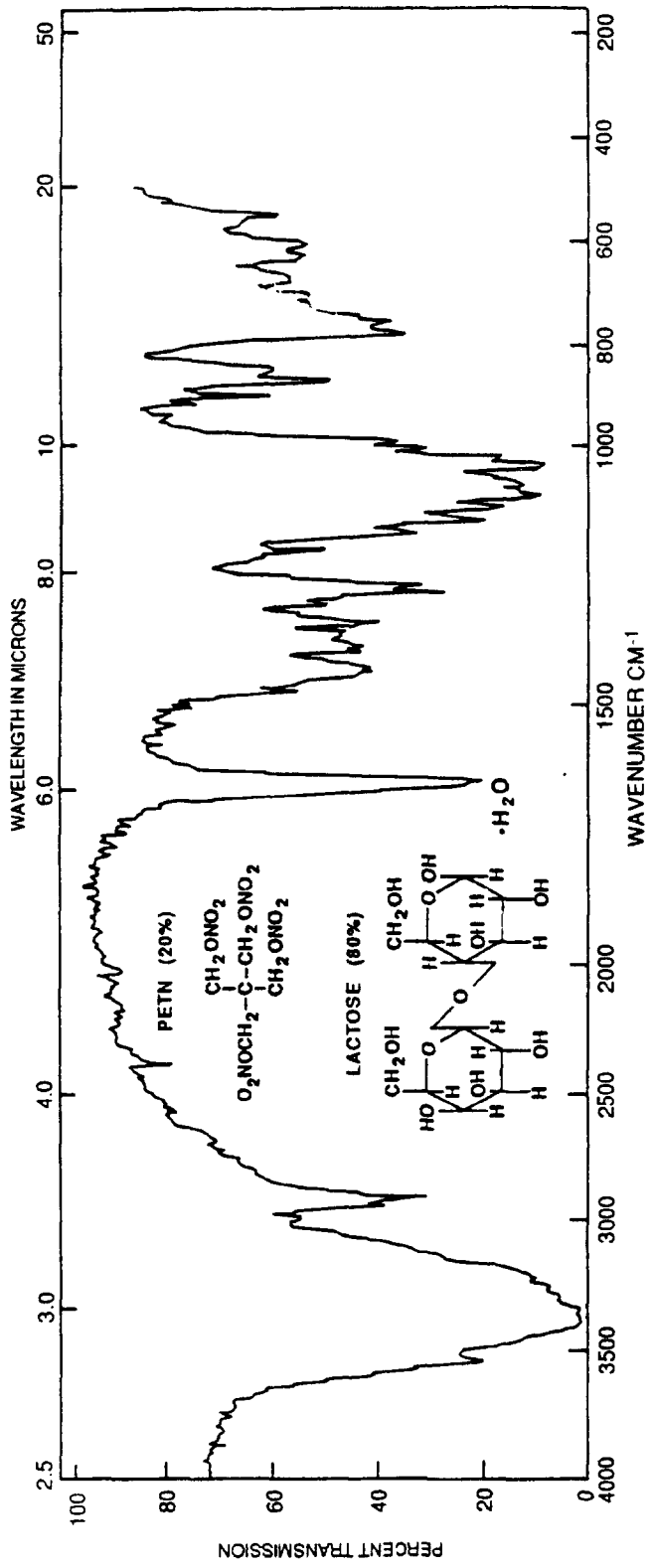

FIGURE 1. INFRARED ABSORPTION SPECTRUM OF PETN, NF (LOT NO. G23-H2)
(3% IN A POTASSIUM BROMIDE DISC)

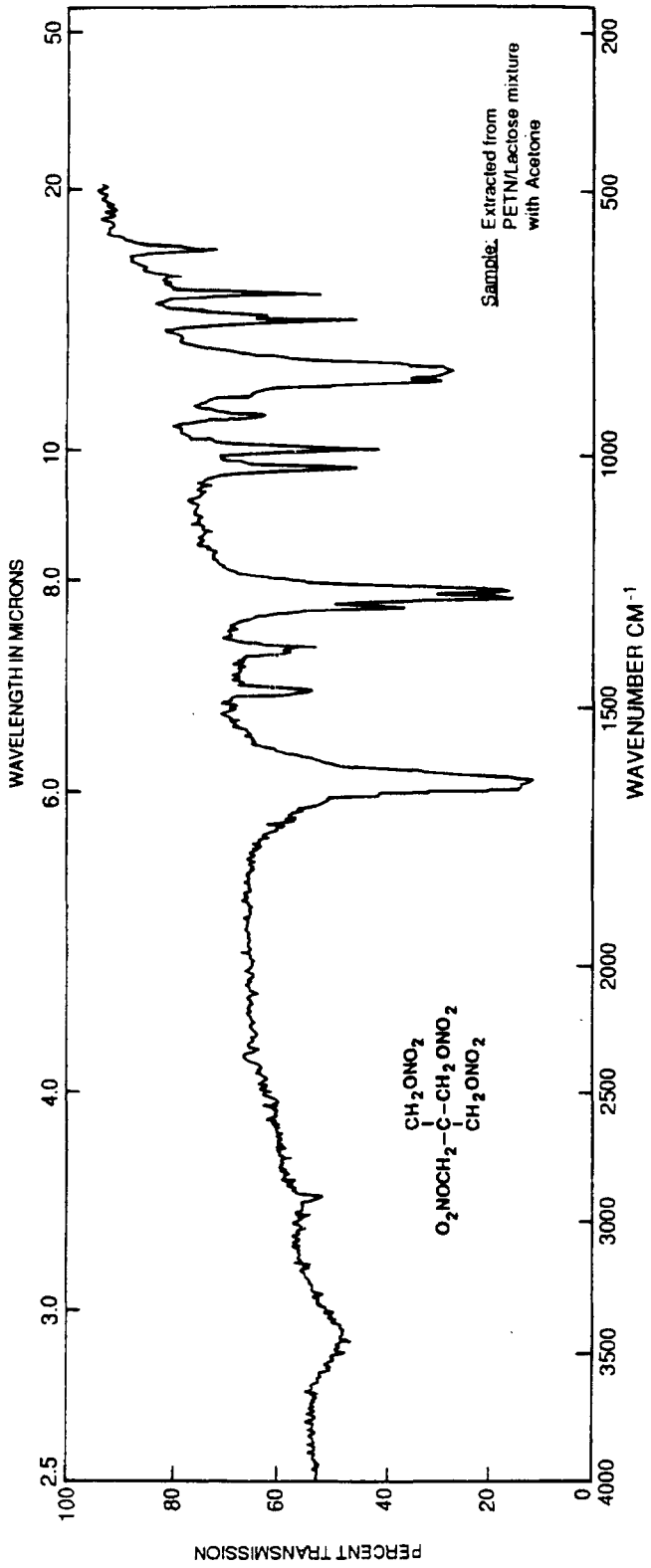

FIGURE 2. INFRARED ABSORPTION SPECTRUM OF PETN, NF (LOT NO. G23-H2)
(1% IN A POTASSIUM BROMIDE DISC)

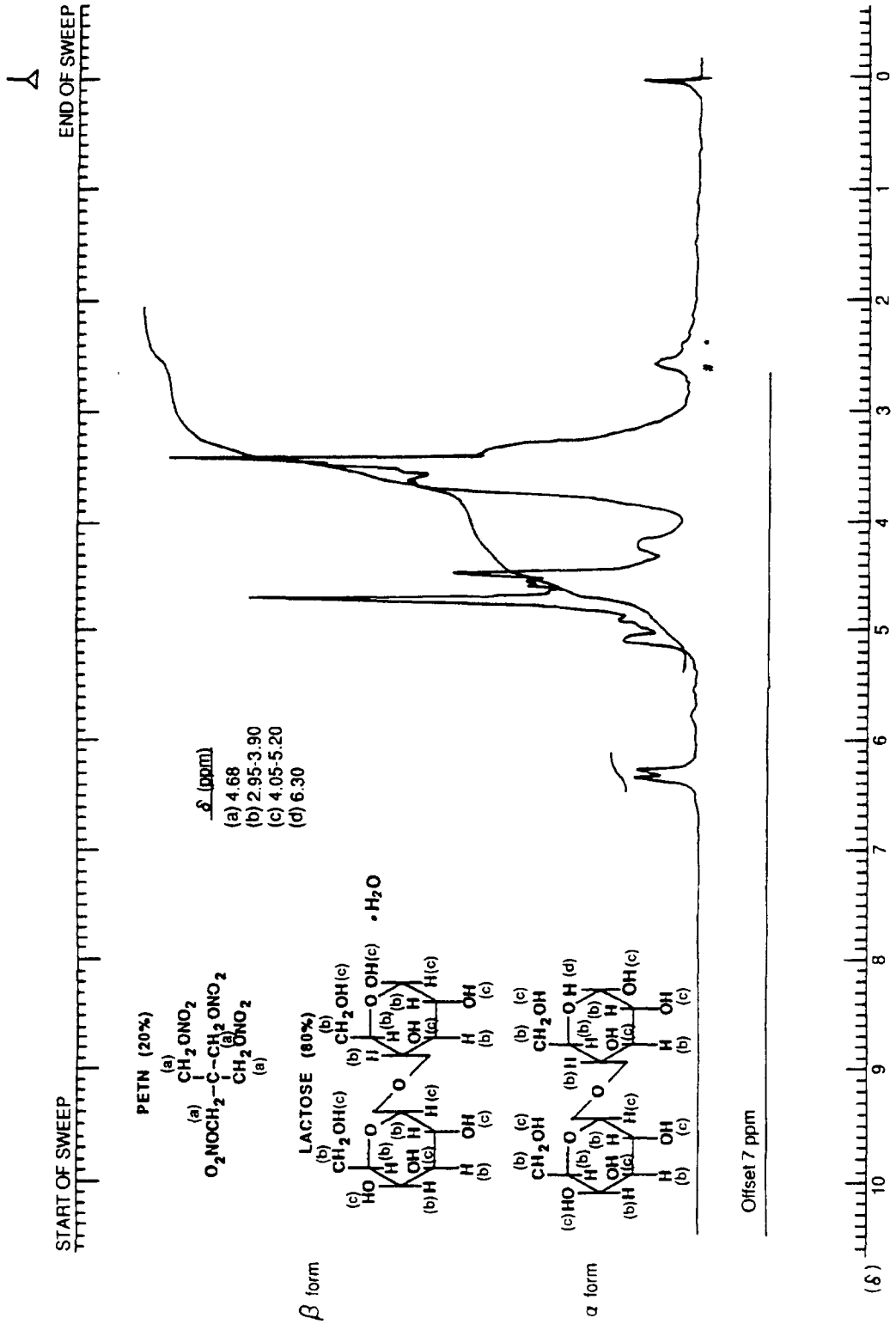

FIGURE 3. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF PETN, NF (LOT NO. G23-H2) IN DIMETHYL SULFOXIDE-d_6

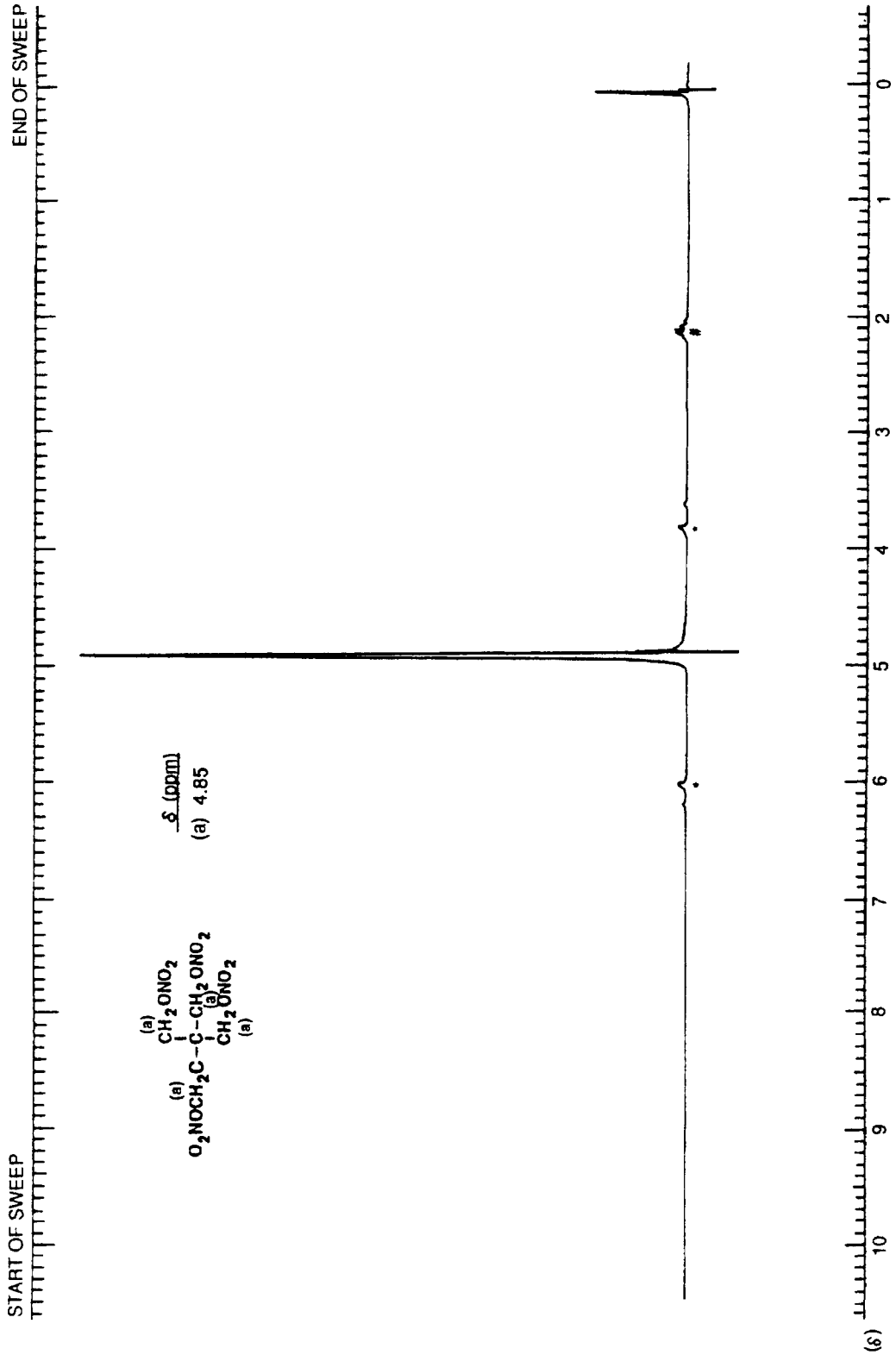

FIGURE 4. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF A PETN ACETONE EXTRACT OF PETN, NF (LOT NO. G23-H2) IN ACETONE-d_6

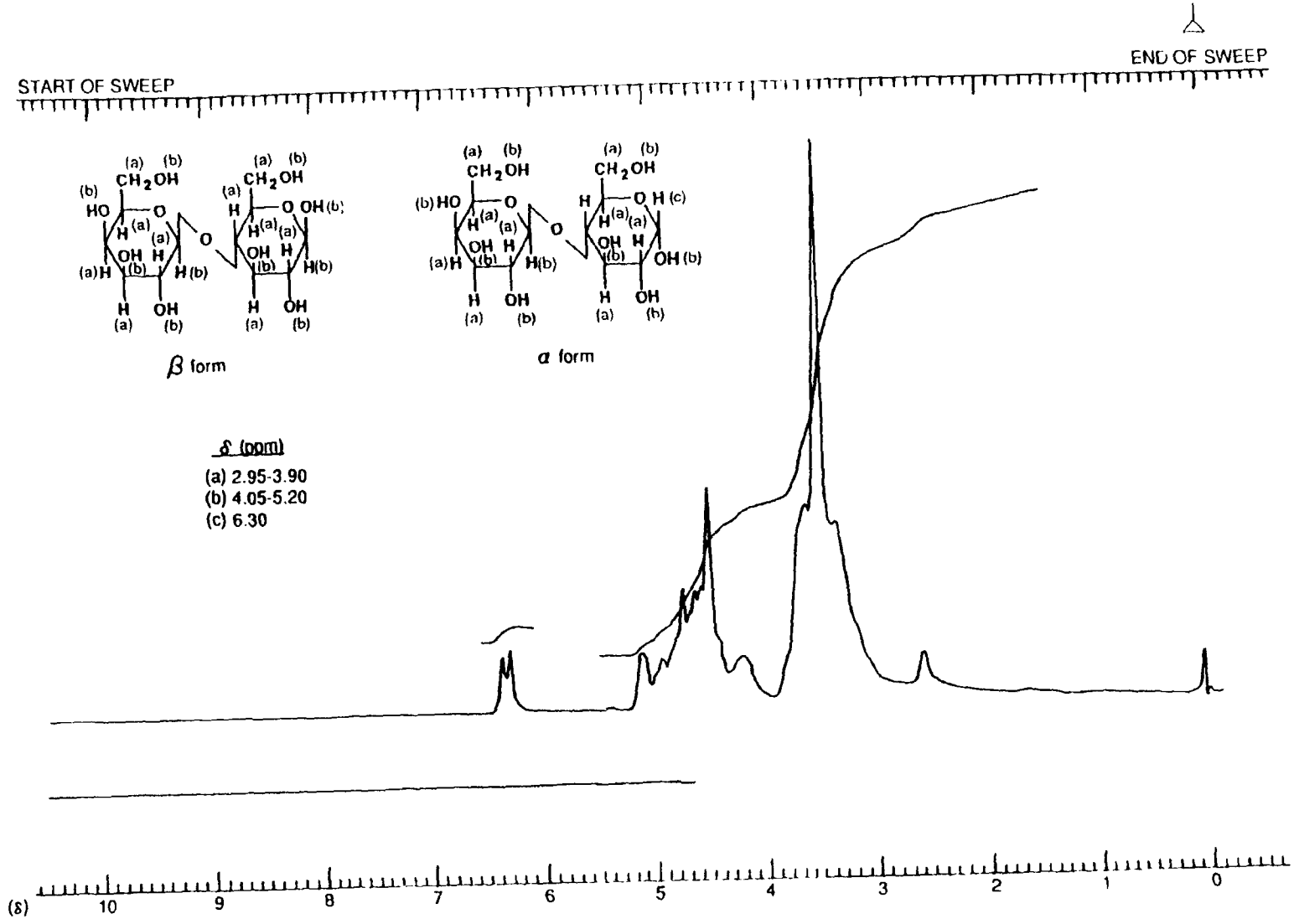

FIGURE 5. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF LACTOSE

II. MATERIALS AND METHODS

Results of elemental analysis of lot no G23-H2 for carbon, hydrogen, and nitrogen were in agreement with the theoretical values based on a 20 80 mixture of PETN and lactose monohydrate Karl Fischer analysis indicated 3 95% water The USP assay determined the PETN concentration to be 21 1% Specific rotation indicated the presence of 79 8% lactose after correction for water content Thin-layer chromatographic analysis indicated only spots for PETN and lactose

Results of elemental analysis of lot no 80124 for carbon, hydrogen, and nitrogen were in agreement with the theoretical values Karl Fischer analysis indicated 4 07% water The USP assay determined the PETN concentration to be 20 6% Specific rotation indicated the presence of 79% lactose after correction for water content Thin-layer chromatographic analysis indicated only spots for PETN and lactose

Results of elemental analysis of lot no 81130 for carbon were slightly high, those for nitrogen were slightly low, and those for hydrogen were in agreement with the theoretical values Karl Fischer analysis indicated 4 1% water The USP assay determined the PETN concentration to be 20 7% Specific rotation data indicated the presence of 72 9% lactose after correction for water content Acetone-insoluble material represented 78 4% of the study material Thin-layer chromatographic analysis by two systems indicated only spots for PETN and lactose High-performance liquid chromatographic analysis indicated that the PETN content of lot nos G23-H2 and 81130 was identical

Stability studies on PETN were performed by extracting PETN from the lactose with acetone containing 0 02% diethyl phthalate as an internal standard, followed by gas chromatographic analysis with nitrogen as the carrier, a flow rate of 70 ml/minute, a 3% SP2100 column, flame ionization detection, and an isothermal oven temperature of 145° C (Decomposition on the column occurred at temperatures above 150° C) PETN was found to be stable as a 20 80 mixture in lactose when stored for 2 weeks, protected from light, at temperatures up to 60° C The bulk chemical was reanalyzed by the study laboratory every 4 months over the course of the studies by infrared spectroscopy, high performance liquid chromatography, and thin-layer and gas chromatography Since no deterioration of the study material was seen by the study lab oratory, it was concluded that PETN remained stable during the studies

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES AND FORMULATED DIETS

Formulated diets were made by preparing a premix of PETN, NF, and feed in a mortar with a pestle and then blending the premix with additional feed in a twin-shell blender for 15 minutes (Table 2). Studies to determine the homogeneity of a formulated diet mixture indicated about a 2% deviation from the theoretical concentration for samples taken from three

TABLE 2. PREPARATION AND STORAGE OF DOSE MIXTURES AND FORMULATED DIETS IN THE STUDIES OF PETN, NF

Fourteen-Day Studies	Thirteen- and Fourteen-Week Studies	Two-Year Studies
Preparation		
Premix of weighed PETN, NF, and feed layered between feed in a Patterson-Kelly V Twin Shell® blender equipped with an intensifier bar, mixed for 15 min	Same as 14-d studies, mixed for 20 min	Same as 14-d studies
Maximum Storage Time		
2 wk	2 wk	2 wk
Storage Conditions		
0° ± 5° C in double plastic bags	0° ± 5° C in double plastic bags	0° ± 5° C in double plastic bags

locations in the blender after 15 minutes of mixing, demonstrating homogeneity by the mixing procedure PETN, NF, at a concentration of 100 ppm in feed, was stable for 2 weeks in the dark at 5° and 25° C and exhibited a loss of approximately 6% after 2 weeks' storage at 45° C During the 2-year studies, formulated diets were stored at 0° ± 5° C for no longer than 2 weeks

