NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 404

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 5,5-DIPHENYLHYDANTOIN
(PHENYTOIN)

(CAS NO. 57-41-0)

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

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE PERINATAL

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P.O. Box 12233
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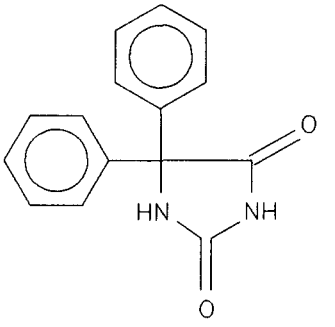

5,5-DIPHENYLHYDANTOIN
(PHENYTOIN)

CAS No. 57-41-0

Chemical Formula: $C_{15}H_{12}N_2O_2$ Molecular Weight: 252.26

Synonyms: Diphenylhydantoin; 5,5-diphenyl-2,4-imidazolidinedione
Trade names: Difhydan; Dihycon; Di-Hydan; Di-Lan; Dilabid; Dilantin; Ekko; Hydantol; Lehydan; Zentropil

5,5-Diphenylhydantoin and its sodium salt are primarily used in the treatment of grand mal and psychomotor seizures, often in combination with other anticonvulsants, including phenobarbital. 5,5-Diphenylhydantoin is a suspected human carcinogen and was one of three compounds selected by the NTP to investigate the potential value of perinatal exposures in assessing chemical carcinogenicity.

Chronic toxicity and carcinogenicity studies of 5,5-diphenylhydantoin were conducted in male and female F344/N rats and B6C3F$_1$ mice. The studies were designed to determine the following: a) the effects of 5,5-diphenylhydantoin in the diet given to rats and mice during the adult (F$_1$) period only (a typical carcinogenicity study), b) the toxic and carcinogenic effects of 5,5-diphenylhydantoin in rats and mice receiving perinatal (F$_0$) exposure only (dietary exposure of dams prior to breeding and throughout gestation and lactation), and c) the effects of combined perinatal and adult exposure to 5,5-diphenylhydantoin. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, and mouse bone marrow cells.

STUDIES IN F344/N RATS

A 13-week toxicity study was conducted to select the exposure levels for adults in the 2-year study. The exposure levels for the 13-week study ranged from 300 to 4,800 ppm 5,5-diphenylhydantoin in the diet. The final mean body weights of males and females exposed to 2,400 or 4,800 ppm were significantly decreased. All groups showed a net weight gain over the study period, although the mean body weight gain of females in the 4,800 ppm group was only one-half that of the controls. Feed consumption also decreased with increasing exposure level. No chemical-related gross lesions were present in the tissues of exposed rats. Microscopically, centrilobular hypertrophy of hepatocytes was observed in the liver of rats in the 4,800 ppm groups. Based on these results, 2,400 ppm was selected as the highest exposure for the adult-only portion of the 2-year carcinogenicity study.

A gestational study was performed to select the exposure levels for the perinatal portion of the 2-year study. The exposure levels ranged from 80 to 2,400 ppm 5,5-diphenylhydantoin in the diet of the dams. The 2,400 ppm exposure level was found to have reproductive and embryotoxic effects, as none of the sperm-positive females delivered litters. In the 800 ppm group, a greater number of pups died between postnatal day 1 and day 28 than in the control group. No gross external malformations were observed among fetuses or pups surviving to term in any exposure group, and no gross or histopathologic lesions were observed in the animals exposed to 800 ppm for 4 weeks following weaning. Based on these results, 630 ppm was selected as the highest exposure level for the perinatal portion of the 2-year carcinogenicity study. The eight F_0:F_1 exposure combinations selected for the 2-year study are listed in the table below.