Periodic analyses of formulated diet mixtures of PETN, NF, were conducted at the study laboratory and the analytical chemistry laboratory Feed samples were extracted with acetonitrile containing acetanilide as an internal standard Extracts were clarified by centrifugation, and PETN, NF, was determined by high-performance liquid chromatographic analysis with a Waters μBondapak C_{18} column, a methanol water solvent system, and ultraviolet detection at 210 nm. Formulated diets were analyzed before the start of, and midway through, the 13- and 14-week studies All results were within specifications and ranged from 93% to 100% of target concentrations (Table 3) During the 2-year studies, the formulated diets were analyzed at approximately 8-week intervals. For the PETN, NF, studies, the mixtures were formulated within ±10% of the target concentrations approximately 98% (55/56) of the time throughout the 2-year studies (Table 4) Results of periodic referee analysis performed by the analytical chemistry laboratory indicated generally good agreement with the results from the study laboratory (Table 5)

TABLE 3. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN- AND FOURTEEN-WEEK FEED STUDIES OF PETN, NF

Date Mixed	Concentration of PETN, NF, in Feed (ppm)		Determined as a Percent of Target
	Target	Determined (a,b)	
12/31/80	3,100	3,100	100
	6,200	6,080	98 1
	12,500	12,200	97 6
	25,000	23,300	93 0
	50,000	46,900	93 8
02/25/81	3,100	3,030	97 7
	6,200	6,000	96 8
	12,500	12,500	100
	25,000	24,000	96 0
	50,000	46,600	93 2

(a) Samples stored for 10 weeks before analysis until method of analysis was developed
(b) Results of duplicate analysis

TABLE 4. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF PETN, NF

Date Mixed	Determined Concentration of PETN, NF, in Feed for Target Concentration (ppm) (a)			
	6,200	12,500	25,000	50,000
12/29/81	6,300	13,100	26,600	51,300
04/08/82	6,400	12,100	25,000	50,200
05/26/82	6,500	13,000	26,100	51,600
06/24/82	6,250	12,900	25,600	50,500
09/16/82	(b) 6,333	12,400	25,500	(b) 50,700
10/21/82	6,250	12,500	25,500	50,700
01/13/83	6,250	12,300	25,100	50,500
02/03/83	6,400	13,000	26,200	50,600
04/14/83	6,700	(c) 14,400	26,200	54,200
04/15/83	--	(d) 12,700	--	--
06/08/83	6,200	12,800	25,000	49,800
08/03/83	6,500	12,700	25,500	51,500
10/05/83	6,200	13,100	26,000	52,600
11/09/83	6,300	12,700	24,900	49,100
12/28/83	5,900	12,600	24,400	51,100
Mean (ppm)	6,320	12,829	25,543	51,029
Standard deviation	184.6	545.5	621 1	1,241.2
Coefficient of variation (percent)	2.9	4.3	2 4	2.4
Range (ppm)	5,900-6,700	12,100-14,400	24,400-26,600	49,100-54,200
Number of samples	14	14	14	14

(a) Results of duplicate analysis
(b) Mean concentration for samples taken from three locations within the blender
(c) Out of specifications; not used in the studies.
(d) Remix; not included in the mean.

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

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory (a)	Referee Laboratory (b)
12/29/81	6,200	6,300	6,200
09/16/82	25,000	25,500	26,500
04/14/83	12,500	14,400	13,300
10/05/83	50,000	52,600	52,300

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

II. MATERIALS AND METHODS

FOURTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and were held for 15 days before the studies began. The rats were 6-7 weeks old when placed on study, and the mice were 7-8 weeks old.

Groups of five rats and five mice of each sex were fed diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm PETN, NF, for 14 consecutive days. Animals were observed two times per day and weighed one time per week. Feed consumption was monitored throughout the studies.

Animals were housed five per cage. Water and feed were available ad libitum. Details of animal maintenance are presented in Table 6. A necropsy was performed on all animals. Histologic examination on the kidneys only was performed on mice fed diets containing 0, 25,000, or 50,000 ppm PETN, NF.

THIRTEEN-WEEK AND FOURTEEN-WEEK STUDIES

Thirteen-week or 14-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to PETN, NF, and to determine the concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and were held for 26-28 days (rats) or 20-22 days (mice) before the studies began. Animals were distributed to weight classes and assigned to cages such that average cage weights for animals of each sex and species were approximately equal. Groups of 10 rats and 10 mice of each sex were fed diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm PETN, NF, for 13 weeks (mice) or 14 weeks (rats).

Rats and mice were housed five per cage. Formulated diets, control diets, and water were available ad libitum. Animals were observed two times per day. Individual animal weights were recorded at day 0, once per week during the studies, and at necropsy. Feed consumption was monitored 2-3 days per week. Further experimental details are summarized in Table 6.

An 18- to 24-hour sample of urine was collected during week 13 of the studies from rats placed in suspended stainless-steel metabolism cages. Only water was provided. Urinary nitrite was determined with an Ames Clini-Tek Semi-Automated Urinalysis Analyzer, Model 5500. At the end of the studies, blood was collected from barbiturate-anesthetized rats and mice by exsanguination from the jugular vein for methemoglobin determination by spectrophotometry. The whole blood specimen was placed in a 2-ml Vacutainer® tube containing K_3EDTA as an anticoagulant. The tubes were inverted several times to ensure proper mixing and then placed over ice for transport to the clinical laboratory. Methemoglobin levels were determined according to the spectrophotometric method outlined by Simmons (1976). A Coleman Junior spectrophotometer (Model 6A) was used to read the samples. As a positive control, several aliquots of blood from control rats were spiked with sodium nitrite to produce an in vitro methemoglobin burden of approximately 7%-8%.

A necropsy was performed on all animals. Weights of the brain, liver, right kidney, thymus, heart, and lungs were recorded. Histologic examinations were performed on all controls and on animals that received 50,000 ppm PETN, NF. Selected tissues were examined from other groups of animals. Tissues and groups examined are listed in Table 6.

TWO-YEAR STUDIES

Study Design

Diets containing 0, 25,000, or 50,000 ppm PETN, NF, were fed to groups of 50 male rats for 103 weeks. Diets containing 0, 6,200, or 12,500 ppm PETN, NF, were fed to groups of 50 female rats on the same schedule. Diets containing 0, 25,000, or 50,000 ppm PETN, NF, were fed to groups of 49 or 50 male mice for 103 weeks and to groups of 50 female mice for 103 weeks.

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF PETN, NF

Fourteen-Day Studies	Thirteen- and Fourteen Week Studies	Two Year Studies
EXPERIMENTAL DESIGN		
Size of Study Groups		
5 males and 5 females of each species	10 males and 10 females of each species	49 or 50 males and 50 females of each species
Doses		
0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm PETN, NF, in feed	Same as 14 d studies	Male rats and all mice 0, 25,000, or 50,000 ppm PETN, NF, in feed, female rats 0, 6,200, or 12,500 ppm
Date of First Dose		
10/15/80	Rats- male 1/12/81, female 1/14/81, mice male 1/26/81, female 1/28/81	Rats male 1/19/82 female 1/26/82, mice male 1/11/82, female 1/4/82
Date of Last Dose		
10/28/80	Rats male 4/20/81, female 4/22/81, mice male 4/27/81, female 4/29/81	Rats male 1/16/84, female 1/23/84, mice male 1/2/84, female 12/26/83
Duration of Dosing		
14 consecutive d	Rats 14 wk, mice 13 wk	Rats 104 wk, mice 103 wk
Type and Frequency of Observation		
Observed 2 × d, weighed initially, 1 × wk, and at the end of the studies	Observed 2 × d, weighed initially 1 × wk, and at the end of the studies, feed consumption measured 2 3 d/wk	Observed 2 × d, weighed initially, 1 × wk for 13 wk and then 1 × mo
Necropsy, Histologic Examinations, and Supplemental Studies		
Necropsy performed on all animals, kidneys of male and female mice from the control, 25,000 , and 50,000-ppm groups examined histologically	Necropsy performed on all animals, the following tissues examined histologically for control and high dose groups adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), gall bladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland (rats), rectum, salivary glands, skin, spinal cord (if neurologic signs present), spleen, sternebrae including marrow, stomach, thymus, thyroid gland, trachea, urinary bladder, and Zymbal gland Tissues examined from 25,000 ppm groups include Zymbal gland for female rats and liver for female mice Organ weights recorded at necropsy include brain, heart, right kidney, liver, lungs, and thymus Urinary nitrite determined for rats and whole blood methemoglobin determined for rats and mice	Necropsy performed on all animals, the following tissues examined histologically for low dose animals that died before month 21 and for all control and high dose animals adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/tunica vaginalis/scrotal sac/prostate/testes or ovaries/uterus, esophagus, femur or sternebrae or vertebrae, gross lesions including marrow, gallbladder (mice) and tissue masses with regional lymph nodes, heart and aorta, ileum, jejunum, kidneys, larynx, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroid glands, pharynx, pituitary gland, preputial or clitoral gland, rectum, salivary glands, skin, spleen, stomach, thymus, thyroid gland, tongue, trachea, urinary bladder, and Zymbal gland Gross lesions examined for all low dose animals Tissues examined for low dose groups include brain, kidneys, liver, pancreas, and testes for male rats, esophagus, kidneys, liver, lungs, thyroid gland, and uterus for female rats, stomach for male mice, and liver, spleen, and stomach for female mice
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species		
F344/N rats, B6C3F$_1$ mice	F344/N rats, B6C3F$_1$ mice	F344/N rats, B6C3F$_1$ mice

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF PETN, NF (Continued)

Fourteen-Day Studies	Thirteen- and Fourteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Animal Source		
Charles River Breeding Laboratories (Portage, MI)	Rats Charles River Breeding Laboratories (Kingston, NY), mice- Charles River Breeding Laboratories (Portage, MI)	Frederick Cancer Research Facility (Frederick, MD)
Study Laboratory		
EG&G Mason Research Institute	EG&G Mason Research Institute	EG&G Mason Research Institute
Method of Animal Identification		
Ear punch	Ear punch	Ear punch
Time Held Before Study		
15 d	Rats--male 26 d, female 28 d, mice male 20 d, female 22 d	Rats--19-20 d; mice- 18-20 d
Age When Placed on Study		
Rats--6-7 wk, mice--7-8 wk	8-9 wk	Rats 8 wk, mice--8-9 wk
Age When Killed		
Rats--8-9 wk, mice 9 10 wk	Rats 22 23 wk, mice 21 22 wk	Rats 112-114 wk, mice 112-113 wk
Necropsy Dates		
10/30/80-11/4/80	Rats 4/21/81 4/24/81, mice 4/28/81 5/1/81	Rats--1/23/84-2/3/84, mice -1/3/84 1/12/84
Method of Animal Distribution		
Assigned to groups such that for a given sex and species all cage weights were approxi mately equal	Same as 14 d studies	Assigned to cages by one table of random numbers and then to groups by another table of random numbers
Feed		
NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc , Gardners, PA); available ad libitum	Same as 14 d studies	Same as 14-d studies
Bedding		
Aspen Bed (American Excelsior, Baltimore, MD)	Same as 14 d studies	Same as 14-d studies
Water		
Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 14-d studies	Same as 14-d studies
Cages		
Polycarbonate (Lab Products, Inc , Rochelle Park, NJ)	Same as 14-d studies	Same as 14-d studies
Cage Filters		
Nonwoven fiber filters (Snow Filtration, Cincinnati, OH)	Same as 14-d studies and nonwoven fiber (Lab Products, Inc , Rochelle Park, NJ)	Same as 14-d studies
Animals per Cage		
5	5	5
Other Chemicals on Study in the Same Room		
None	None	None

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF PETN, NF (Continued)

Fourteen-Day Studies	Thirteen- and Fourteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Animal Room Environment		
Temp 20° 24°C, hum -42%-77%, fluorescent light 12 h/d, 10 12 room air changes/h	Temp 16 1° 26 7°C, hum 25% 65%, fluorescent light 12 h/d, more than 12 room air changes/h	Temp- 19° 27°C, hum 4% 62%, fluorescent light 12 h/d, 13 room air change/h

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 Frederick Cancer Research Facility under a contract to the Carcinogenesis Program Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms Rats were shipped to the study laboratory at 5 weeks, and mice at 5-6 weeks of age The animals were quarantined at the study laboratory for 18-20 days Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status The rats were placed on study at 8 weeks of age, and the mice at 8-9 weeks 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 rotated during the studies Further details of animal maintenance are given in Table 6

Clinical Examinations and Pathology

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

During necropsy, all organs and tissues were examined for grossly visible lesions Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin Histopathologic examination of tissues was performed according to an "inverse pyramid" design (McConnell, 1983a,b) That is, complete histopathologic examinations (Table 6) were performed on all high dose and control animals and on low dose animals dying before 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 group were examined histopathologically

When the pathology evaluation was completed by the laboratory pathologist and the pathology data entered into the Toxicology Data Management System, 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, which included the laboratory pathologist, without knowledge of previously rendered diagnoses. The PWG Chairperson selected a subset of slides for PWG review which included all diagnosed Zymbal gland lesions, all available pancreata with diagnosed acinar cell proliferative lesions, and other selected lesions of the liver, kidney, pituitary gland, uterus, prostate, forestomach, lung, and ovary of rats. For mice, selected lesions were examined from the stomach, adrenal gland, ovary, urinary bladder, and the vascular system. 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

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

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

Analysis of Tumor Incidence: The majority of tumors in this study were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated

II. MATERIALS AND METHODS

if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

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

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

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 Haworth et al. (1983) and Mortelmans et al. (1986). Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). The study chemical was incubated with the *Salmonella typhimurium* tester strains (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 four strains; if all results were negative, the chemical was retested in all strains.

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.

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

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

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

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

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

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

III. RESULTS

RATS

FOURTEEN-DAY STUDIES

FOURTEEN-WEEK STUDIES

TWO-YEAR STUDIES

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

MICE

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

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

GENETIC TOXICOLOGY

III. RESULTS: RATS

FOURTEEN-DAY STUDIES

All rats lived to the end of the studies (Table 7) Final mean body weights of dosed and control rats were comparable. Feed consumption by dosed male rats was lower than that by controls. No clinical signs or toxic lesions were related to PETN, NF, administration.

FOURTEEN-WEEK STUDIES

All rats lived to the end of the studies (Table 8) No clinical signs were attributed to the chemical Final mean body weights of dosed and control male rats were similar The final mean body weight of female rats that received 25,000 or 50,000 ppm was 6% or 7% lower than that of controls. The relative brain and kidney weights for female rats that received 50,000 ppm were marginally higher than those for controls (Table 9). Nitrite was detected in the urine of one male rat in the 6,200-ppm group, one female rat in the 25,000-ppm group, and one female rat in the 50,000-ppm group. Methemoglobin levels in whole blood were not affected by administration of PETN, NF (Table 10). An adenoma of the Zymbal gland was seen in one female rat that received 50,000 ppm.

Dose Selection Rationale. Because of the absence of toxic effects in males in the 14-week study, the highest dietary concentrations recommended for a 2-year study (25,000 ppm and 50,000 ppm PETN, NF) were selected for male rats. Because of lower mean body weight gain (−17% to −18%) by female rats at higher concentrations in the 14-week study, dietary concentrations of PETN, NF, selected for female rats for the 2-year study were 6,200 ppm and 12,500 ppm.

TABLE 7. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FOURTEEN-DAY FEED STUDIES OF PETN, NF

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	139 ± 3	208 ± 7	+69 ± 8		27	25
3,100	5/5	133 ± 5	207 ± 5	+74 ± 2	100	18	18
6,200	5/5	133 ± 5	213 ± 5	+80 ± 1	102	18	20
12,500	5/5	132 ± 4	213 ± 7	+81 ± 4	102	24	20
25,000	5/5	132 ± 4	209 ± 5	+77 ± 2	100	23	19
50,000	5/5	132 ± 4	209 ± 7	+77 ± 3	100	19	19
FEMALE							
0	5/5	104 ± 2	145 ± 3	+41 ± 2		15	16
3,100	5/5	107 ± 2	144 ± 2	+37 ± 1	99	16	17
6,200	5/5	106 ± 2	145 ± 2	+39 ± 2	100	17	17
12,500	5/5	106 ± 1	147 ± 1	+41 ± 1	101	18	15
25,000	5/5	107 ± 2	145 ± 2	+38 ± 1	100	16	17
50,000	5/5	107 ± 2	138 ± 1	+31 ± 1	95	16	15

(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 8. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FOURTEEN-WEEK FEED STUDIES OF PETN, NF

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	183 ± 6	339 ± 9	+156 ± 6		55	47
3,100	10/10	184 ± 6	331 ± 8	+147 ± 10	98	63	48
6,200	10/10	183 ± 6	335 ± 7	+152 ± 6	99	71	46
12,500	10/10	183 ± 6	351 ± 6	+168 ± 6	104	76	54
25,000	10/10	183 ± 6	336 ± 8	+153 ± 5	99	66	36
50,000	10/10	183 ± 6	336 ± 6	+153 ± 5	99	63	36
FEMALE							
0	10/10	139 ± 4	215 ± 2	+76 ± 3		66	76
3,100	10/10	140 ± 4	210 ± 3	+70 ± 2	98	69	69
6,200	10/10	139 ± 4	211 ± 3	+72 ± 1	98	69	66
12,500	10/10	139 ± 4	206 ± 4	+67 ± 2	96	80	74
25,000	10/10	140 ± 3	203 ± 4	+63 ± 2	94	74	74
50,000	10/10	139 ± 4	201 ± 4	+62 ± 2	93	83	66

(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 kilogram of body weight per day, not corrected for scatter

TABLE 9. ORGAN WEIGHT TO NECROPSY BODY WEIGHT RATIOS FOR RATS IN THE FOURTEEN-WEEK FEED STUDIES OF PETN, NF (a)

	0 ppm	3,100 ppm	6,200 ppm	12,500 ppm	25,000 ppm	50,000 ppm
MALE						
Necropsy body weight (grams)	364 ± 5 5	344 ± 7 8	352 ± 6 8	349 ± 5 7	341 ± 8 2	344 ± 6 4
Brain	(b) 5 2 ± 0 15	(b) 5 5 ± 0 11	(b) 5 5 ± 0 12	(b) 5 1 ± 0 49	(b) 5 4 ± 0 53	(b) 5 6 ± 0 12
Liver	37 0 ± 0 88	(c) 30 6 ± 0 98	(d) 32 5 ± 1 24	32 9 ± 1 61	33 1 ± 0 90	32 7 ± 1 52
Right kidney	4 6 ± 0 55	4 5 ± 0 60	4 4 ± 0 54	4 6 ± 0 61	4 5 ± 0 52	4 6 ± 0 55
Thymus	1 0 ± 0 16	1 0 ± 0 29	0 8 ± 0 10	1 0 ± 0 16	0 7 ± 0 02	0 8 ± 0 12
Heart	2 7 ± 0 06	2 7 ± 0 08	2 7 ± 0 08	2 9 ± 0 25	2 7 ± 0 08	2 6 ± 0 07
Lungs	(e) 4 1 ± 0 12	(b) 3 7 ± 0 10	(b) 3 9 ± 0 2	(b) 3 8 ± 0 18	(e) 3 7 ± 0 10	(b) 3 9 ± 0 09
FEMALE						
Necropsy body weight (grams)	217 ± 2 5	211 ± 3 4	208 ± 3 8	205 ± 3 8	(d) 201 ± 3 9	(d) 201 ± 4 0
Brain	8 3 ± 0 09	8 6 ± 0 15	8 7 ± 0 12	(d) 8 8 ± 0 13	(c) 9 0 ± 0 13	(c) 9 0 ± 0 19
Liver	32 9 ± 0 41	32 5 ± 0 63	31 1 ± 0 70	32 3 ± 0 74	31 9 ± 0 85	33 1 ± 0 78
Right kidney	3 1 ± 0 05	3 1 ± 0 05	3 1 ± 0 05	3 2 ± 0 06	3 2 ± 0 05	(d) 3 3 ± 0 06
Thymus	1 1 ± 0 10	1 0 ± 0 03	1 1 ± 0 02	1 0 ± 0 04	1 0 ± 0 04	0 9 ± 0 05
Heart	2 8 ± 0 04	2 8 ± 0 05	2 8 ± 0 08	2 9 ± 0 06	2 9 ± 0 07	3 0 ± 0 06
Lungs	4 8 ± 0 11	4 6 ± 0 08	4 7 ± 0 10	4 8 ± 0 08	4 8 ± 0 11	4 7 ± 0 12

(a) Mean ± standard error in milligrams per gram for groups of 10 unless otherwise specified, P values vs the controls by Dunnett's test (Dunnett, 1955)
(b) Six were weighed
(c) $P<0.01$
(d) $P<0.05$
(e) Five were weighed

TABLE 10. METHEMOGLOBIN LEVELS FOR RATS IN THE FOURTEEN-WEEK FEED STUDIES OF PETN, NF (a)

Concentration (ppm)	Male	Female
0	(b) 0 56 ± 0 08	0 51 ± 0 05
3,100	0 72 ± 0 11	(c) 0 90 ± 0 09
6,200	0 78 ± 0 12	0 64 ± 0 09
12,500	0 57 ± 0 08	0 57 ± 0 08
25,000	0 62 ± 0 12	0 44 ± 0 03
50,000	0 59 ± 0 07	0 66 ± 0 12

(a) Percent, mean ± standard error for groups of 10 unless otherwise specified, P values vs the controls by Dunnett's test (Dunnett, 1955)
(b) Nine were examined
(c) P<0 01

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of high dose male rats were 2% 9% lower than those of controls throughout the study (Table 11 and Figure 6) Mean body weights of high dose female rats were within 5% of those of controls throughout the study The average daily feed consumption by low dose or high dose rats was 98% or 97% that by controls for males and 97% or 103% for females (Tables F1 and F2) The average amount of PETN, NF, consumed per day was approximately 1,200 or 2,500 mg/kg for low or high dose male rats and 400 or 830 mg/kg for low or high dose female rats No compound-related clinical signs were observed

TABLE 11. MEAN BODY WEIGHTS OF RATS IN THE TWO-YEAR FEED STUDIES OF PETN, NF

Weeks on Study	Control Av. Wt. (grams)	Control No. Weighed	Low Dose Av. Wt. (grams)	Low Dose Wt. (percent of controls)	Low Dose No. Weighed	High Dose Av. Wt. (grams)	High Dose Wt. (percent of controls)	High Dose No. Weighed
MALE				**25,000 ppm**			**50,000 ppm**	
1	165	50	160	97	50	160	97	50
2	208	50	197	95	50	195	94	50
3	235	50	222	94	50	217	92	50
4	259	50	240	93	50	237	92	50
5	278	50	262	94	50	258	93	50
6	298	50	280	94	50	278	93	50
7	316	50	297	94	50	293	93	50
8	319	50	311	97	50	309	97	50
9	335	50	324	97	50	317	95	50
10	341	50	329	96	50	326	96	50
11	355	50	339	95	50	335	94	50
12	368	50	343	93	50	335	91	50
13	369	50	351	95	50	343	93	50
17	386	50	375	97	50	370	96	50
21	405	50	396	98	50	390	96	50
25	415	50	407	98	50	402	97	50
29	418	50	419	100	50	406	97	50
33	431	50	430	100	50	409	95	50
37	437	50	435	100	50	415	95	50
41	449	50	447	100	50	430	96	50
45	450	50	455	101	50	434	96	50
49	467	50	463	99	49	447	96	50
53	460	50	468	102	49	449	98	50
57	470	50	472	100	49	448	95	50
61	472	49	472	100	49	451	96	50
65	475	49	479	101	49	458	96	50
69	477	49	479	100	49	455	95	50
73	479	48	484	101	48	462	96	50
77	471	48	477	101	48	457	97	50
81	467	47	472	101	47	455	97	49
85	472	44	462	98	46	449	95	47
89	463	44	458	99	46	447	97	47
93	468	34	460	98	40	438	94	45
97	465	30	457	98	38	436	94	43
101	449	28	454	101	34	423	94	39
105	441	22	428	97	(a) 28	409	93	29
FEMALE				**6,200 ppm**			**12,500 ppm**	
1	119	50	120	101	50	120	101	50
2	141	50	141	100	50	141	100	50
3	152	50	150	99	50	152	100	50
4	163	50	161	99	50	161	99	50
5	173	50	168	97	50	169	98	50
6	179	50	174	97	50	175	98	50
7	185	50	180	97	50	180	97	50
8	190	50	186	98	50	184	97	50
9	196	50	188	96	50	189	96	50
10	200	50	193	97	50	191	96	50
11	202	50	196	97	50	195	97	50
12	206	50	201	98	50	200	97	50
13	205	50	200	98	50	201	98	50
17	217	50	212	98	50	212	98	50
21	225	50	219	97	(a) 49	224	100	50
25	235	50	228	97	50	227	97	50
29	239	50	233	97	50	236	99	50
33	250	50	243	97	49	246	98	50
37	255	50	252	99	49	253	99	50
41	267	50	260	97	49	263	99	50
45	275	50	274	100	49	278	101	50
49	289	50	287	99	49	292	101	50
53	299	50	297	99	49	299	100	50
57	308	50	304	99	49	306	99	50
61	318	50	313	98	49	316	99	50
65	329	49	323	98	48	329	100	48
69	340	49	332	98	48	333	98	48
73	342	48	335	98	48	336	98	48
77	342	48	335	98	48	339	99	46
81	350	47	342	98	(a) 46	346	99	46
85	355	45	338	95	43	345	97	45
89	359	44	341	95	43	343	96	44
93	364	40	348	96	40	351	96	42
97	367	38	352	96	38	350	95	39
101	365	37	348	95	36	349	96	34
105	365	33	352	96	33	349	96	31

(a) The number of animals weighed was lower than the number of animals surviving

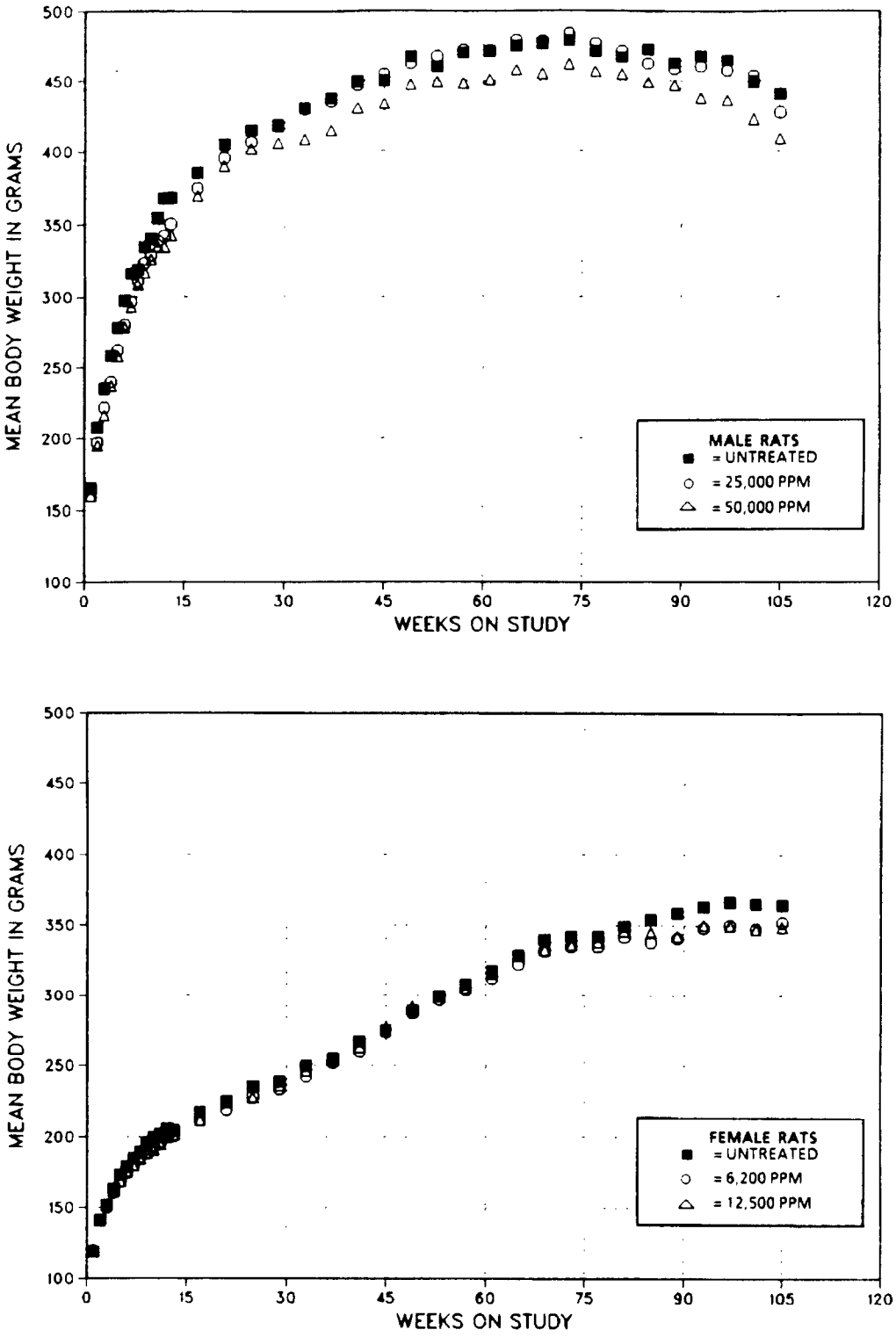

FIGURE 6. GROWTH CURVES FOR RATS FED DIETS CONTAINING PETN, NF, FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats fed diets containing PETN, NF, at the concentrations used in these studies and for controls are shown in Table 12 and in the Kaplan and Meier curves in Figure 7. No significant differences in survival were observed between any groups of either sex.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the Zymbal gland, thyroid gland, and hematopoietic system.

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 12. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF PETN, NF

	Control	Low Dose	High Dose
MALE (a)		**25,000 ppm**	**50,000 ppm**
Animals initially in study	50	50	50
Natural deaths	8	5	5
Moribund kills	20	17	16
Animals surviving until study termination	22	(b) 29	29
Survival P values (c)	0.086	0.177	0.099
FEMALE (a)		**6,200 ppm**	**12,500 ppm**
Animals initially in study	50	50	50
Natural deaths	4	2	1
Moribund kills	13	15	18
Animals surviving until study termination	33	33	31
Survival P values (c)	0.774	0.885	0.846

(a) First day of termination period: 735
(b) One animal died or was killed in a moribund condition and was 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.

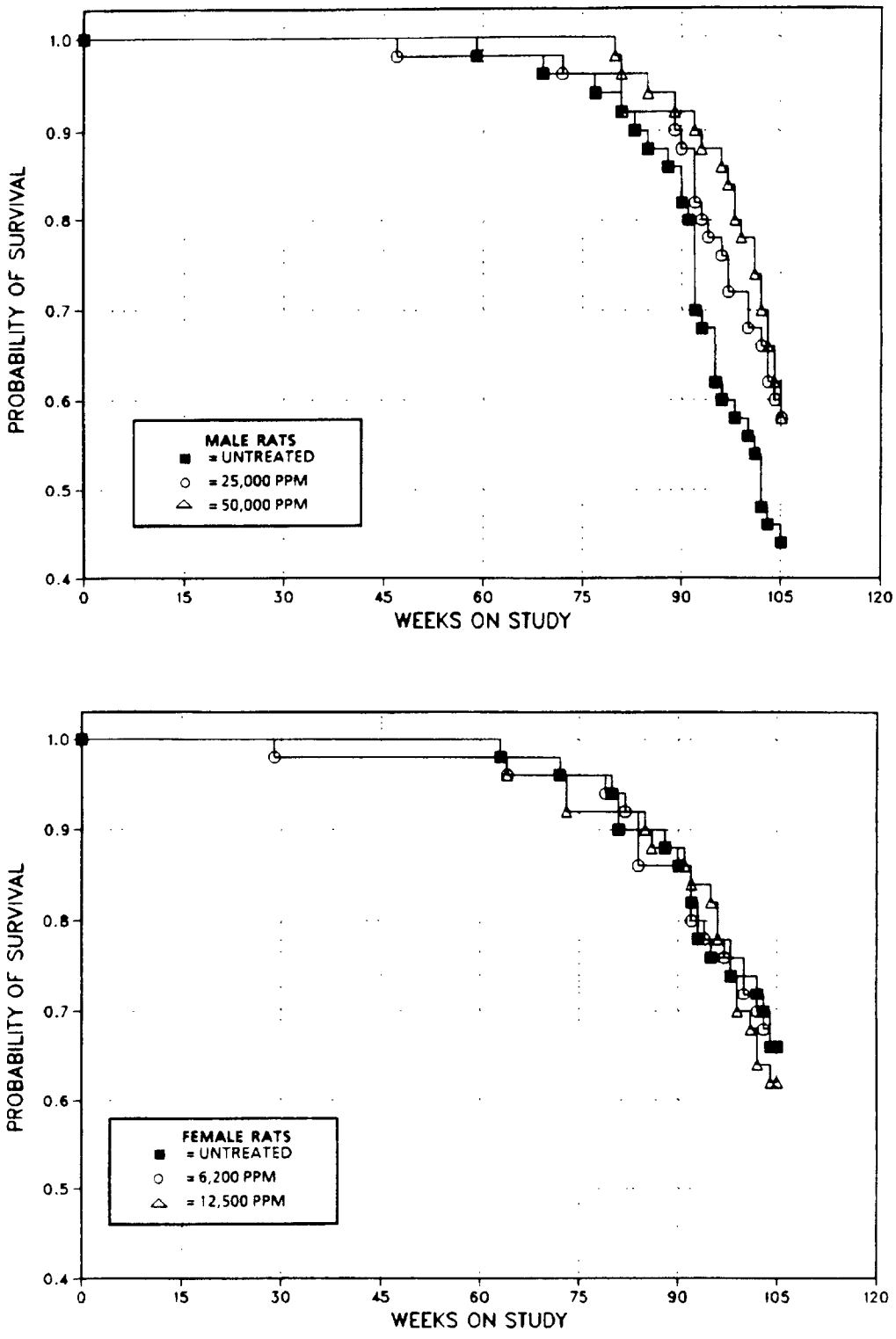

FIGURE 7. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING PETN, NF, FOR TWO YEARS

Zymbal Gland: Adenomas or carcinomas occurred only in dosed male and female rats (Table 13). Carcinomas were visible grossly as ulcerated masses on the side of the head just below the ear canal. Histologically, they appeared as typical squamous cell carcinomas with extension to the subcutaneous tissue and replacement of the epidermis. Adenomas lacked squamous metaplasia and occurred as well-defined nodules of differentiated sebaceous-type cells with fewer undifferentiated reserve-type cells. In contrast to normal acini with a pattern of differentiation to sebaceous cells from the periphery to the center, sebaceous differentiation in the adenoma is more random and appears within multiple foci within the nodule. Adenomas were larger than adjacent normal lobules/acini, with numerous hyperchromatic cells. One adenoma was cystic with focal, disoriented proliferations of cells at the margin, and one had a thin fibrous capsule. The hyperplasia was smaller than the adenomas and exhibited less cellular proliferation. With the exception of one adenoma, all tumors were visible upon gross examination. Special efforts were made to collect and evaluate microscopically the Zymbal gland from all animals.

TABLE 13. ZYMBAL GLAND LESIONS IN RATS IN THE TWO-YEAR FEED STUDIES OF PETN, NF (a,b)

	Control	Low Dose	High Dose
MALE		**25,000 ppm**	**50,000 ppm**
Hyperplasia			
Overall Rates	0/49 (0%)	1/45 (2%)	0/41 (0%)
Adenoma			
Overall Rates	0/49 (0%)	1/45 (2%)	0/41 (0%)
Carcinoma			
Overall Rates	0/49 (0%)	2/45 (4%)	2/41 (4%)
Adenoma or Carcinoma (c)			
Overall Rates	0/49 (0%)	3/45 (7%)	2/41 (7%)
Adjusted Rates	0.0%	8.3%	6.9%
Terminal Rates	0/22 (0%)	1/24 (4%)	1/22 (5%)
Day of First Observation		562	687
Life Table Tests	P=0.231	P=0.138	P=0.275
Logistic Regression Tests	P=0.135	P=0.108	P=0.219
FEMALE		**6,200 ppm**	**12,500 ppm**
Hyperplasia			
Overall Rates	1/36 (3%)	0/37 (0%)	0/35 (0%)
Adenoma			
Overall Rates	0/36 (0%)	0/37 (0%)	2/35 (6%)
Carcinoma			
Overall Rates	0/36 (0%)	1/37 (3%)	1/35 (3%)
Adenoma or Carcinoma (d)			
Overall Rates	0/36 (0%)	1/37 (3%)	3/35 (9%)
Adjusted Rates	0.0%	4.3%	9.1%
Terminal Rates	0/24 (0%)	1/23 (4%)	1/23 (4%)
Day of First Observation		735	634
Life Table Tests	P=0.060	P=0.492	P=0.121
Logistic Regression Tests	P=0.055	P=0.492	P=0.116

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table A3 (footnotes).
(b) The estimated dose in milligrams per kilograms per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.
(c) Historical incidence at the study laboratory (mean ± SD): 4/599 (0.7% ± 1%); historical incidence in NTP studies: 19/1,936 (1% ± 2%)
(d) Historical incidence at the study laboratory (mean ± SD): 1/649 (0.2% ± 0.6%); historical incidence in NTP studies: 11/1,983 (0.6% ± 1%)

III. RESULTS: RATS

Unless enlarged with a tumor, however, this organ is small and is not collected as part of the typical sections taken from the head; therefore, sampling was incomplete in several groups.

Thyroid Gland: Follicular cell adenomas or carcinomas (combined) in female rats occurred with a significant positive trend (control, 0/50; low dose, 0/48; high dose, 3/50) (Table B3); although the incidence in the high dose group was not significantly greater than that in the controls, it exceeded the highest incidence observed in NTP untreated control female F344/N rats (2/49). Follicular cell adenomas or carcinomas (combined) were seen in 1/49 control, 2/15 low dose, and 0/50 high dose male rats.

Hematopoietic System: Mononuclear leukemia in male rats occurred with a significant negative trend; the incidence in the high dose group was significantly lower than that in the controls (Table 14).

TABLE 14. MONONUCLEAR LEUKEMIA IN MALE RATS IN THE TWO-YEAR FEED STUDY OF PETN, NF (a)

	Control	25,000 ppm	50,000 ppm
Overall Rates	29/50 (58%)	(b) 27/50 (54%)	20/50 (40%)
Adjusted Rates	70.9%	62.0%	54.4%
Terminal Rates	11/22 (50%)	13/29 (45%)	13/29 (45%)
Day of First Observation	577	501	589
Life Table Tests	P=0.009N	P=0.157N	P=0.011N
Logistic Regression Tests	P=0.035N	P=0.405N	P=0.036N

(a) Historical incidence of leukemia at study laboratory (mean ± SD): 145/599 (24% ± 9%); historical incidence in NTP studies: 636/1,936 (33% ± 15%)
(b) Gross lesions and target organs in low dose animals were examined according to protocol (see Table 6); 36 spleens were examined microscopically.

III. RESULTS: MICE

FOURTEEN-DAY STUDIES

All mice lived to the end of the studies (Table 15). The final mean body weight of female mice that received 50,000 ppm was 13% lower than that of controls. Feed consumption by dosed and control mice was similar. No compound-related clinical signs or histopathologic lesions were observed.

THIRTEEN-WEEK STUDIES

All mice lived to the end of the studies (Table 16). No compound-related clinical signs were observed. Final mean body weights of dosed and control mice were similar. Feed consumption by dosed male mice was lower than that by controls. The relative liver and kidney weights for female mice that received 50,000 ppm were slightly greater than those for controls (Table 17). No compound-related increases in methemoglobin levels were observed (Table 18). A hepatocellular adenoma was seen in 1/10 female mice that received 50,000 ppm.

TABLE 15. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE FOURTEEN-DAY FEED STUDIES OF PETN, NF

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	22.9 ± 0.9	25.6 ± 1.0	+2.7 ± 0.3		6.5	6.5
3,100	5/5	22.9 ± 0.9	25.1 ± 1.1	+2.2 ± 0.4	98.0	6.7	6.5
6,200	5/5	22.6 ± 0.9	25.6 ± 0.8	+3.0 ± 0.3	100.0	6.0	5.9
12,500	5/5	23.0 ± 0.9	26.1 ± 1.0	+3.1 ± 0.5	102.0	5.3	6.3
25,000	5/5	22.8 ± 1.0	27.1 ± 1.0	+4.3 ± 0.3	105.9	6.9	6.8
50,000	5/5	22.6 ± 0.7	25.9 ± 0.8	+3.3 ± 0.2	101.2	6.3	7.1
FEMALE							
0	5/5	18.0 ± 0.3	20.6 ± 0.4	+2.6 ± 0.1		5.9	6.1
3,100	5/5	18.2 ± 0.4	20.2 ± 0.5	+2.0 ± 0.3	98.1	5.8	8.3
6,200	5/5	18.2 ± 0.2	20.1 ± 0.4	+1.9 ± 0.2	97.6	8.6	8.9
12,500	5/5	18.3 ± 0.4	20.1 ± 0.2	+1.8 ± 0.4	97.6	5.4	7.2
25,000	5/5	18.6 ± 0.4	19.7 ± 0.4	+1.1 ± 0.4	95.6	6.9	6.4
50,000	5/5	17.6 ± 0.7	18.0 ± 0.9	+0.4 ± 0.7	87.4	4.5	7.5

(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.

TABLE 16. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PETN, NF

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	24.1 ± 0.4	30.9 ± 0.3	+6.8 ± 0.5		193	214
3,100	10/10	24.0 ± 0.4	32.0 ± 0.6	+8.0 ± 0.4	103.6	178	159
6,200	10/10	23.9 ± 0.4	30.0 ± 0.8	+6.1 ± 0.7	97.1	244	168
12,500	10/10	24.0 ± 0.4	32.7 ± 0.8	+8.7 ± 0.5	105.8	145	143
25,000	10/10	23.8 ± 0.4	31.6 ± 0.6	+7.8 ± 0.4	102.3	185	144
50,000	10/10	24.0 ± 0.4	31.1 ± 0.6	+7.1 ± 0.7	100.6	214	132
FEMALE							
0	10/10	19.8 ± 0.3	27.3 ± 0.6	+7.5 ± 0.5		230	200
3,100	10/10	20.2 ± 0.3	29.0 ± 0.7	+8.8 ± 0.6	106.2	277	226
6,200	10/10	20.1 ± 0.2	29.1 ± 0.7	+9.0 ± 0.6	106.6	247	187
12,500	10/10	20.5 ± 0.3	27.4 ± 0.6	+6.9 ± 0.5	100.4	253	191
25,000	10/10	20.4 ± 0.3	28.3 ± 0.7	+7.9 ± 0.7	103.7	267	183
50,000	10/10	20.0 ± 0.3	27.7 ± 0.8	+7.7 ± 0.5	101.5	312	195

(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 kilogram of body weight per day; not corrected for scatter.

TABLE 17. ORGAN WEIGHT TO NECROPSY BODY WEIGHT RATIOS FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PETN, NF (a)

	0 ppm	3,100 ppm	6,200 ppm	12,500 ppm	25,000 ppm	50,000 ppm
MALE						
Necropsy body weight (grams)	31.0 ± 0.49	31.4 ± 0.82	31.2 ± 0.82	31.5 ± 0.89	30.5 ± 0.48	29.8 ± 0.61
Brain	14.6 ± 0.34	14.5 ± 0.56	14.3 ± 0.40	14.1 ± 0.37	14.8 ± 0.34	15.2 ± 0.34
Liver	52.9 ± 1.01	55.1 ± 0.97	56.3 ± 1.13	53.7 ± 1.49	55.4 ± 2.13	52.3 ± 1.34
Right kidney	8.8 ± 0.27	8.9 ± 0.25	8.8 ± 0.38	8.7 ± 0.21	8.4 ± 0.16	9.1 ± 0.21
Thymus	1.3 ± 0.13	1.2 ± 0.11	1.3 ± 0.26	1.2 ± 0.17	1.2 ± 0.11	1.3 ± 0.24
Heart	4.8 ± 0.15	(b) 4.8 ± 0.11	4.8 ± 0.14	4.5 ± 0.12	4.7 ± 0.15	4.8 ± 0.11
Lung	5.9 ± 0.19	6.2 ± 0.30	6.0 ± 0.33	6.0 ± 0.38	6.1 ± 0.23	6.3 ± 0.25
FEMALE						
Necropsy body weight (grams)	26.4 ± 0.52	28.0 ± 0.78	27.8 ± 0.62	27.1 ± 0.52	27.3 ± 0.70	26.1 ± 0.65
Brain	17.9 ± 0.35	17.4 ± 0.50	17.4 ± 0.41	17.7 ± 0.46	17.4 ± 0.46	18.0 ± 0.41
Liver	50.2 ± 0.71	51.7 ± 1.16	49.8 ± 0.94	51.7 ± 0.75	52.5 ± 1.10	(c) 53.8 ± 0.71
Right kidney	6.4 ± 0.10	6.8 ± 0.21	6.3 ± 0.14	6.6 ± 0.11	6.7 ± 0.15	(c) 6.9 ± 0.12
Thymus	1.8 ± 0.16	1.6 ± 0.12	1.8 ± 0.19	1.8 ± 0.21	1.8 ± 0.15	2.0 ± 0.14
Heart	4.5 ± 0.13	4.3 ± 0.10	4.1 ± 0.10	4.4 ± 0.17	4.4 ± 0.15	4.5 ± 0.10
Lungs	7.2 ± 0.27	6.9 ± 0.24	6.3 ± 0.33	6.5 ± 0.31	6.6 ± 0.32	6.8 ± 0.28

(a) Mean ± standard error in milligrams per gram for groups of 10 unless otherwise specified; P values vs. the controls by Dunnett's test (Dunnett, 1955).
(b) Nine were weighed.
(c) $P < 0.05$

TABLE 18. METHEMOGLOBIN LEVELS FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PETN, NF (a)

Concentration (ppm)	Male	Female
0	0.58 ± 0.08	0.79 ± 0.13
3,100	0.61 ± 0.08	0.79 ± 0.10
6,200	0.48 ± 0.03	0.84 ± 0.12
12,500	0.57 ± 0.08	0.72 ± 0.12
25,000	0.56 ± 0.08	0.60 ± 0.09
50,000	0.73 ± 0.09	0.73 ± 0.14

(a) Percent, mean ± standard error for groups of 10; no significant differences vs. the controls were obtained by Dunnett's test (Dunnett, 1955).

Dose Selection Rationale: Because of the absence of toxic effects in the 13-week studies, the highest dietary concentrations recommended for 2-year studies (25,000 ppm and 50,000 ppm PETN, NF) were selected for mice.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of dosed and control mice were generally similar throughout the studies (Table 19 and Figure 8). The average daily feed consumption per mouse by low dose or high dose mice was 100% or 98% that by controls for males and 100% and 98% for females (Tables F3 and F4). The average amount of PETN, NF, consumed per day was approximately 4,000 or 8,100 mg/kg for low dose or high dose male mice and 5,100 or 9,700 mg/kg for low dose or high dose female mice. No compound-related clinical signs were observed.

TABLE 19. MEAN BODY WEIGHTS OF MICE IN THE TWO-YEAR FEED STUDIES OF PETN, NF

Week on Study	Control Av. Wt. (grams)	Control No. Weighed	25,000 ppm Av. Wt. (grams)	25,000 ppm Wt. (percent of controls)	25,000 ppm No. Weighed	50,000 ppm Av. Wt. (grams)	50,000 ppm Wt. (percent of controls)	50,000 ppm No. Weighed
MALE								
1	22.3	50	22.4	100.4	50	22.3	100.0	50
2	23.7	50	24.2	102.1	50	23.4	98.7	50
3	25.4	50	25.6	100.8	50	25.1	98.8	50
4	26.5	50	26.0	98.1	50	25.9	97.7	50
5	27.6	(a) 48	27.3	98.9	50	26.1	94.6	(a) 49
6	27.6	(a) 48	28.1	101.8	50	28.2	102.2	(a) 48
7	27.7	(a) 48	28.7	103.6	50	27.9	100.7	(a) 48
8	29.7	(a) 48	29.8	100.3	50	29.3	98.7	(a) 48
9	30.6	(a) 48	30.3	99.0	50	29.8	97.4	(a) 48
10	29.1	(a) 48	28.7	98.6	50	28.0	96.2	(a) 47
11	31.3	(a) 48	31.2	99.7	50	30.6	97.8	(a) 47
12	30.4	(a) 48	31.3	103.0	50	30.8	101.3	(a) 47
13	32.0	(a) 48	31.5	98.4	50	31.7	99.1	(a) 47
17	33.1	(a) 48	33.0	99.7	50	32.7	98.8	(a) 47
21	35.3	(a) 48	35.3	100.0	50	35.0	99.2	(a) 47
25	35.8	(a) 48	35.7	99.7	50	35.8	100.0	(a) 47
29	35.8	(a) 47	36.6	102.2	50	36.2	101.1	(a) 46
37	36.7	46	37.5	102.2	50	35.5	96.7	48
41	36.8	46	38.4	104.3	49	38.9	105.7	48
45	38.8	46	39.5	101.8	49	39.1	100.8	48
49	39.8	46	40.7	102.3	49	39.4	99.0	48
53	39.4	46	39.8	101.0	49	39.3	99.7	48
57	40.8	46	40.8	100.0	49	40.1	98.3	48
61	40.4	45	39.9	98.8	49	39.1	96.8	(a) 43
65	41.8	45	40.5	96.9	49	40.4	96.7	48
69	42.4	43	42.2	99.5	48	41.4	97.6	48
73	42.1	43	41.5	98.6	47	40.8	96.9	48
77	40.2	43	40.6	101.0	47	38.7	96.3	48
81	41.5	41	41.6	100.2	46	40.6	97.8	48
85	41.7	38	41.3	99.0	46	39.4	94.5	47
89	41.6	36	39.1	94.0	45	40.0	96.2	45
93	40.2	35	39.3	97.8	44	39.3	97.8	45
97	41.0	32	40.5	98.8	41	40.0	97.6	43
101	40.1	29	40.1	100.0	39	38.5	96.0	39
105	39.0	26	39.3	100.8	38	38.0	97.4	38
FEMALE								
1	18.4	50	17.9	97.3	50	18.3	99.5	50
2	19.0	50	18.8	98.9	49	19.1	100.5	50
3	18.9	50	17.9	94.7	49	18.8	99.5	50
4	20.3	50	20.4	100.5	49	20.1	99.0	50
5	20.9	50	20.9	100.0	49	21.0	100.5	50
6	21.5	50	21.7	100.9	49	21.7	100.9	50
7	22.0	50	21.9	99.5	49	22.1	100.5	50
8	21.0	50	22.2	105.7	49	22.3	106.2	50
9	23.1	50	22.7	98.3	49	23.2	100.4	50
10	23.5	50	23.0	97.9	49	23.5	100.0	50
11	23.5	50	23.2	98.7	49	23.3	99.1	50
12	24.1	50	23.6	97.9	49	24.3	100.8	50
13	24.8	50	24.5	98.8	49	24.9	100.4	50
17	27.3	50	26.0	95.2	49	27.3	100.0	50
21	28.9	50	27.7	95.8	49	29.3	101.4	50
25	30.1	50	29.8	99.0	49	30.9	102.7	50
29	31.6	50	30.6	96.8	49	32.3	102.2	50
33	31.8	50	29.6	93.1	49	33.0	103.8	50
37	33.7	50	31.6	93.8	49	34.0	100.9	50
41	34.9	50	33.2	95.1	49	35.3	101.1	49
45	35.3	50	34.2	96.9	49	36.8	104.2	49
49	38.4	50	35.9	93.5	49	38.8	101.0	49
53	38.5	49	37.5	97.4	49	39.2	101.8	49
57	40.0	49	38.3	95.8	49	40.0	100.0	49
61	40.7	48	37.7	92.6	48	39.7	97.5	49
65	42.5	48	38.4	90.4	48	41.3	97.2	49
69	44.5	48	40.6	91.2	48	43.4	97.5	48
73	45.4	48	41.5	91.4	48	43.3	95.4	48
77	44.0	47	41.8	95.0	46	44.1	100.2	48
81	44.6	46	42.4	95.1	46	44.5	99.8	48
85	44.9	46	43.6	97.1	42	44.5	99.1	47
89	41.5	46	42.0	101.2	42	43.3	104.3	45
93	41.5	45	41.8	100.7	40	42.9	103.4	45
97	43.0	43	42.5	98.8	38	44.5	103.5	41
101	41.6	40	42.3	101.7	34	44.7	107.5	39
105	41.6	38	40.7	97.8	30	43.3	104.1	38

(a) The number of animals weighed was lower than the number of animals surviving.

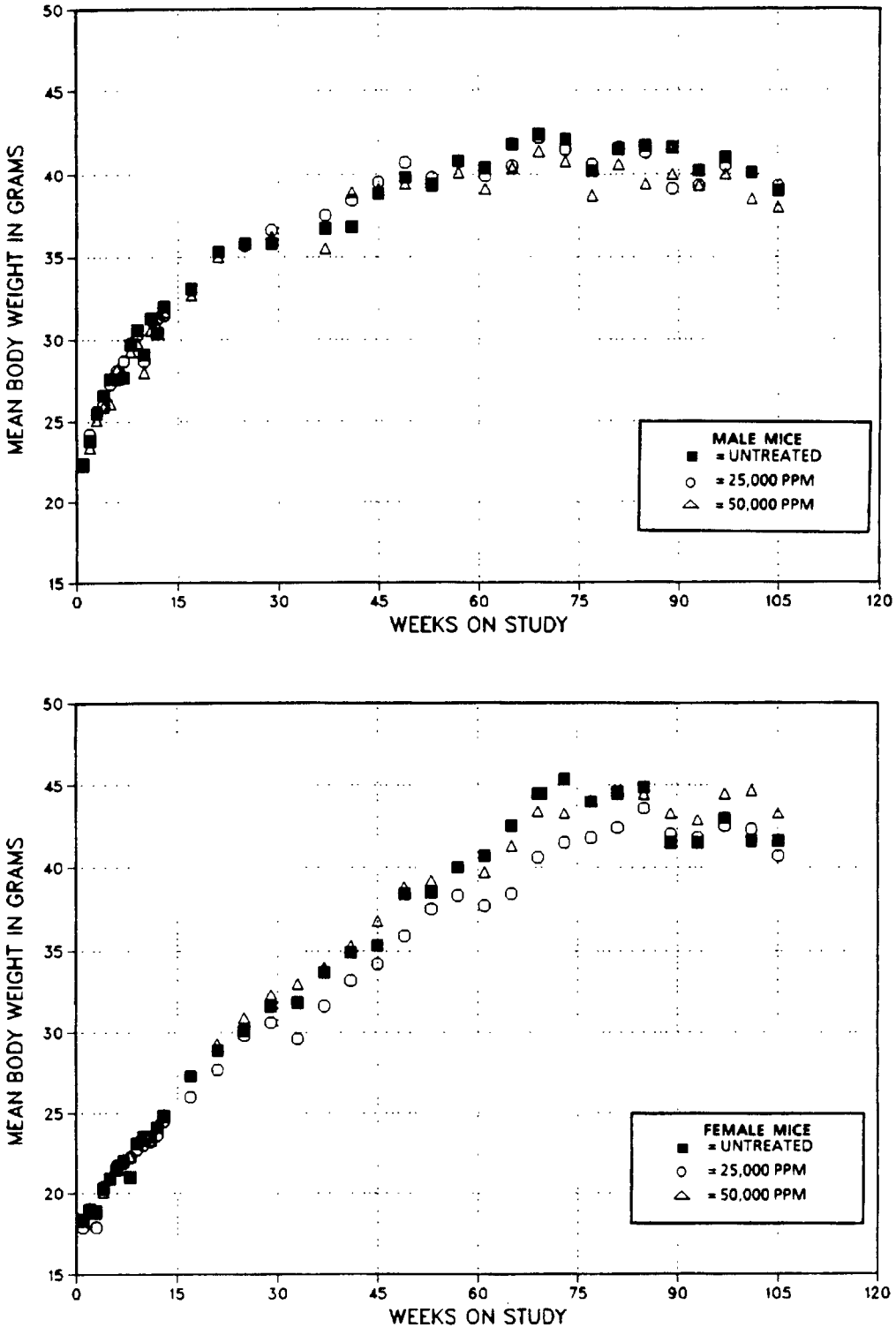

FIGURE 8. GROWTH CURVES FOR MICE FED DIETS CONTAINING PETN, NF, FOR TWO YEARS

III. RESULTS: MICE

Survival

Estimates of the probabilities of survival for male and female mice fed diets containing PETN, NF, at the concentrations used in these studies and for controls are shown in Table 20 and in the Kaplan and Meier curves in Figure 9. The survival of the control group of male mice was significantly lower than that of both the low and high dose groups after day 715. No significant differences in survival were observed between any groups of female mice.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy decreases in the incidences of mice with neoplastic lesions of the subcutaneous tissue and liver. At no site was a significantly increased incidence of neoplasms observed in dosed mice.

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.

Subcutaneous Tissue: Fibromas, fibrosarcomas, neurofibrosarcomas, or sarcomas (combined) in dosed male mice occurred with a significant negative trend; the incidences in the dosed groups were significantly lower than that in controls (Table 21). The incidence in the controls is nearly six times greater than the mean historical incidence (Table C4).

Liver: Hepatocellular adenomas or carcinomas (combined) in female mice occurred with a significant negative trend ($P < 0.05$); the incidences in the dosed groups were not significantly lower than that in the controls (control, 6/49; low dose, 2/50; high dose, 1/49).

TABLE 20. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF PETN, NF

	Control	25,000 ppm	50,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Natural deaths	8	6	3
Moribund kills	15	6	9
Animals missexed	1	0	0
Animals surviving until study termination	26	38	38
Survival P values (b)	0.014	0.025	0.023
FEMALE (a)			
Animals initially in study	50	50	50
Natural deaths	7	12	7
Moribund kills	5	8	5
Animals surviving until study termination	38	30	38
Survival P values (b)	0.960	0.127	0.870

(a) First day of termination period: male--729; female--730
(b) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

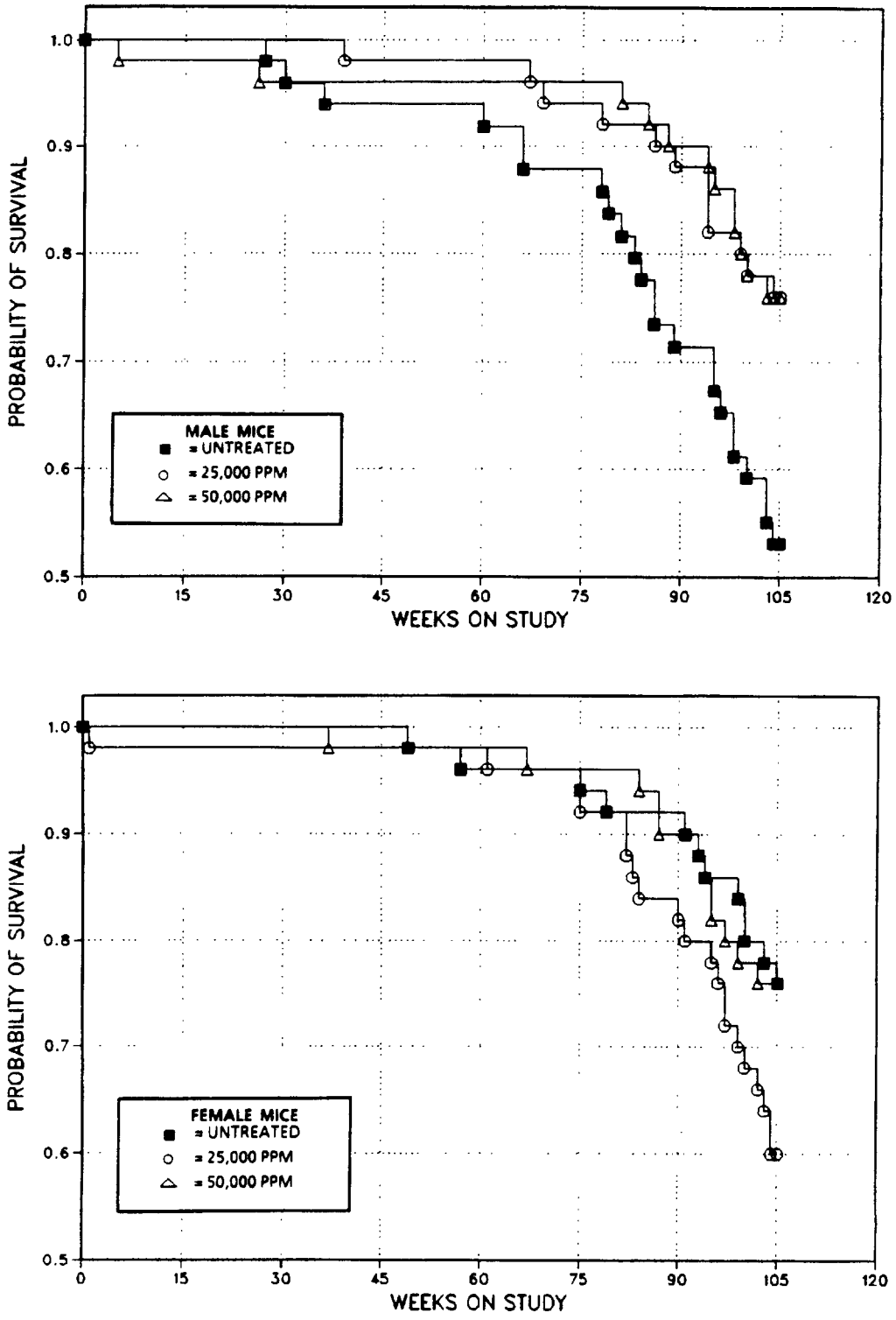

FIGURE 9. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING PETN, NF, FOR TWO YEARS

TABLE 21 SUBCUTANEOUS TISSUE TUMORS IN MALE MICE IN THE TWO YEAR FEED STUDY OF PETN, NF (a)

	Control	25,000 ppm (b)	50,000 ppm (b)
Fibroma			
Overall Rates	5/49 (10%)	2/50 (4%)	1/50 (2%)
Adjusted Rates	18 0%	5 3%	2 6%
Terminal Rates	4/26 (15%)	2/38 (5%)	1/38 (3%)
Day of First Observation	682	729	729
Life Table Tests	P=0 021N	P=0 098N	P=0 041N
Logistic Regression Tests	P=0 027N	P=0 125N	P=0 053N
Fibrosarcoma			
Overall Rates	14/49 (29%)	2/50 (4%)	8/50 (16%)
Adjusted Rates	35 8%	4 9%	17 8%
Terminal Rates	4/26 (15%)	0/38 (0%)	3/38 (8%)
Day of First Observation	456	656	564
Life Table Tests	P=0 026N	P<0 001N	P=0 042N
Logistic Regression Tests	P=0 077N	P=0 002N	P=0 121N
Fibroma or Fibrosarcoma			
Overall Rates	19/49 (39%)	4/50 (8%)	9/50 (18%)
Adjusted Rates	49 2%	9 9%	20 1%
Terminal Rates	8/26 (31%)	2/38 (5%)	4/38 (11%)
Day of First Observation	456	656	564
Life Table Tests	P=0 002N	P<0 001N	P=0 005N
Logistic Regression Tests	P=0 009N	P<0 001N	P=0 019N
Sarcoma			
Overall Rates	2/49 (4%)	2/50 (4%)	0/50 (0%)
Neurofibrosarcoma			
Overall Rates	0/49 (0%)	1/50 (2%)	0/50 (0%)
Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates	15/49 (31%)	5/50 (10%)	8/50 (16%)
Adjusted Rates	37 5%	12 1%	17 8%
Terminal Rates	4/26 (15%)	2/38 (5%)	3/38 (8%)
Day of First Observation	456	656	564
Life Table Tests	P=0 016N	P=0 004N	P=0 027N
Logistic Regression Tests	P=0 053N	P=0 013N	P=0 084N
Fibroma, Sarcoma, Fibrosarcoma, or Neurofibrosarcoma (c)			
Overall Rates	20/49 (41%)	7/50 (14%)	9/50 (18%)
Adjusted Rates	50 5%	17 0%	20 1%
Terminal Rates	8/26 (31%)	4/38 (11%)	4/38 (11%)
Day of First Observation	456	656	564
Life Table Tests	P=0 001N	P<0 001N	P=0 003N
Logistic Regression Tests	P=0 006N	P=0 002N	P=0 012N

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table C3 (footnotes)
(b) The estimated dose in milligrams per kilograms per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F
(c) Historical incidence at study laboratory (mean ± SD) 45/746 (6% ± 5%), historical incidence in NTP studies 178/2,040 (9% ± 8%)

III. RESULTS: GENETIC TOXICOLOGY

PETN, NF, was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in a preincubation protocol with doses up to 10 mg/plate with or without Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Mortelmans et al., 1986; Table 22). PETN, NF, induced an increase in sister chromatid exchanges (SCEs) in cultured Chinese hamster ovary (CHO) cells over a dose range of 160-2,500 μg/ml in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 (Table 23). The level of increased SCEs did not appear to correlate with dose, and the chemical did not induce cell cycle delay. Precipitation of the chemical was observed at concentrations of 500 μg/ml and above, which may account for the fluctuation in the magnitude of the responses at higher concentrations. PETN, NF, did not induce chromosomal aberrations in CHO cells when tested over a similar dose range with and without S9 (Table 24).

TABLE 22. MUTAGENICITY OF PETN, NF, IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b) −S9 Trial 1	−S9 Trial 2	+S9 (hamster) Trial 1	+S9 (hamster) Trial 2	+S9 (rat) Trial 1	+S9 (rat) Trial 2
TA100	0	79 ± 10	140 ± 15	78 ± 38	185 ± 68	70 ± 25	118 ± 41
	100	75 ± 60	139 ± 81	84 ± 54	151 ± 61	76 ± 68	121 ± 112
	333	76 ± 75	136 ± 66	83 ± 43	146 ± 29	71 ± 85	110 ± 71
	1,000	81 ± 98	161 ± 15	85 ± 31	164 ± 38	85 ± 85	114 ± 17
	3,333	71 ± 28	123 ± 91	83 ± 46	161 ± 91	82 ± 50	121 ± 61
	(c) 10,000	73 ± 104	137 ± 88	89 ± 78	169 ± 27	91 ± 58	115 ± 105
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (d)		477 ± 27	505 ± 101	819 ± 989	2,617 ± 1066	499 ± 495	697 ± 674
TA1535	0	6 ± 15	16 ± 20	7 ± 12	8 ± 09	4 ± 06	9 ± 18
	100	8 ± 19	24 ± 26	12 ± 20	8 ± 15	5 ± 15	7 ± 12
	333	8 ± 38	26 ± 12	10 ± 18	9 ± 15	5 ± 17	8 ± 17
	1,000	7 ± 06	21 ± 09	7 ± 12	7 ± 26	6 ± 33	8 ± 13
	3,333	8 ± 13	20 ± 19	8 ± 07	8 ± 20	6 ± 03	9 ± 22
	(c) 10,000	11 ± 29	19 ± 27	10 ± 15	7 ± 26	7 ± 15	10 ± 35
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (d)		300 ± 62	523 ± 125	192 ± 215	597 ± 39	260 ± 154	265 ± 107
TA1537	0	5 ± 09	5 ± 06	3 ± 03	5 ± 06	2 ± 03	6 ± 03
	100	5 ± 21	7 ± 07	9 ± 23	5 ± 06	4 ± 06	6 ± 17
	333	3 ± 15	7 ± 03	5 ± 10	9 ± 23	4 ± 13	10 ± 17
	1 000	5 ± 21	5 ± 03	4 ± 09	6 ± 09	6 ± 12	8 ± 06
	3,333	6 ± 03	5 ± 00	5 ± 07	7 ± 09	6 ± 13	9 ± 15
	(c) 10,000	4 ± 15	5 ± 03	6 ± 26	7 ± 09	7 ± 12	7 ± 15
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (d)		123 ± 222	162 ± 711	62 ± 149	455 ± 412	124 ± 132	203 ± 234
TA98	0	13 ± 06	35 ± 40	18 ± 09	47 ± 12	17 ± 15	36 ± 47
	100	8 ± 07	33 ± 60	21 ± 50	37 ± 41	17 ± 18	37 ± 38
	333	6 ± 03	27 ± 58	20 ± 26	41 ± 39	14 ± 09	32 ± 23
	1,000	11 ± 23	29 ± 46	23 ± 19	32 ± 36	14 ± 25	36 ± 58
	3,333	14 ± 15	23 ± 31	17 ± 15	35 ± 44	14 ± 29	40 ± 19
	(c) 10,000	16 ± 35	28 ± 19	21 ± 27	35 ± 21	14 ± 12	43 ± 19
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (d)		848 ± 267	848 ± 373	415 ± 50	1,769 ± 569	216 ± 152	532 ± 345

(a) Study performed at SRI International The detailed protocol is presented by Haworth et al (1983), and the data are presented in 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) Precipitate on plate at highest dose

(d) Positive control, 2 aminoanthracene was used on all strains in the presence of S9 In the absence of metabolic activation, 4 nitro *o* phenylenediamine was used with TA98 sodium azide was used with TA100 and TA1535, and 9 aminoacridine was used with TA1537

TABLE 23. INDUCTION OF SISTER-CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY PETN, NF (a)

	Dose (µg/ml)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hours in BrdU	Relative SCEs/cell (percent) (b)
-S9 (c)- Summary Positive								
Dimethyl sulfoxide		50	1,050	437	0 42	8 7	26 0	
PETN, NF	160	50	1,047	567	0 54	11 3	26 0	129 9
	(e) 500	50	1,050	527	0 50	10 5	26 0	120 7
	1,600	50	1,047	525	0 50	10 5	26 0	120 7
	2,500	50	1,050	512	0 49	10 2	26 0	117 2
Mitomycin C	0 0005	50	1,051	577	0 55	11 5	26 0	132 2
	0 005	10	210	290	1 38	29 0	26 0	333 3
+S9 (d)								
Trial 1 Summary Positive								
Dimethyl sulfoxide		50	1,047	422	0 4	8 4	26 0	
PETN, NF	160	50	1,053	566	0 54	11 3	26 0	134 5
	(e) 500	50	1,045	543	0 52	10 9	26 0	129 8
	1,600	50	1,050	595	0 57	11 9	26 0	141 7
	2,500	50	1,048	587	0 56	11 7	26 0	139 3
Cyclophosphamide	0 15	50	1,046	534	0 51	10 7	26 0	127 4
	0 6	10	210	282	1 34	28 2	26 0	335 7
Trial 2 Summary Positive								
Dimethyl sulfoxide		50	1,049	425	0 41	8 5	26 0	
PETN, NF	160	50	1,050	528	0 50	10 6	26 0	124 7
	(e) 500	50	1,050	614	0 58	12 3	26 0	144 7
	1,600	50	1,050	551	0 52	11 0	26 0	129 4
	2,500	50	1,050	598	0 57	12 0	26 0	141 2
Cyclophosphamide	0 1	50	1,049	576	0 55	11 5	26 0	135 3
	0 6	10	210	247	1 18	24 7	26 0	290 6

(a) Study performed at Environmental Health Research and Testing, Inc SCE = sister chromatid exchange, BrdU = bromo deoxyuridine A detailed description of the SCE protocol is presented by Galloway et al (1985, 1987) Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as described in (c) and (d) below and cultured for sufficient time to reach second metaphase division Cells were then collected by mitotic shake off, fixed, air dried, and stained

(b) SCEs/cell of culture exposed to study chemical relative to those of culture exposed to solvent

(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C Then BrdU was added, and incubation was continued for 24 hours Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2 3 hours

(d) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C Cells were then washed, and medium containing BrdU was added Cells were incubated for a further 26 hours, with colcemid present for the final 2 3 hours S9 was from the liver of Aroclor 1254 induced male Sprague Dawley rats

(e) Precipitate formed at this and higher concentrations

TABLE 24. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY PETN, NF (a)

−S9 (b) Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	+S9 (c) Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Harvest time 12 5 hours					Harvest time 13 hours				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	200	3	0 02	1 5		200	2	0 01	1 0
PETN, NF					PETN, NF				
1,000	200	6	0 03	3 0	1,000	200	6	0 03	3 0
1,600	200	0	0 00	0 0	1,600	200	9	0 05	4 0
2,500	200	4	0 02	2 0	2,500	200	8	0 04	3 5
Mitomycin C					Cyclophosphamide				
0 0625	200	31	0 16	13 5	2 5	200	26	0 13	12 5
0 25	50	21	0 42	34 0	7 5	50	26	0 52	34 0
Summary Negative					Summary Negative				

(a) Study performed at Environmental Health Research and Testing Abs = aberrations A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al (1985, 1987) Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as indicated in (b) and (c) Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake off, fixed, and stained in 6% Giemsa

(b) In the absence of S9, cells were incubated with study compound or solvent for 8 10 hours at 37° C Cells were then washed, and fresh medium containing colcemid was added for an additional 2 3 hours followed by harvest

(c) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C Cells were then washed, medium was added, and incubation was continued for 8 10 hours Colcemid was added for the last 2 3 hours of incubation before harvest S9 was from the liver of Aroclor 1254 induced male Sprague Dawley rats

IV. DISCUSSION AND CONCLUSIONS

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Pentaerythritol tetranitrate (PETN) is an organic nitrate used in munitions and as a drug for the prevention of angina pectoris. Toxicity and carcinogenicity studies were carried out by incorporation of PETN into feed given to F344/N rats and B6C3F$_1$ mice of each sex. The PETN formulation used contained lactose as a stabilizing agent (PETN:lactose, 1:4) and thus was similar to formulations used therapeutically. The dietary concentrations used in these studies ranged up to 5% by weight of the PETN/lactose mixture; thus, the maximum PETN concentration was about 1%, and the maximum lactose concentration was 4%. Lactose is normally present in the NIH 07 diet at a concentration of about 1%.

PETN, NF, was found to be essentially nontoxic to both rats and mice in 14-day and in 13- or 14-week studies. No effects were seen on survival, clinical signs, or body weight gains, except for female rats, which showed weight gains of 83% and 82% those of controls during the 14-week study at dietary concentrations of 25,000 ppm and 50,000 ppm. No nonneoplastic lesions were attributed to PETN administration in the short-term studies, but a Zymbal gland adenoma was seen in one high dose female rat and a hepatocellular adenoma was observed in one high dose female mouse.

Although denitration is a recognized pathway for metabolism of PETN, urinary nitrite levels were not consistently increased at any dose in rats or mice. Comparable data on human urinary nitrite levels following PETN administration were not found in the literature. In other studies, denitration reactions of organic nitrates were shown to lead to increased nitrate and nitrite levels in the urine of rats (unspecified strain) administered 60 mg/kg ethylene glycol dinitrate (Litchfield, 1973), and somewhat increased nitrite levels were found in the blood of dogs administered oral doses of PETN at 5 mg/kg (von Oettingen et al., 1944). No increases in methemoglobin were noted in any group of rats or mice, suggesting the lack of significant blood nitrite levels during the studies or that the capacity of methemoglobin reductase was sufficient to prevent the accumulation of increased levels of methemoglobin in erythrocytes. No reports of increased methemoglobin in human users of PETN were found in the literature. The typical therapeutic doses for humans are in the range of 2-3 mg/kg per day (PDR, 1987). Top doses for rats and mice in the short-term studies ranged from 3 to 15 g/kg per day of the PETN/lactose mixture, or about 0.6-3 g/kg per day of PETN.

Since there was no evidence of toxicity of PETN, NF, in the 14-day and 13- or 14-week studies, doses for the 2-year studies were based on the maximum dietary concentrations recommended for 2-year studies (NCI, 1976). Thus, doses of 25,000 ppm and 50,000 ppm were chosen for male rats and male and female mice. Doses for female rats were set at 6,200 ppm and 12,500 ppm because higher doses resulted in a somewhat lower weight gain than for controls in the 14-week study, although final weights were within 7% of those of controls.

In the 2-year studies, body weights of high dose male rats were up to 9% lower than those of controls; body weights of female rats were similar to those of controls. Feed consumption by the dosed groups was 97%-103% that by the controls, and the estimated amounts of PETN, NF, consumed per day for low and high dose groups were 1,200 and 2,500 mg/kg for males and 400 and 830 mg/kg for females.

No compound-related clinical signs were observed. Survival of dosed male rats was somewhat greater than that of controls and was typical of that for current NTP 2-year studies. Survival of control male rats fell below that of the dosed groups after about week 90; no reasons for this were apparent. Survival of female rats was also typical and not affected by PETN, NF, administration.

No nonneoplastic lesions in rats were attributed to PETN, NF, administration. It is likely, therefore, that female rats could have been given doses of PETN, NF, as high as those administered to males, which were limited by the convention of not exceeding 5% in a dietary admixture.

Neoplasms of the Zymbal gland were observed in three low and two high dose male rats and in one low and three high dose female rats. The

incidences did not reach statistical significance when compared with the zero incidences in each control group. The incidences did exceed the mean historical incidences for each sex but were within the upper ranges previously seen in control groups (male: mean, 1%; range, 0%-8%; female: mean, 0.6%; range, 0%-6%). The incidences of hyperplasia did not suggest an increase in proliferative lesions in the Zymbal gland. Nonetheless, the occurrence of nine neoplasms in dosed rats compared with none in controls, coupled with the observation of a Zymbal gland tumor in a high dose female rat in the 13-week study, suggests a possible chemical-related effect.

Ashby and Tennant (1988) have reported that compounds found to induce neoplasms in the Zymbal gland of male and female rats (9 of 222 chemicals studied by the NCI/NTP) are all mutagens or show genotoxic activity. Seven (3-amino-9-ethylcarbazole hydrochloride, C.I. Basic Red 9 monohydrochloride, cupferron, 2,4-diaminoanisole sulfate, hydrazobenzene, 5-nitro-o-anisidine, 4,4'-thiodianiline) of these nine chemicals contain aromatic amino or aromatic nitro groups, and eight (the seven mentioned plus benzene) are carcinogenic for both rats and mice. A similar relationship with genotoxic activity was observed in the chemicals reported to cause Zymbal gland neoplasms recorded in the Carcinogenic Potency Database (Gold et al., 1984; L. Gold, personal communication to J. Bucher, NIEHS, 1988). These chemicals include the nonaromatic compounds acrylonitrile and vinyl chloride. In the current studies, PETN, NF, was found to be at most very weakly genotoxic, and a very marginal response was noted in Zymbal gland neoplasms in male and female rats.

Thyroid gland follicular cell adenomas or carcinomas occurred in three high dose female rats. The incidence is greater than the average historical incidence in NTP studies (1%, Table B4), but follicular cell hyperplasia and follicular cell tumors were not increased in male rats. For these reasons, this marginal increase is not considered related to PETN, NF.

In the 2-year studies with mice, mean body weights of dosed and control mice were similar, and no chemically related clinical signs were noted. Feed consumption by the dosed groups was 98%-100% that of the controls, and the estimated amounts of PETN, NF, consumed per day for low and high dose mice were 4,000 and 8,100 mg/kg for males and 5,100 and 9,700 mg/kg for females. Survival of dosed male mice was significantly greater than that of controls, and survival of dosed female mice was similar to that of controls.

No increases in neoplastic or nonneoplastic lesions were observed in dosed mice. Combined tumors of the subcutaneous tissues occurred with a negative trend in male mice, and the incidences in the low and high dose groups were significantly lower than that in the controls. Subcutaneous tumors occurred in the control group at a rate nearly six times that customarily seen in historical control male mice (Tables C3 and C4). The reasons for this are not clear; the presence of these masses could account for the higher number of animals killed in a moribund condition in this group compared with the number in the dosed groups, and this may be the primary reason for the reduced survival of the control group.

No reports of long-term studies with other organic nitrates were found in the literature. Studies of many chemicals containing aromatic nitro groups have resulted in positive indications of carcinogenicity in animals (Haseman et al., 1987; IARC, 1987), and alkyl nitroso compounds, including nitrosamines and nitrosoamides, are among the most widely recognized and studied classes of chemical carcinogens (Weisburger and Williams, 1980). Aromatic nitro compounds are usually mutagenic in Salmonella unless the reactivity of the nitro group is reduced by steric hindrance, and certain alkyl nitrosamines or aryl nitroso compounds are considered structural alerts for potential genotoxic activity (Ashby and Tennant, 1988). PETN, NF, was found to be negative in the Salmonella mutagenicity assay with or without metabolic activation and did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells. PETN, NF, produced a small increase in sister chromatid exchanges in CHO cells, but the response was not dose related. Thus, if the results of these studies with PETN, NF, apply to other organic nitrates, it would appear that these chemicals have a much lower potential for

IV. DISCUSSION AND CONCLUSIONS

genotoxic or carcinogenic activity than the alkyl nitrosamines or aryl nitroso compounds

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

Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** of PETN, NF, for male and female F344/N rats, based on a marginal increase in neoplasms of the Zymbal gland Female rats might have tolerated a higher dose There was *no evidence of carcinogenic activity* of PETN, NF, for male or female $B6C3F_1$ mice fed diets containing 25,000 or 50,000 ppm for 2 years No nonneoplastic lesions were attributed to PETN, NF, administration

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

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF PETN, NF

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

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine large, cecum	(46)	*(50)	(47)
Leukemia mononuclear		1 (2%)	
Intestine large, colon	(47)	*(50)	(48)
Leukemia mononuclear	1 (2%)		
Intestine small, duodenum	(48)	*(50)	(46)
Leukemia mononuclear		1 (2%)	
Intestine small, ileum	(47)	*(50)	(47)
Leukemia mononuclear	1 (2%)		
Intestine small, jejunum	(45)	*(50)	(46)
Leukemia mononuclear	1 (2%)		
Liver	(49)	(50)	(50)
Hepatocellular carcinoma	1 (2%)		
Leukemia mononuclear	29 (59%)	27 (54%)	20 (40%)
Neoplastic nodule	2 (4%)		1 (2%)
Mesentery	*(50)	*(50)	*(50)
Leukemia mononuclear		2 (4%)	
Mesothelioma malignant	1 (2%)		
Pancreas	(49)	(49)	(48)
Leukemia mononuclear	4 (8%)	4 (8%)	3 (6%)
Mesothelioma malignant	1 (2%)		
Mixed tumor benign	1 (2%)		
Acinus, adenoma	1 (2%)	4 (8%)	3 (6%)
Acinus, adenoma, multiple		1 (2%)	
Salivary glands	(48)	*(50)	(49)
Leukemia mononuclear	1 (2%)		
Stomach	(49)	*(50)	(49)
Leukemia mononuclear	1 (2%)		2 (4%)
Stomach, forestomach	(49)	*(50)	(49)
Leukemia mononuclear			1 (2%)
Stomach, glandular	(48)	*(50)	(47)
Leukemia mononuclear	1 (2%)	1 (2%)	1 (2%)
Tongue	*(50)	*(50)	*(50)
Papilloma squamous	2 (4%)	1 (2%)	
CARDIOVASCULAR SYSTEM			
Heart	(49)	*(50)	(50)
Leukemia mononuclear	11 (22%)	7 (14%)	8 (16%)
ENDOCRINE SYSTEM			
Adrenal gland	(49)	*(50)	(48)
Leukemia mononuclear	1 (2%)		1 (2%)
Adrenal gland, cortex	(49)	*(50)	(48)
Leukemia mononuclear	7 (14%)	6 (12%)	5 (10%)
Adrenal gland, medulla	(49)	*(50)	(48)
Leukemia mononuclear	7 (14%)	6 (12%)	5 (10%)
Pheochromocytoma malignant		2 (4%)	3 (6%)
Pheochromocytoma benign	12 (24%)	8 (16%)	11 (23%)
Bilateral, pheochromocytoma benign	7 (14%)		4 (8%)
Islets, pancreatic	(49)	*(50)	(47)
Adenoma	6 (12%)	2 (4%)	1 (2%)
Adenoma, multiple		1 (2%)	
Carcinoma			1 (2%)

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

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
Pituitary gland	(49)	*(50)	(50)
Leukemia mononuclear	5 (10%)	4 (8%)	1 (2%)
Pars distalis, adenoma	13 (27%)	11 (22%)	11 (22%)
Pars distalis, adenoma, multiple		1 (2%)	1 (2%)
Thyroid gland	(49)	*(50)	(50)
Leukemia mononuclear	1 (2%)	1 (2%)	1 (2%)
C-cell, adenoma	5 (10%)	2 (4%)	7 (14%)
C-cell, carcinoma			2 (4%)
Follicular cell, adenoma		1 (2%)	
Follicular cell, carcinoma	1 (2%)	1 (2%)	
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Epididymis	(48)	*(50)	(50)
Mesothelioma malignant	1 (2%)		
Preputial gland	(46)	*(50)	(50)
Adenoma	11 (24%)	10 (20%)	13 (26%)
Carcinoma	3 (7%)	1 (2%)	1 (2%)
Bilateral, adenoma		1 (2%)	
Bilateral, carcinoma		1 (2%)	
Prostate	(48)	*(50)	(50)
Adenocarcinoma			1 (2%)
Leukemia mononuclear	3 (6%)	1 (2%)	1 (2%)
Seminal vesicle	(49)	*(50)	(50)
Leukemia mononuclear	3 (6%)	1 (2%)	1 (2%)
Testes	(49)	*(50)	(50)
Leukemia mononuclear		1 (2%)	
Bilateral, interstitial cell, adenoma	39 (80%)	36 (72%)	42 (84%)
Interstitial cell, adenoma	8 (16%)	8 (16%)	7 (14%)
Tunic, mesothelioma malignant	1 (2%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	*(50)	(50)
Leukemia mononuclear	4 (8%)	1 (2%)	1 (2%)
Lymph node	(48)	*(50)	(50)
Axillary, leukemia mononuclear	1 (2%)		
Bronchial, leukemia mononuclear	1 (2%)		
Iliac, leukemia mononuclear		1 (2%)	
Lumbar, leukemia mononuclear	1 (2%)	3 (6%)	1 (2%)
Mediastinal, leukemia mononuclear	6 (13%)	11 (22%)	7 (14%)
Pancreatic, leukemia mononuclear	4 (8%)	6 (12%)	2 (4%)
Renal, leukemia mononuclear	1 (2%)	2 (4%)	1 (2%)
Lymph node, mandibular	(46)	*(50)	(49)
Leukemia mononuclear	9 (20%)	5 (10%)	7 (14%)
Liposarcoma, metastatic, skin	1 (2%)		
Lymph node, mesenteric	(47)	*(50)	(48)
Leukemia mononuclear	9 (19%)	6 (12%)	8 (17%)
Spleen	(48)	*(50)	(50)
Leukemia mononuclear	28 (58%)	25 (50%)	20 (40%)
Thymus	(36)	*(50)	(45)
Leukemia mononuclear	5 (14%)	7 (14%)	6 (13%)

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

	Untreated Control	Low Dose	High Dose
INTEGUMENTARY SYSTEM			
Mammary gland	(37)	*(50)	(38)
Adenocarcinoma	1 (3%)		1 (3%)
Fibroadenoma	3 (8%)	1 (2%)	1 (3%)
Fibroadenoma, multiple	1 (3%)		
Skin	(48)	*(50)	(50)
Basal cell adenoma			1 (2%)
Keratoacanthoma	3 (6%)		1 (2%)
Papilloma squamous	1 (2%)	1 (2%)	1 (2%)
Squamous cell carcinoma		1 (2%)	
Scrotal, fibroma		1 (2%)	
Subcutaneous tissue, fibroma	4 (8%)	2 (4%)	1 (2%)
Subcutaneous tissue, fibroma, multiple			1 (2%)
Subcutaneous tissue, fibrosarcoma			2 (4%)
Subcutaneous tissue, leukemia mononuclear			1 (2%)
Subcutaneous tissue, liposarcoma	1 (2%)		
Subcutaneous tissue, sarcoma	1 (2%)		
Tail, keratoacanthoma	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
None			
NERVOUS SYSTEM			
Brain	(49)	(48)	(50)
Astrocytoma malignant			2 (4%)
Leukemia mononuclear	2 (4%)	4 (8%)	
Cerebellum, carcinoma, metastatic, Zymbal gland			1 (2%)
RESPIRATORY SYSTEM			
Lung	(49)	*(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	3 (6%)	4 (8%)
Alveolar/bronchiolar carcinoma	1 (2%)		
Carcinoma, metastatic, Zymbal gland		1 (2%)	
Carcinoma adenosquamous	1 (2%)		
Leukemia mononuclear	15 (31%)	12 (24%)	10 (20%)
Liposarcoma, metastatic, skin	1 (2%)		
Nose	(47)	*(50)	(50)
Chondroma			1 (2%)
SPECIAL SENSES SYSTEM			
Ear	*(50)	*(50)	*(50)
Sarcoma		1 (2%)	
Eye	*(50)	*(50)	*(50)
Leukemia mononuclear		1 (2%)	
Zymbal gland	*(50)	*(50)	*(50)
Adenoma		1 (2%)	
Carcinoma		2 (4%)	2 (4%)
URINARY SYSTEM			
Kidney	(49)	(50)	(50)
Leukemia mononuclear	6 (12%)	18 (36%)	4 (8%)
Renal tubule, adenoma			1 (2%)
Renal tubule, carcinoma			1 (2%)
Urinary bladder	(46)	*(50)	(48)
Adenocarcinoma, metastatic, prostate			1 (2%)
Leukemia mononuclear	2 (4%)	1 (2%)	2 (4%)

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

	Untreated Control	Low Dose	High Dose
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Mesothelioma malignant	1 (2%)	1 (2%)	1 (2%)
Leukemia mononuclear	29 (58%)	27 (54%)	20 (40%)
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Terminal sacrifice	22	28	29
Moribund	20	17	16
Dead	8	5	5
TUMOR SUMMARY			
Total animals with primary neoplasms **	49	49	50
Total primary neoplasms	163	133	151
Total animals with benign neoplasms	49	48	50
Total benign neoplasms	123	96	114
Total animals with malignant neoplasms	33	32	31
Total malignant neoplasms	40	37	37
Total animals with secondary neoplasms ***	1	1	2
Total secondary neoplasms	2	1	2

* Number of animals receiving complete necropsy examination, all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** 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 PETN, NF: UNTREATED CONTROL

WEEKS ON STUDY	056	069	077	081	083	085	088	090	090	091	092	092	092	092	092	093	095	095	095	096	098	100	101	102	102
CARCASS ID	0655	0555	0855	0844	0544	0255	0955	0455	0833	0444	0533	0433	0224	0755	0944	0933	0155	0344	0355	0155	0333	0744	0332	0522	0732
ALIMENTARY SYSTEM																									
Esophagus	M	+	+	M	+	+	+	+	–	+	M	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Intestine large	M	+	+	+	A	+	A	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	M	+	A	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	M	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Intestine large, rectum	M	+	A	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	M	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Intestine small, jejunum	M	+	+	+	A	+	A	+	A	+	+	+	+	+	+	+	+	+	+	–	+	M	+	+	+
Leukemia mononuclear																									
Liver	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Hepatocellular carcinoma																									
Leukemia mononuclear					X	X	X	X	X	X			X	X	X		X	X	X	X	X	X			X
Neoplastic nodule																									X
Mesentery																						+			
Mesothelioma malignant																									
Pancreas	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X	X		X															
Mesothelioma malignant																									
Mixed tumor benign									X																
Acinus, adenoma																									
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X																	
Stomach	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X																	
Stomach, forestomach	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	M	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Tongue									+																
Papilloma squamous									X																
CARDIOVASCULAR SYSTEM																									
Heart	M	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+
Leukemia mononuclear								X	X	X				X					X		X				X
ENDOCRINE SYSTEM																									
Adrenal gland	M	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X																
Adrenal gland, cortex	M	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X		X				X					X						X
Adrenal gland, medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X		X				X					X						X
Pheochromocytoma benign		X					X														X				
Bilateral, pheochromocytoma benign																X	X								X
Islets, pancreatic	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X						
Parathyroid gland	M	M	M	M	+	+	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X		X									X						X
Pars distalis, adenoma			X		X	X					X				X										
Thyroid gland	M	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Leukemia mononuclear																									
C cell, adenoma						X																X			
Follicular cell, carcinoma														X											
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Coagulating gland		+	+																						
Epididymis	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	–	+	+
Mesothelioma malignant																									
Preputial gland	M	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	–	–	+	+	+
Adenoma				X			X						X		X	X		X						X	
Carcinoma		X										X								X					
Prostate	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+
Leukemia mononuclear								X																	X
Seminal vesicle	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X		X															
Testes	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X	X				X		X	X	X	X	X	X	X	X		X		X	X	X	X	X	
Interstitial cell, adenoma				X		X		X									X								X
Tunic, mesothelioma malignant																									

+ Tissue examined microscopically
Not examined
I Insufficient tissue

M Missing
A Autolysis precludes examination
X Incidence of listed morphology

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

WEEKS ON STUDY	102	103	105	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	TOTAL
CARCASS ID	082	104	072	011	112	133	144	021	022	041	042	061	062	063	064	071	081	091	101	023	031	051	092	102	103	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leukemia mononuclear		X																								1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leukemia mononuclear		X																								1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear		X																								1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma				X																						1
Leukemia mononuclear		X	X					X	X	X	X	X	X	X		X	X							X	X	29
Neoplastic nodule	X																									2
Mesentery				+											+		−									4
Mesothelioma malignant				X																						1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	49
Leukemia mononuclear		X																								4
Mesothelioma malignant				X																						1
Mixed tumor benign																										1
Acinus, adenoma																		X								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Leukemia mononuclear																										1
Stomach	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	49
Leukemia mononuclear																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear		X																								1
Tongue																+										2
Papilloma squamous																X										2
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear		X	X								X													X		11
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear			X																					X		7
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear			X																					X		7
Pheochromocytoma benign	X		X				X	X		X					X		X						X		X	12
Bilateral, pheochromocytoma benign				X												X		X						X		7
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma										X		X							X	X				X		6
Parathyroid gland	+	M	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	M	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear		X																								5
Pars distalis, adenoma						X								X	X		X	X				X	X		X	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear		X																								1
C cell, adenoma						X							X							X						5
Follicular cell, carcinoma																										1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Coagulating gland		+				+			+						+		+							+	+	9
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesothelioma malignant				X																						1
Preputial gland	+	+	+	+	+	+	+	+	+		+	+	+	−	+	M	+	+	+	+	−	+	+	+	+	46
Adenoma	X		X																X				X			11
Carcinoma																										3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	18
Leukemia mononuclear										X																3
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	49
Leukemia mononuclear		X																								3
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	49
Bilateral, interstitial cell, adenoma	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	39
Interstitial cell, adenoma						X								X								X				8
Tunic, mesothelioma malignant				X																						1

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

WEEKS ON STUDY	059	069	077	081	083	085	088	090	090	091	092	092	092	092	092	093	095	095	095	096	098	100	101	102
CARCASS ID	065	055	085	084	054	025	095	045	083	044	053	043	024	075	094	093	015	034	035	055	033	074	032	073
HEMATOPOIETIC SYSTEM																								
Bone marrow	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X						X										X
Lymph node	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Axillary, leukemia mononuclear								X																
Bronchial, leukemia mononuclear																								
Lumbar, leukemia mononuclear														X										
Mediastinal, leukemia mononuclear								X	X					X				X			X			X
Pancreatic, leukemia mononuclear									X					X										
Renal, leukemia mononuclear														X										
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X	X				X			X		X					X
Liposarcoma, metastatic, skin				X																				
Lymph node, mesenteric	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X	X				X			X		X					X
Spleen	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X		X	X	X			X	X	X		X	X	X	X	X	X		X
Thymus	M	+	M	+	+	+	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+		M	+
Leukemia mononuclear								X		X									X					X
INTEGUMENTARY SYSTEM																								
Mammary gland	M	+	+	+	M	+	M	M	+	M	+	+	+	+	+	+	+	M	+	+	+	M	M	+	+
Adenocarcinoma																								
Fibroadenoma			X																					
Fibroadenoma, multiple																								
Skin	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																				X		X		
Papilloma squamous																								
Subcutaneous tissue, fibroma																		X			X	X	X	
Subcutaneous tissue, liposarcoma				X																				
Subcutaneous tissue, sarcoma																			X					
Tail, keratoacanthoma																						X		
MUSCULOSKELETAL SYSTEM																								
Bone	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																								
Brain	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X		X														
RESPIRATORY SYSTEM																								
Lung	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X																			
Alveolar/bronchiolar carcinoma																								
Carcinoma adenosquamous																				X				
Leukemia mononuclear							X	X	X	X				X	X		X		X		X	X		X
Liposarcoma, metastatic, skin				X																				
Nose	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																								
Eye					+							+		+										
Zymoal gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																								
Kidney	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X	X														X
Urinary bladder	M	+	+	+	+	+	M	+	+	+	+	+	–	+	+	+	+	+	M	+	M	+	+	+
Leukemia mononuclear								X		X														

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

WEEKS ON STUDY	102	103	105	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	
CARCASS ID	0821	1044	0722	0111	0112	0113	0114	0221	0222	0441	0442	0611	0622	0633	0644	0711	0811	0911	1011	0231	0331	0551	0992	1022	1033	TOTAL TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear		X																								4
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+		48
Axillary, leukemia mononuclear																										1
Bronchial, leukemia mononuclear		X																								1
Lumbar, leukemia mononuclear																										1
Mediastinal, leukemia mononuclear																										6
Pancreatic, leukemia mononuclear		X									X															4
Renal, leukemia mononuclear																										1
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	M	+	+	46
Leukemia mononuclear		X									X															9
Liposarcoma, metastatic, skin																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	47
Leukemia mononuclear		X									X															9
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear		X	X					X	X	X	X	X	X	X		X	X							X	X	28
Thymus	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	M	M	M	M	+	+	+	M	36
Leukemia mononuclear		X																								5
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	M	+	M	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	37
Adenocarcinoma				X																						1
Fibroadenoma										X											X					3
Fibroadenoma, multiple			X																							1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Keratoacanthoma		X																								3
Papilloma squamous				X																						1
Subcutaneous tissue, fibroma																										4
Subcutaneous tissue, liposarcoma																										1
Subcutaneous tissue, sarcoma																										1
Tail, keratoacanthoma																										1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										2
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma																			X	X						3
Alveolar/bronchiolar carcinoma													X													1
Carcinoma adenosquamous																										1
Leukemia mononuclear		X	X								X													X		15
Liposarcoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SPECIAL SENSES SYSTEM																										
Eye				+										+												5
Zymbal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear		X									X															6
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Leukemia mononuclear																										2

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

WEEKS ON STUDY	047	072	081	089	090	092	093	092	093	094	096	097	097	100	100	102	103	103	104	105	105	106	106	106
CARCASS ID	1855	1955	2055	1555	1155	1545	1255	1355	1114	1633	1775	1774	1665	2044	1344	1733	1533	1333	1934	1453	1552	1444	1111	1123	1133
ALIMENTARY SYSTEM																								
Esophagus	+	M	+	+	+	+	+	+	+	I	+			+		+						+		
Intestine large	+	+	+	+	+	+	+	+	+	+														
Intestine large, cecum	A	+	+	+	+	M	+	+	+	+														
Leukemia mononuclear				X																				
Intestine large, colon	+	+	+	+	+	+	+	+	+	+														
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+														
Intestine small	A	+	+	+	+	+	+	+	+	+														
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+														
Leukemia mononuclear				X																				
Intestine small, ileum	A	+	A	M	+	+	+	+	+	+														
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+														
Liver	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X	X	X			X	X			X	X	X		X	X	X	X	X		
Mesentery														+										
Leukemia mononuclear														X										
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X								X	X							X		
Acinus, adenoma																								
Acinus, adenoma, multiple																								
Salivary glands	+	+	+	+	+	+	+	+	+	+														
Stomach	+	+	+	+	+	+	+	+	+	+								+				+		
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+								+						
Stomach, glandular	A	+	+	+	+	+	+	+	+	+								+				+		
Leukemia mononuclear						X																		
Tongue																								
Papilloma squamous																								
CARDIOVASCULAR SYSTEM																								
Heart	+	+	+	+	+	+	+	+	+	+								+						
Leukemia mononuclear		X		X	X	X												X						
ENDOCRINE SYSTEM																								
Adrenal gland	+	+	+	+	+	+	+	+	-	+	+			+										+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	-	+	+			+										+
Leukemia mononuclear		X		X	X	X								X										
Adrenal gland, medulla	+	+	+	+	+	+	+	+	-	+	+			+										+
Leukemia mononuclear		X		X	X	X								X										
Pheochromocytoma malignant																								
Pheochromocytoma benign									X	X	X			X										X
Islets, pancreatic	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Adenoma, multiple																X								
Parathyroid gland	+	M	M	+	+	M	+	M	M	M	+			+		+						+		
Pituitary gland	+	+	+	+	+	+	+	+	+	+				+					+	+				
Leukemia mononuclear		X		X										X				X						
Pars distalis, adenoma		X			X				X									X	X					
Pars distalis, adenoma, multiple																								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+			+		+						+		
Leukemia mononuclear						X																		
C cell, adenoma										X	X													
Follicular cell, adenoma																						X		
Follicular cell, carcinoma																X								
GENERAL BODY SYSTEM																								
None																								
GENITAL SYSTEM																								
Epididymis	+	+	+	+	+	+	+	+	+	+				+								+	+	
Preputial gland	M	+	+	+	+	+	+	+	+	+	+	+			+	+					+	+		
Adenoma					X		X	X	X		X				X									
Carcinoma																								
Bilateral, adenoma																								
Bilateral, carcinoma												X												
Prostate	+	+	+	+	+	+	+	+	+	+								+				+		
Leukemia mononuclear																		X						
Seminal vesicle	+	+	+	+	+	+	+	+	+	+				+			+						+	
Leukemia mononuclear														X										
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X																				
Bilateral, interstitial cell, adenoma			X			X	X	X			X					X	X	X	X	X		X	X	X	X
Interstitial cell, adenoma									X	X		X	X	X	X						X				
Tunic, mesothelioma malignant																								

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

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	TOTAL
CARCASS ID	121	122	123	124	131	132	142	143	141	151	161	162	164	171	172	181	182	183	184	191	192	193	201	202	203	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus															+											14
Intestine large																										10
Intestine large, cecum																										8
Leukemia mononuclear																										1
Intestine large, colon																										10
Intestine large, rectum																										9
Intestine small																										9
Intestine small, duodenum																										9
Leukemia mononuclear																										1
Intestine small, ileum																										7
Intestine small, jejunum																										9
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X	X	X					X	X			X		X	X	X							X	X	27
Mesentery			+																							2
Leukemia mononuclear			X																							2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										4
Acinus, adenoma				X			X			X	X															4
Acinus, adenoma, multiple																							X			1
Salivary glands		+																+	+							13
Stomach																+	+	+		+		+		+		18
Stomach, forestomach																	+					+		+		14
Stomach, glandular																+		+		+		+		+		15
Leukemia mononuclear																										1
Tongue								+																		1
Papilloma squamous								X																		1
CARDIOVASCULAR SYSTEM																										
Heart			+													+					+					14
Leukemia mononuclear			X													X										7
ENDOCRINE SYSTEM																										
Adrenal gland	+		+		+	+									+											18
Adrenal gland, cortex	+		+		+	+									+											18
Leukemia mononuclear			X																							6
Adrenal gland, medulla	+		+		+	+									+											18
Leukemia mononuclear			X																							6
Pheochromocytoma malignant	X				X																					2
Pheochromocytoma benign			X			X									X											8
Islets, pancreatic	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+			+	+	+	45
Adenoma			X								X															2
Adenoma, multiple																										1
Parathyroid gland															+											9
Pituitary gland			+				+		+			+	+		+							+				20
Leukemia mononuclear																										4
Pars distalis, adenoma			X				X		X			X	X									X				11
Pars distalis, adenoma, multiple															X											1
Thyroid gland															+											15
Leukemia mononuclear																										1
C cell, adenoma																										2
Follicular cell, adenoma																										1
Follicular cell, carcinoma																										1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis																										13
Preputial gland						+		+				+	+		+		+									22
Adenoma								X					X		X					X						10
Carcinoma																	X									1
Bilateral, adenoma						X																				1
Bilateral, carcinoma																										1
Prostate			+																							13
Leukemia mononuclear																										1
Seminal vesicle			+	+	+			+			+					+	+					+	+		+	23
Leukemia mononuclear																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	36
Interstitial cell, adenoma																			X							8
Tunic, mesothelioma malignant	X																									1

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

WEEKS ON STUDY	047	072	081	081	089	090	092	092	092	093	094	096	097	097	100	100	102	103	103	103	104	105	105	106	106	106
CARCASS ID	185	195	205	155	115	154	125	135	114	163	175	174	165	204	134	173	153	133	194	145	152	144	111	112	113
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+																
Leukemia mononuclear				X																						
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+							
Iliac, leukemia mononuclear																										
Lumbar, leukemia mononuclear														X												
Mediastinal, leukemia mononuclear					X	X				X	X			X				X	X							
Pancreatic, leukemia mononuclear					X									X				X	X							
Renal, leukemia mononuclear														X												
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+		+		+	+			+	+							
Leukemia mononuclear						X								X				X	X							
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+				+				+								
Leukemia mononuclear					X	X								X				X								
Spleen	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+				-
Leukemia mononuclear		X		X	X	X	X			X	X			X	X	X		X	X	X	X	X				
Thymus	+	+	+	+	M	+	M	+	M	+	+			+				+	+							+
Leukemia mononuclear				X		X					X			X				X	X							
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	M	M	M	+	+	+	+	+																
Fibroadenoma																										
Skin	+	+	+	+	+	+	+	+	+	+							+									
Papilloma squamous																										
Squamous cell carcinoma										X																
Scrotal, fibroma																										
Subcutaneous tissue, fibroma									X																	
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+																
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+		
Leukemia mononuclear		X		X		X																	X			
Spinal cord			+																							
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+		+			+		-				+	-		
Alveolar/bronchiolar adenoma																	X									
Carcinoma, metastatic, Zymbal gland			X																							
Leukemia mononuclear		X		X	X	X				X	X			X					X							
Nose	+	+	+	+	+	+	+	-	+	+																
Trachea	+	+	+	+	+	+	+	-	+		+	+		+			+		+				+	+		
SPECIAL SENSES SYSTEM																										
Ear																										
Sarcoma																										
Eye			+						+			+	+					+				+		+		
Leukemia mononuclear																		X								
Harderian gland			+									+	+					+				+		+		
Zymbal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	
Adenoma																										
Carcinoma			X					X																		
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Leukemia mononuclear		X		X		X				X	X			X	X	X		X	X	X						
Urinary bladder	+	+	A	+	-	+	+	+	+	+																
Leukemia mononuclear				X																						

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

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	
CARCASS ID	121	122	123	124	131	132	142	143	141	151	161	162	164	171	172	181	182	183	184	191	192	193	201	202	203	TOTAL TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow																										10
Leukemia mononuclear																										1
Lymph node		+	+	+		+	+				+				+	+	+	+	+		+			+		30
Iliac, leukemia mononuclear			X																							1
Lumbar, leukemia mononuclear		X	X																							3
Mediastinal, leukemia mononuclear			X	X												X	X									11
Pancreatic, leukemia mononuclear																X	X									6
Renal, leukemia mononuclear		X																								2
Lymph node, mandibular		+					-											+	+					+		20
Leukemia mononuclear		X																								5
Lymph node, mesenteric		+	+												+											15
Leukemia mononuclear		X	X																							6
Spleen	+	+	+	+	+	+			+		+			+	+	+	+		+					+	+	36
Leukemia mononuclear	X	X	X	X					X						X	X	X							X	X	25
Thymus			+			+	+										+				+					17
Leukemia mononuclear			X																							7
INTEGUMENTARY SYSTEM																										
Mammary gland									+						+											9
Fibroadenoma															X											1
Skin	+						+	+	+				+		+				+							18
Papilloma squamous								X																		1
Squamous cell carcinoma																										1
Scrotal, fibroma													X													1
Subcutaneous tissue, fibroma							X																			2
MUSCULOSKELETAL SYSTEM																										
Bone																										10
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	48
Leukemia mononuclear																										4
Spinal cord																										1
RESPIRATORY SYSTEM																										
Lung		+	+	+			+	+			+						+				+	+	+		+	28
Alveolar/bronchiolar adenoma											X											X				3
Carcinoma, metastatic, Zymbal gland																										1
Leukemia mononuclear		X	X	X													X									12
Nose																										10
Trachea		+	+	+			+	+			+						+				+	+	+		+	27
SPECIAL SENSES SYSTEM																										
Ear												+														1
Sarcoma												X														1
Eye									+													+				9
Leukemia mononuclear																										1
Harderian gland																						+				7
Zymbal gland	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	45
Adenoma								X																		1
Carcinoma																										2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X	X	X											X	X	X									18
Urinary bladder					+																					10
Leukemia mononuclear																										1

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

WEEKS ON STUDY	080	081	085	089	092	093	096	097	098	099	101	101	102	102	103	103	104	104	105	105	105	105	105	105
CARCASS ID	215	225	242	214	255	285	245	224	223	235	254	244	265	243	234	305	275	222	274	273	284	211	212	213	221
ALIMENTARY SYSTEM																									
Esophagus	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	−	+	+
Intestine large	A	+	+	−	+	+	+	+	+	−	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	−	A	+	+	+	+	+	+	+	+	+	+	+	−
Intestine large, rectum	A	+	+	−	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	−
Intestine small	A	+	+	−	+	−	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	-	+	+	+	+	+	−	+	+	A	+	+	+	+	+	+	+	+	-	+	+	+
Intestine small, ileum	A	+	+	−	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	A	+	+	+	+	+
Liver	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X					X			X	X				X		X	X			
Neoplastic nodule																									
Mesentery	+																						+		
Pancreas	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																			X						
Acinus, adenoma							X																		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Stomach	A	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Leukemia mononuclear															X										
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Leukemia mononuclear												X													
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	−	+	+	+	+
Leukemia mononuclear												X													
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X					X			X	X				X						
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Leukemia mononuclear															X										
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	M
Leukemia mononuclear			X			X					X			X											
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	M
Leukemia mononuclear			X			X					X			X											
Pheochromocytoma malignant	X			X					X																
Pheochromocytoma benign			X																						
Bilateral, pheochromocytoma benign					X								X	X											
Islets, pancreatic	M	+	+	+	+	+	+	+	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma															X										
Parathyroid gland	M	+	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X													
Pars distalis, adenoma				X	X			X				X	X				X				X			X	
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
C cell, adenoma														X					X						
C cell, carcinoma					X																				X
GENERAL BODY SYSTEM																									
Tissue, NOS		+																							
GENITAL SYSTEM																									
Coagulating gland										+		+			+				+						
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X	X			X										X		X	X	X		X
Carcinoma										X															
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma		X																							
Leukemia mononuclear																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	−	+	+	+	X	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X													
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X				X	X	X	X	X		X		X	X		X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma	X			X	X			X			X		X			X									
Tunic, mesothelioma malignant									X																

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

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	
CARCASS ID	231	232	233	241	251	252	253	262	263	264	261	271	272	281	282	283	291	292	293	294	295	301	302	303	304	TOTAL TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X						X		X	X	X			X		X		X			X	X	X		X	20
Neoplastic nodule												X														1
Mesentery			+			+		+								+										8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear								X															X			3
Acinus, adenoma																		X							X	3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear								X																		2
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Stomach, glandular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leukemia mononuclear																										1
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear								X															X			8
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		48
Leukemia mononuclear																										1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		48
Leukemia mononuclear								X																		5
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		48
Leukemia mononuclear								X																		5
Pheochromocytoma malignant																										3
Pheochromocytoma benign	X					X						X	Y			X			X	X	X					11
Bilateral, pheochromocytoma benign																										4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma								X																		1
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Pars distalis, adenoma							X									X									X	11
Pars distalis, adenoma, multiple						X																				1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																							X			1
C cell, adenoma							X									X						X		X		7
C cell, carcinoma						X																				2
GENERAL BODY SYSTEM																										
Tissue, NOS																										1
GENITAL SYSTEM																										
Coagulating gland																		+							+	6
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X		X			X					X	X												13
Carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										1
Leukemia mononuclear																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	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	42
Interstitial cell, adenoma																										1
Tunic, mesothelioma malignant																										1

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

WEEKS ON STUDY	080	081	085	089	092	093	096	097	098	099	101	101	102	102	103	103	104	104	105	105	105	105	105	105
CARCASS ID	215	225	242	214	255	285	245	224	223	235	254	244	265	243	234	305	275	222	274	273	284	211	212	213	221
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X													
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Lumbar, leukemia mononuclear																									
Mediastinal, leukemia mononuclear			X										X	X				X							
Pancreatic, leukemia mononuclear																									
Renal, leukemia mononuclear																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	
Leukemia mononuclear			X			X						X		X	X										
Lymph node, mesenteric	+	+	+	+	+	+	+	+	M	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	
Leukemia mononuclear			X			X						X		X				X							
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Leukemia mononuclear			X			X						X		X	X			X							
Thymus	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	X	+	+	X	X	+	+	
Leukemia mononuclear						X						X		X				X					M		
INTEGUMENTARY SYSTEM																									
Mammary gland	M	M	+	M	M	M	M	+	+	M	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+
Adenocarcinoma			X																						
Fibroadenoma																	X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																									
Papilloma squamous																									
Subcutaneous tissue, fibroma									X																
Subcutaneous tissue, fibroma, multiple																									
Subcutaneous tissue, fibrosarcoma				X			X																		
Subcutaneous tissue, leukemia mononuclear																									
Tail, keratoacanthoma		X																							
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																		X		X					
Cerebellum, carcinoma, metastatic, Zymbal gland											X														
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X		X			X												
Leukemia mononuclear			X			X						X		X	X				X						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chondroma								X																	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Ear			+																						
Eye			+	+								+					+			+					
Harderian gland												+													
Zymbal gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	-	+	+	M	+	+
Carcinoma											X											X			
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X																			
Renal tubule, adenoma																									
Renal tubule, carcinoma																									
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, prostate		X																							
Leukemia mononuclear											X														

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

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	106	106	106	106	106	106	106	106	106	106	106	106	106	106	
CARCASS ID	231	232	233	241	251	252	253	262	263	264	261	271	272	281	282	283	291	292	293	294	295	301	302	303	304	TOTAL: TISSUES / TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lumbar, leukemia mononuclear									X																	1
Mediastinal, leukemia mononuclear									X													X	X			7
Pancreatic, leukemia mononuclear									X														X			2
Renal, leukemia mononuclear									X																	1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	49
Leukemia mononuclear									X														X			7
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear									X													X	X			8
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X						X		X	X	X			X		X		X			X	X	X		X	20
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	45
Leukemia mononuclear									X														X			6
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	M	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	−	+	+	+	+	+	38
Adenocarcinoma																										1
Fibroadenoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	50
Basal cell adenoma																										1
Keratoacanthoma					X																	X				1
Papilloma squamous																										1
Subcutaneous tissue, fibroma																						X				1
Subcutaneous tissue, fibroma, multiple							X																			1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, leukemia mononuclear																										2
Tail, keratoacanthoma																							X			1
MUSCULOSKELETAL SYSTEM																										1
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										2
Cerebellum, carcinoma, metastatic, Zymbal gland																										1
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X																								4
Leukemia mononuclear								X	X																	10
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	X	X			50
Chondroma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Ear										+																3
Eye											+									+						6
Harderian gland											+									+						4
Zymbal gland	+	+	+	+	+	+	+	+	M	M	+	M	M	+	+	+	+	+	M	+	+	+	+	+	M	41
Carcinoma																										2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X													X			4
Renal tubule, adenoma														X												1
Renal tubule, carcinoma															X											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma, metastatic, prostate									X																	1
Leukemia mononuclear																										2

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

	Control	25,000 ppm	50,000 ppm
Adrenal Medulla: Pheochromocytoma			
Overall Rates (a)	19/49 (39%)	(b) 8/18 (44%)	15/48 (31%)
Adjusted Rates (c)	61 3%		43.1%
Terminal Rates (d)	11/22 (50%)		9/27 (33%)
Day of First Observation	479		589
Life Table Test (e)			P=0.092N
Logistic Regression Test (e)			P=0.198N
Fisher Exact Test (e)			P=0.287N
Adrenal Medulla: Malignant Pheochromocytoma			
Overall Rates (a)	0/49 (0%)	(b) 2/18 (11%)	3/48 (6%)
Adjusted Rates (c)	0 0%		6 4%
Terminal Rates (d)	0/22 (0%)		0/27 (0%)
Day of First Observation			560
Life Table Test (e)			P=0.154
Logistic Regression Test (e)			P=0 032
Fisher Exact Test (e)			P=0.117
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	19/49 (39%)	(b) 10/18 (56%)	18/48 (38%)
Adjusted Rates (c)	61.3%		46.7%
Terminal Rates (d)	11/22 (50%)		9/27 (33%)
Day of First Observation	479		560
Life Table Test (e)			P=0.230N
Logistic Regression Test (e)			P=0.504N
Fisher Exact Test (e)			P=0 532N
Preputial Gland: Adenoma			
Overall Rates (a)	11/46 (24%)	(b) 11/22 (50%)	13/50 (26%)
Adjusted Rates (c)	32 6%		36.7%
Terminal Rates (d)	2/20 (10%)		8/29 (28%)
Day of First Observation	564		641
Life Table Test (e)			P=0.442N
Logistic Regression Test (e)			P=0.484
Fisher Exact Test (e)			P=0.501
Preputial Gland: Carcinoma			
Overall Rates (a)	3/46 (7%)	(b) 2/22 (9%)	1/50 (2%)
Adjusted Rates (c)	7 8%		2 4%
Terminal Rates (d)	0/20 (0%)		0/29 (0%)
Day of First Observation	479		681
Life Table Test (e)			P=0 230N
Logistic Regression Test (e)			P=0.510N
Fisher Exact Test (e)			P=0.278N
Preputial Gland: Adenoma or Carcinoma			
Overall Rates (a)	14/46 (30%)	(b) 13/22 (59%)	14/50 (28%)
Adjusted Rates (c)	37.9%		38.2%
Terminal Rates (d)	2/20 (10%)		8/29 (28%)
Day of First Observation	479		641
Life Table Test (e)			P=0.266N
Logistic Regression Test (e)			P=0.563
Fisher Exact Test (e)			P=0.485N
Pancreas: Adenoma			
Overall Rates (a)	1/49 (2%)	5/49 (10%)	3/48 (6%)
Adjusted Rates (c)	4 5%	17.2%	9 0%
Terminal Rates (d)	1/22 (5%)	5/29 (17%)	2/29 (7%)
Day of First Observation	735	735	669
Life Table Tests (e)	P=0.382	P=0.172	P=0.416
Logistic Regression Tests (e)	P=0.376	P=0.172	P=0.366
Cochran-Armitage Trend Test (e)	P=0.255		
Fisher Exact Test (e)		P=0.102	P=0.301

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

	Control	25,000 ppm	50,000 ppm
Pancreatic Islets: Adenoma			
Overall Rates (a)	6/49 (12%)	3/45 (7%)	1/47 (2%)
Adjusted Rates (c)	25 0%	10 3%	3 4%
Terminal Rates (d)	5/22 (23%)	2/26 (8%)	1/29 (3%)
Day of First Observation	660	696	735
Life Table Tests (e)	P=0 014N	P=0 165N	P=0 024N
Logistic Regression Tests (e)	P=0 017N	P=0 198N	P=0 028N
Cochran Armitage Trend Test (e)	P=0 102N		
Fisher Exact Test (e)		P=0 288N	P=0 062N
Pancreatic Islets: Adenoma or Carcinoma			
Overall Rates (a)	6/49 (12%)	3/45 (7%)	2/47 (4%)
Adjusted Rates (c)	25 0%	10 3%	6 1%
Terminal Rates (d)	5/22 (23%)	2/26 (8%)	1/29 (3%)
Day of First Observation	660	696	711
Life Table Tests (e)	P=0 041N	P=0 165N	P=0 064N
Logistic Regression Tests (e)	P=0 051N	P=0 198N	P=0 075N
Cochran Armitage Trend Test (e)	P=0 102N		
Fisher Exact Test (e)		P=0 288N	P=0 148N
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	3/49 (6%)	0/50 (0%)	1/50 (2%)
Adjusted Rates (c)	11 6%	0 0%	3 4%
Terminal Rates (d)	1/22 (5%)	0/29 (0%)	1/29 (3%)
Day of First Observation	711		735
Life Table Tests (e)	P=0 120N	P=0 083N	P=0 213N
Logistic Regression Tests (e)	P=0 126N	P=0 094N	P=0 220N
Cochran Armitage Trend Test (e)	P=0 171N		
Fisher Exact Test (e)		P=0 117N	P=0 301N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	3/49 (6%)	(b) 3/28 (11%)	4/50 (8%)
Adjusted Rates (c)	11 1%		10 4%
Terminal Rates (d)	2/22 (9%)		1/29 (3%)
Day of First Observation	577		674
Life Table Test (e)			P=0 641
Logistic Regression Test (e)			P=0 486
Fisher Exact Test (e)			P=0 511
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	5/49 (10%)	(b) 3/28 (11%)	4/50 (8%)
Adjusted Rates (c)	18 2%		10 4%
Terminal Rates (d)	3/22 (14%)		1/29 (3%)
Day of First Observation	577		674
Life Table Test (e)			P=0 337N
Logistic Regression Test (e)			P=0 503N
Fisher Exact Test (e)			P=0 487N
Mammary Gland: Fibroadenoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (c)	14 9%	3 4%	2 9%
Terminal Rates (d)	2/22 (9%)	1/29 (3%)	0/29 (0%)
Day of First Observation	534	735	718
Life Table Tests (e)	P=0 063N	P=0 125N	P=0 120N
Logistic Regression Tests (e)	P=0 093N	P=0 172N	P=0 182N
Cochran Armitage Trend Test (e)	P=0 101N		
Fisher Exact Test (e)		P=0 181N	P=0 181N

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

	Control	25,000 ppm	50,000 ppm
Mammary Gland: Fibroadenoma or Adenocarcinoma			
Overall Rates (a)	5/50 (10%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (c)	19.1%	3.4%	5.0%
Terminal Rates (d)	3/22 (14%)	1/29 (3%)	0/29 (0%)
Day of First Observation	534	735	589
Life Table Tests (e)	P=0.085N	P=0.062N	P=0.144N
Logistic Regression Tests (e)	P=0.139N	P=0.091N	P=0.248N
Cochran-Armitage Trend Test (e)	P=0.133N		
Fisher Exact Test (e)		P=0.102N	P=0 218N
Pancreas: Adenoma			
Overall Rates (a)	1/49 (2%)	3/49 (6%)	0/48 (0%)
Adjusted Rates (c)	4.5%	10.3%	0.0%
Terminal Rates (d)	1/22 (5%)	3/29 (10%)	0/29 (0%)
Day of First Observation	735	735	
Life Table Tests (e)	P=0.293N	P=0.407	P=0.445N
Logistic Regression Tests (e)	P=0.293N	P=0.407	P=0 445N
Cochran-Armitage Trend Test (e)	P=0.384N		
Fisher Exact Test (e)		P=0 309	P=0 505N
Pituitary Gland/Pars Distalis: Adenoma			
Overall Rates (a)	13/49 (27%)	(b) 12/20 (60%)	12/50 (24%)
Adjusted Rates (c)	43.5%		31.1%
Terminal Rates (d)	8/22 (36%)		5/29 (17%)
Day of First Observation	534		622
Life Table Test (e)			P=0.257N
Logistic Regression Test (e)			P=0.517N
Fisher Exact Test (e)			P=0.477N
Skin: Keratoacanthoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted Rates (c)	10.5%	0.0%	5.4%
Terminal Rates (d)	0/22 (0%)	0/29 (0%)	1/29 (3%)
Day of First Observation	672		562
Life Table Tests (e)	P=0.305N	P=0.084N	P=0.386N
Logistic Regression Tests (e)	P=0.430N	P=0.115N	P=0.568N
Cochran-Armitage Trend Test (e)	P=0.390N		
Fisher Exact Test (e)		P=0.121N	P=0.500N
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	4/50 (8%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (c)	12.8%	9.1%	5.8%
Terminal Rates (d)	0/22 (0%)	2/29 (7%)	1/29 (3%)
Day of First Observation	660	641	681
Life Table Tests (e)	P=0.168N	P=0.394N	P=0.220N
Logistic Regression Tests (e)	P=0.254N	P=0.489N	P=0.336N
Cochran-Armitage Trend Test (e)	P=0.264N		
Fisher Exact Test (e)		P=0.500N	P=0.339N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted Rates (c)	12.8%	9.1%	9.9%
Terminal Rates (d)	0/22 (0%)	2/29 (7%)	1/29 (3%)
Day of First Observation	660	641	622
Life Table Tests (e)	P=0.433N	P=0.394N	P=0.488N
Logistic Regression Tests (e)	P=0.551	P=0.489N	P=0.590
Cochran-Armitage Trend Test (e)	P=0.576		
Fisher Exact Test (e)		P=0.500N	P=0.643

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

	Control	25,000 ppm	50,000 ppm
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	5/50 (10%)	3/50 (6%)	4/50 (8%)
Adjusted Rates (c)	15 4%	9 1%	9 9%
Terminal Rates (d)	0/22 (0%)	2/29 (7%)	1/29 (3%)
Day of First Observation	660	641	622
Life Table Tests (e)	P=0 290N	P=0 263N	P=0 341N
Logistic Regression Tests (e)	P=0 462N	P=0 352N	P=0 578N
Cochran Armitage Trend Test (e)	P=0 427N		
Fisher Exact Test (e)		P=0 357N	P=0 500N
Testis: Interstitial Cell Adenoma			
Overall Rates (a)	47/49 (96%)	44/50 (88%)	49/50 (98%)
Adjusted Rates (c)	100 0%	95 6%	100 0%
Terminal Rates (d)	22/22 (100%)	27/29 (93%)	29/29 (100%)
Day of First Observation	479	562	560
Life Table Tests (e)	P=0 076N	P=0 034N	P=0 082N
Logistic Regression Tests (e)	P=0 507N	P=0 101N	P=0 734
Cochran Armitage Trend Test (e)	P=0 407		
Fisher Exact Test (e)		P=0 141N	P=0 492
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	5/49 (10%)	(b) 2/15 (13%)	7/50 (14%)
Adjusted Rates (c)	18 5%		22 1%
Terminal Rates (d)	3/22 (14%)		5/29 (17%)
Day of First Observation	589		709
Life Table Test (e)			P=0 571
Logistic Regression Test (e)			P=0 512
Fisher Exact Test (e)			P=0 394
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	5/49 (10%)	(b) 2/15 (13%)	9/50 (18%)
Adjusted Rates (c)	18 5%		26 9%
Terminal Rates (d)	3/22 (14%)		6/29 (21%)
Day of First Observation	589		641
Life Table Test (e)			P=0 368
Logistic Regression Test (e)			P=0 279
Fisher Exact Test (e)			P=0 205
Zymbal Gland: Adenoma or Carcinoma			
Overall Rates (a)	0/49 (0%)	3/45 (7%)	2/41 (7%)
Adjusted Rates (c)	0 0%	8 3%	6 9%
Terminal Rates (d)	0/22 (0%)	1/24 (4%)	1/22 (5%)
Day of First Observation		562	687
Life Table Tests (e)	P=0 231	P=0 138	P=0 275
Logistic Regression Tests (e)	P=0 135	P=0 108	P=0 219
Cochran Armitage Trend Test (e)	P=0 157		
Fisher Exact Test (e)		P=0 106	P=0 205
Hematopoietic System: Mononuclear Leukemia			
Overall Rates (a)	29/50 (58%)	(f) 27/50 (54%)	20/50 (40%)
Adjusted Rates (c)	70 9%	62 0%	54 4%
Terminal Rates (d)	11/22 (50%)	13/29 (45%)	13/29 (45%)
Day of First Observation	577	501	589
Life Table Tests (e)	P=0 009N	P=0 157N	P=0 011N
Logistic Regression Tests (e)	P=0 035N	P=0 405N	P=0 036N
Cochran Armitage Trend Test (e)	P=0 045N		
Fisher Exact Test (e)		P=0 420N	P=0 055N

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

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

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

Study	Incidence of Carcinomas in Controls
Historical Incidence at EG&G Mason Research Institute	
4,4' Methylenedianiline dihydrochloride	0/50
C I Basic Red 9 monohydrochloride	1/50
Monuron	0/50
8 Hydroxyquinoline	(b) 1/50
Di(2 ethylhexyl)phthalate	0/50
Di(2-ethylhexyl)adipate	0/49
Guar gum	(c) 1/50
Locust bean gum	0/50
Gum arabic	0/50
Agar	0/50
Tara gum	0/50
2 Biphenylamine hydrochloride	(b) 1/50
TOTAL	4/599 (0 7%)
SD (d)	0 98%
Range (e)	
High	1/50
Low	0/50
Overall Historical Incidence at All Laboratories	
TOTAL	(f) 19/1,936 (1 0%)
SD (d)	1.71%
Range (e)	
High	4/50
Low	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks, no benign tumors have been observed
(b) Squamous cell carcinoma
(c) Ceruminous carcinoma
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals
(f) Includes nine squamous cell carcinomas and one ceruminous carcinoma

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

Study	Incidence of Leukemia in Controls
Historical Incidence at EG&G Mason Research Institute	
4,4'-Methylenedianiline dihydrochloride	12/50
C.I Basic Red 9 monohydrochloride	7/50
Monuron	5/50
8-Hydroxyquinoline	17/50
Di(2-ethylhexyl)phthalate	13/50
Di(2 ethylhexyl)adipate	9/49
Guar gum	13/50
Locust bean gum	21/50
Gum arabic	10/50
Agar	9/50
Tara gum	14/50
2-Biphenylamine hydrochloride	15/50
TOTAL	145/599 (24 2%)
SD (b)	8 86%
Range (c)	
High	21/50
Low	5/50
Overall Historical Incidence at All Laboratories	
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 PETN, NF

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine large, colon	(47)	(10)	(48)
Parasite	6 (13%)	2 (20%)	14 (29%)
Intestine large, rectum	(46)	(9)	(48)
Parasite	1 (2%)		2 (4%)
Intestine small, ileum	(47)	(7)	(47)
Lymphoid tissue, hyperplasia			1 (2%)
Liver	(49)	(50)	(50)
Angiectasis	5 (10%)	7 (14%)	8 (16%)
Basophilic focus	25 (51%)	19 (38%)	25 (50%)
Clear cell focus	2 (4%)	4 (8%)	3 (6%)
Congestion	1 (2%)		1 (2%)
Degeneration, cystic	3 (6%)	6 (12%)	6 (12%)
Developmental malformation		2 (4%)	1 (2%)
Eosinophilic focus	1 (2%)	1 (2%)	
Fatty change, diffuse	3 (6%)	1 (2%)	1 (2%)
Fatty change, focal	7 (14%)	4 (8%)	5 (10%)
Focal cellular change	2 (4%)		8 (16%)
Hemorrhage			2 (4%)
Hepatodiaphragmatic nodule	4 (8%)	1 (2%)	
Hyperplasia	3 (6%)	2 (4%)	
Hyperplasia, focal	1 (2%)	1 (2%)	5 (10%)
Hyperplasia, multifocal		1 (2%)	1 (2%)
Inflammation			1 (2%)
Inflammation, granulomatous		1 (2%)	
Mixed cell focus	4 (8%)	9 (18%)	3 (6%)
Necrosis	3 (6%)	1 (2%)	4 (8%)
Pigmentation		1 (2%)	
Thrombus		2 (4%)	
Vacuolization, cytoplasmic	1 (2%)		
Bile duct, hyperplasia	39 (80%)	31 (62%)	35 (70%)
Oval cell, hyperplasia	1 (2%)		
Mesentery	(4)	(1)	(8)
Hemorrhage			1 (13%)
Fat, mineralization	2 (50%)		1 (13%)
Fat, necrosis	2 (50%)	1 (100%)	6 (75%)
Fat, pigmentation			3 (38%)
Pancreas	(49)	(49)	(48)
Fibrosis			1 (2%)
Acinus, atrophy	1 (2%)	2 (4%)	
Acinus, hyperplasia	3 (6%)	6 (12%)	2 (4%)
Acinus, hyperplasia, focal		1 (2%)	1 (2%)
Artery, hemorrhage		1 (2%)	
Artery, inflammation, chronic active		2 (4%)	
Artery, mineralization	1 (2%)		
Artery, necrosis, fibrinoid	4 (8%)	2 (4%)	
Duct, hyperplasia			1 (2%)
Pharynx			1 (2%)
Palate, epithelium, hyperplasia			(1)
Salivary glands	(48)	(13)	1 (100%)
Inflammation, chronic active		1 (8%)	(49)
Stomach, forestomach	(49)	(14)	
Acanthosis	3 (6%)	4 (29%)	(48)
Edema	1 (2%)		2 (4%)
Fibrosis	1 (2%)		1 (2%)
Hyperkeratosis	4 (8%)		7 (14%)

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

	Untreated Control	Low Dose	High Dose
ALIMENTARY SYSTEM			
Stomach, forestomach (Continued)	(49)	(14)	(48)
Hyperplasia, basal cell			1 (2%)
Inflammation, chronic active	2 (4%)		1 (2%)
Necrosis	2 (4%)		
Ulcer		2 (14%)	
Stomach, glandular	(48)	(15)	(47)
Degeneration			1 (2%)
Erosion		3 (20%)	
Hyperplasia			3 (6%)
Infiltration cellular, lymphocytic		2 (13%)	2 (4%)
Inflammation, chronic		2 (13%)	
Mineralization	1 (2%)		2 (4%)
Necrosis	1 (2%)	1 (7%)	1 (2%)
Pigmentation			1 (2%)
Tooth	(1)		
Gingiva, inflammation, chronic	1 (100%)		
Tongue	(2)	(1)	
Hyperkeratosis	1 (50%)		
CARDIOVASCULAR SYSTEM			
Heart	(49)	(15)	(50)
Cardiomyopathy	37 (76%)	13 (87%)	47 (94%)
Inflammation, acute			1 (2%)
Inflammation, chronic active	1 (2%)		
Mineralization			1 (2%)
Thrombus		3 (20%)	
Artery, mineralization	2 (4%)		
ENDOCRINE SYSTEM			
Adrenal gland, cortex	(49)	(18)	(48)
Hemorrhage			1 (2%)
Hyperplasia	1 (2%)		2 (4%)
Hypertrophy		1 (6%)	
Vacuolization cytoplasmic, diffuse			1 (2%)
Adrenal gland, medulla	(49)	(18)	(48)
Hyperplasia	25 (51%)	5 (28%)	21 (44%)
Islets, pancreatic	(49)	(45)	(47)
Hyperplasia		2 (4%)	
Hyperplasia, focal		1 (2%)	
Parathyroid gland	(40)	(9)	(42)
Hyperplasia	2 (5%)		
Pituitary gland	(49)	(20)	(50)
Pars distalis, angiectasis	12 (24%)	11 (55%)	6 (12%)
Pars distalis, cyst	2 (4%)		
Pars distalis, hyperplasia	10 (20%)	3 (15%)	21 (42%)
Pars distalis, hyperplasia, focal	1 (2%)		
Pars intermedia, hyperplasia	1 (2%)	1 (5%)	3 (6%)
Thyroid gland	(49)	(15)	(50)
C cell, hyperplasia	4 (8%)		6 (12%)
Follicle, cyst			1 (2%)
Follicular cell, hyperplasia	1 (2%)		
GENERAL BODY SYSTEM			
Tissue, NOS			(1)
Necrosis			1 (100%)

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

	Untreated Control	Low Dose	High Dose
GENITAL SYSTEM			
Epididymis	(48)	(13)	(50)
Mineralization	4 (8%)		
Preputial gland	(46)	(22)	(50)
Dilatation	1 (2%)		1 (2%)
Hyperplasia			1 (2%)
Inflammation, acute		2 (9%)	
Inflammation, chronic active	2 (4%)		3 (6%)
Necrosis	7 (15%)	1 (5%)	7 (14%)
Prostate	(48)	(13)	(50)
Inflammation			1 (2%)
Inflammation, chronic active	12 (25%)	3 (23%)	9 (18%)
Mineralization			1 (2%)
Necrosis			1 (2%)
Pigmentation	1 (2%)		
Epithelium, hyperplasia	1 (2%)		4 (8%)
Seminal vesicle	(49)	(23)	(50)
Atrophy	3 (6%)		
Testes	(49)	(50)	(50)
Interstitial cell, hyperplasia	28 (57%)	23 (46%)	25 (50%)
Seminiferous tubule, atrophy	43 (88%)	32 (64%)	44 (88%)
Seminiferous tubule, mineralization	4 (8%)		2 (4%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(10)	(50)
Hyperplasia, reticulum cell			1 (2%)
Lymph node	(48)	(30)	(50)
Axillary, infiltration cellular, plasma cell		1 (3%)	
Inguinal, infiltration cellular, plasma cell		1 (3%)	
Lumbar, congestion	1 (2%)		
Lumbar, infiltration cellular, plasma cell	1 (2%)		
Lumbar, infiltration cellular, histiocytic		1 (3%)	
Lumbar, pigmentation			1 (2%)
Mediastinal, angiectasis	2 (4%)	2 (7%)	
Mediastinal, congestion			1 (2%)
Mediastinal, depletion lymphoid			1 (2%)
Mediastinal, infiltration cellular, histiocytic		1 (3%)	
Mediastinal, pigmentation	3 (6%)	1 (3%)	2 (4%)
Pancreatic, angiectasis		1 (3%)	
Pancreatic, cyst			1 (2%)
Pancreatic, depletion lymphoid			1 (2%)
Pancreatic, hematocyst			1 (2%)
Pancreatic, hemorrhage			1 (2%)
Pancreatic, infiltration cellular, plasma cell	1 (2%)		1 (2%)
Pancreatic, infiltration cellular, histiocytic		3 (10%)	1 (2%)
Renal, infiltration cellular, histiocytic		2 (7%)	1 (2%)
Renal, pigmentation	1 (2%)		2 (4%)
Lymph node, mandibular	(46)	(20)	(49)
Angiectasis		1 (5%)	
Cyst	4 (9%)		2 (4%)
Degeneration, cystic		1 (5%)	1 (2%)
Erythrophagocytosis	1 (2%)		
Hemorrhage	2 (4%)		
Infiltration cellular, plasma cell		2 (10%)	
Lymph node, mesenteric	(47)	(15)	(48)
Angiectasis		1 (7%)	1 (2%)
Degeneration, cystic	1 (2%)		2 (4%)
Hemorrhage	1 (2%)	1 (7%)	1 (2%)

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

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM			
Lymph node, mesenteric (Continued)	(47)	(15)	(48)
Infiltration cellular, plasma cell	1 (2%)	1 (7%)	
Infiltration cellular, histiocytic			2 (4%)
Necrosis			1 (2%)
Spleen	(48)	(36)	(50)
Atrophy			1 (2%)
Congestion	2 (4%)		
Depletion lymphoid	1 (2%)	1 (3%)	2 (4%)
Fibrosis	1 (2%)	2 (6%)	2 (4%)
Hematopoietic cell proliferation	26 (54%)	14 (39%)	28 (56%)
Hemorrhage		4 (11%)	
Mineralization		1 (3%)	
Necrosis	1 (2%)	1 (3%)	
Pigmentation	6 (13%)	2 (6%)	15 (30%)
Thrombus		1 (3%)	
Thymus	(36)	(17)	(45)
Depletion lymphoid	3 (8%)	1 (6%)	3 (7%)
Epithelial cell, hyperplasia	1 (3%)		
INTEGUMENTARY SYSTEM			
Mammary gland	(37)	(9)	(38)
Galactocele	4 (11%)		6 (16%)
Acinus, hyperplasia	1 (3%)		1 (3%)
Skin	(48)	(18)	(50)
Acanthosis	3 (6%)	4 (22%)	4 (8%)
Cyst epithelial inclusion		2 (11%)	
Erosion			1 (2%)
Hemorrhage			1 (2%)
Hyperkeratosis	4 (8%)	4 (22%)	5 (10%)
Inflammation, chronic active			1 (2%)
Necrosis	2 (4%)		2 (4%)
MUSCULOSKELETAL SYSTEM			
Bone	(49)	(10)	(50)
Tarsal, hyperostosis			1 (2%)
NERVOUS SYSTEM			
Brain	(49)	(48)	(50)
Hemorrhage	3 (6%)	1 (2%)	2 (4%)
Spinal cord		(1)	
Hemorrhage		1 (100%)	
RESPIRATORY SYSTEM			
Lung	(49)	(28)	(50)
Atelectasis	1 (2%)	1 (4%)	
Congestion	10 (20%)	10 (36%)	11 (22%)
Edema	3 (6%)	1 (4%)	2 (4%)
Embolus tumor	1 (2%)		
Fibrosis	1 (2%)		
Hemorrhage	6 (12%)	2 (7%)	6 (12%)
Infiltration cellular, histiocytic	4 (8%)	1 (4%)	11 (22%)
Inflammation, acute	1 (2%)		
Inflammation, chronic active	1 (2%)		5 (10%)
Alveolar epithelium, hyperplasia	2 (4%)		1 (2%)

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

	Untreated Control	Low Dose	High Dose
RESPIRATORY SYSTEM (Continued)			
Nose	(47)	(10)	(50)
Hemorrhage			1 (2%)
Inflammation, acute	2 (4%)		8 (16%)
Inflammation, chronic active	1 (2%)		1 (2%)
Metaplasia, osseous			1 (2%)
SPECIAL SENSES SYSTEM			
Eye	(5)	(9)	(6)
Cornea, inflammation, acute	1 (20%)		
Lens, cataract	1 (20%)	3 (33%)	1 (17%)
Zymbal gland	(49)	(45)	(41)
Cyst	6 (12%)	1 (2%)	
Hyperplasia		1 (2%)	
Inflammation, granulomatous	1 (2%)		
URINARY SYSTEM			
Kidney	(49)	(50)	(50)
Angiectasis		1 (2%)	
Cyst	4 (8%)	1 (2%)	1 (2%)
Fibrosis			1 (2%)
Hydronephrosis			2 (4%)
Necrosis		1 (2%)	
Nephropathy	49 (100%)	49 (98%)	49 (98%)
Capsule, fibrosis			1 (2%)
Cortex, mineralization	5 (10%)		7 (14%)
Papilla, mineralization	4 (8%)	1 (2%)	9 (18%)
Renal tubule, dilatation			1 (2%)
Renal tubule, hyperplasia		3 (6%)	
Renal tubule, necrosis	1 (2%)		1 (2%)
Renal tubule, pigmentation	3 (6%)	1 (2%)	5 (10%)
Transitional epithelium, hyperplasia	2 (4%)	8 (16%)	3 (6%)
Urinary bladder	(46)	(10)	(48)
Calculus gross observation		1 (10%)	1 (2%)
Calculus micro observation only	1 (2%)	2 (20%)	1 (2%)
Fibrosis			1 (2%)
Hemorrhage			1 (2%)
Inflammation, chronic active			1 (2%)
Artery, necrosis, fibrinoid	1 (2%)		

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF PETN, NF

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

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine large, colon	(48)	*(50)	(50)
Leukemia mononuclear		1 (2%)	
Intestine small, duodenum	(48)	*(50)	(49)
Adenocarcinoma			1 (2%)
Leukemia mononuclear		1 (2%)	
Intestine small, ileum	(46)	*(50)	(49)
Leukemia mononuclear		1 (2%)	
Liver	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)	
Leukemia mononuclear	14 (28%)	14 (28%)	9 (18%)
Pancreas	(50)	*(50)	(49)
Leukemia mononuclear	3 (6%)		1 (2%)
Acinus, adenoma		1 (2%)	1 (2%)
Pharynx	*(50)	*(50)	*(50)
Palate, squamous cell carcinoma	1 (2%)		
Salivary glands	(50)	*(50)	(49)
Leukemia mononuclear		1 (2%)	
Sarcoma, metastatic, skin			1 (2%)
Stomach	(50)	*(50)	(50)
Leukemia mononuclear	1 (2%)		
CARDIOVASCULAR SYSTEM			
Heart	(50)	*(50)	(50)
Leukemia mononuclear	5 (10%)	3 (6%)	6 (12%)
ENDOCRINE SYSTEM			
Adrenal gland, cortex	(49)	*(50)	(49)
Leukemia mononuclear	7 (14%)	2 (4%)	2 (4%)
Adrenal gland, medulla	(49)	*(50)	(49)
Leukemia mononuclear	7 (14%)	2 (4%)	2 (4%)
Pheochromocytoma benign	1 (2%)		4 (8%)
Bilateral, pheochromocytoma benign	1 (2%)		
Islets, pancreatic	(49)	*(50)	(48)
Adenoma	1 (2%)	1 (2%)	
Parathyroid gland	(43)	*(50)	(44)
Adenoma			1 (2%)
Pituitary gland	(50)	*(50)	(49)
Leukemia mononuclear	4 (8%)	1 (2%)	3 (6%)
Pars distalis, adenoma	16 (32%)	13 (26%)	18 (37%)
Pars distalis, adenoma, multiple	3 (6%)	1 (2%)	5 (10%)
Pars distalis, carcinoma			1 (2%)
Pars distalis, leukemia mononuclear			1 (2%)
Thyroid gland	(50)	(48)	(50)
Sarcoma, metastatic, skin			1 (2%)
Bilateral, C-cell, adenoma	2 (4%)		
C-cell, adenoma	5 (10%)	3 (6%)	4 (8%)
C-cell, carcinoma	1 (2%)	1 (2%)	3 (6%)
Follicular cell, adenoma			1 (2%)
Follicular cell, carcinoma			2 (4%)
GENERAL BODY SYSTEM			
None			

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO YEAR FEED STUDY OF PETN, NF (Continued)

	Untreated Control	Low Dose	High Dose
GENITAL SYSTEM			
Clitoral gland	(39)	*(50)	(41)
Adenoma	5 (13%)	4 (8%)	6 (15%)
Carcinoma		1 (2%)	1 (2%)
Bilateral, adenoma		2 (4%)	
Ovary	(50)	*(50)	(50)
Granulosa cell tumor benign		1 (2%)	
Leukemia mononuclear	5 (10%)	2 (4%)	3 (6%)
Uterus	(50)	(48)	(50)
Leiomyosarcoma			1 (2%)
Leukemia mononuclear	2 (4%)	2 (4%)	1 (2%)
Polyp stromal	8 (16%)	9 (19%)	12 (24%)
Bilateral, polyp stromal		1 (2%)	
HEMATOPOIETIC SYSTEM			
Bone marrow	(50)	*(50)	(50)
Leukemia mononuclear	1 (2%)		1 (2%)
Lymph node	(50)	*(50)	(49)
Axillary, leukemia mononuclear	3 (6%)		
Deep cervical, leukemia mononuclear	1 (2%)		
Inguinal, leukemia mononuclear	1 (2%)		
Lumbar, leukemia mononuclear	1 (2%)		
Mediastinal, leukemia mononuclear	6 (12%)	1 (2%)	5 (10%)
Pancreatic, leukemia mononuclear	4 (8%)		1 (2%)
Lymph node, mandibular	(46)	*(50)	(47)
Leukemia mononuclear	7 (15%)	2 (4%)	4 (9%)
Lymph node, mesenteric	(49)	*(50)	(48)
Leukemia mononuclear	7 (14%)	3 (6%)	5 (10%)
Spleen	(49)	*(50)	(50)
Leukemia mononuclear	13 (27%)	14 (28%)	9 (18%)
Thymus	(46)	*(50)	(44)
Leukemia mononuclear	7 (15%)	1 (2%)	6 (14%)
INTEGUMENTARY SYSTEM			
Mammary gland	(46)	*(50)	(48)
Adenocarcinoma		1 (2%)	2 (4%)
Adenoma	1 (2%)		
Fibroadenoma	18 (39%)	15 (30%)	18 (38%)
Fibroadenoma, multiple	9 (20%)	8 (16%)	11 (23%)
Leukemia mononuclear	2 (4%)		
Skin	(49)	*(50)	(50)
Keratoacanthoma	1 (2%)		
Subcutaneous tissue, fibroma	4 (8%)		1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (2%)	
Subcutaneous tissue, fibrous histiocytoma		1 (2%)	
Subcutaneous tissue, hemangioma		1 (2%)	
Subcutaneous tissue, sarcoma			1 (2%)
MUSCULOSKELETAL SYSTEM			
None			
NERVOUS SYSTEM			
Brain	(50)	*(50)	(50)
Astrocytoma malignant		1 (2%)	
Leukemia mononuclear	3 (6%)	2 (4%)	3 (6%)
Spinal cord	*(50)	*(50)	*(50)
Leukemia mononuclear		1 (2%)	1 (2%)