In the 2-year study, male and female rats in the 630:2,400 ppm groups evaluated at 9 months had increased relative liver weights. Hematologic evaluations indicated mild but consistent chemical-related increases in erythrocyte and platelet counts in male and female rats. Mild decreases in triglyceride concentrations and alanine aminotransferase enzyme activity were seen generally in the high-exposure groups. In the 2-year study, the survival of exposed rats was similar to that of the controls. However, body weights of exposed rats were lower than those of the controls, and body weights were 11% to 35% lower in rats receiving adult exposure of 2,400 ppm 5,5-diphenylhydantoin. Feed consumption was similar for exposed and control groups.

Hepatocellular neoplasms, primarily adenomas, occurred with a positive trend in male rats fed 5,5-diphenylhydantoin only as adults (0:0 ppm, 0/50; 0:800 ppm, 2/50; 0:2,400 ppm, 4/50). There were no increased neoplasm incidences at other sites in exposed males or at any site in exposed females. Perinatal-only or combined perinatal and adult exposure to 5,5-diphenylhydantoin did not enhance the overall incidences of liver neoplasms in male or female rats. However, the finding of 5/49 hepatocellular adenomas in the 630:2,400 male rat group was consistent with the marginally elevated liver neoplasm rate observed in the 0:2,400 group. Decreased incidences of a number of different neoplasms in exposed groups were most likely related to the lower body weights.

F_1 Concentration[b] (ppm)	Exposure Groups and Numbers of Rats[a] F_0 Concentration[c] (ppm)			
	0	63	210	630
0	60	–	–	60
240	–	60	–	–
800	60	–	60	60
2,400	60	–	–	60

[a] Ten rats from each group were evaluated at 9 months.
[b] Concentration of 5,5-diphenylhydantoin in feed given to rats beginning at 8 weeks of age for 2 years
[c] Concentration of 5,5-diphenylhydantoin in feed through breeding, gestation, and lactation until pups were 8 weeks of age

STUDIES IN B6C3F$_1$ MICE

A 13-week toxicity study was conducted to select the exposure levels for adults in the 2-year study. The exposure levels for the 13-week study ranged from 75 to 1,200 ppm 5,5-diphenylhydantoin in the diet. With the exception of one male, all mice exposed to 1,200 ppm died before the end of the study. No other chemical-related deaths occurred. All groups of mice except the 1,200 ppm groups gained weight over the 13-week period; however, an exposure-related decrease in body weight gain was seen in males and females. Feed consumption by exposed and control groups was generally similar. Chemical-related histomorphologic lesions were present in the liver of exposed mice, particularly 600 ppm males, and consisted of centrilobular hypertrophy of hepatocytes. Females appeared to be less sensitive than males to the effects of 5,5-diphenylhydantoin on growth and on histomorphologic liver lesions. Based on these results, 300 ppm (males) and 600 ppm (females) were selected as the highest exposure levels for the adult-only portion of the 2-year carcinogenicity study.

A gestational study was performed to select the exposure levels for the perinatal portion of the 2-year study. The exposure levels for males and females ranged from 20 to 600 ppm 5,5-diphenylhydantoin in the diet. In general, reproductive performance and maternal care were poor in all groups, including the controls, thus restricting the sample size and sensitivity of this evaluation. There were no litters in the 600 ppm group, and maternal weight gain was depressed. There were no gross external malformations among pups surviving to term, and no gross or histopathologic lesions were observed in any mice exposed for 4 weeks following weaning. Based on these results, 210 ppm was selected as the highest exposure level for the perinatal portion of the 2-year carcinogenicity study. The F_0:F_1 exposure combinations selected for the 2-year study are listed in the following table.

F_1 Concentration[b] (ppm)	Exposure Groups and Numbers of Mice[a] F_0 Concentration[c] (ppm)			
	0	21	70	210
Male				
0	60	–	–	60
30	–	60	–	–
100	60	–	60	60
300	60	–	–	60
Female				
0	60	–	–	60
60	–	60	–	–
200	60	–	60	60
600	60	–	–	60

[a] Ten mice from each group were evaluated at 9 months.
[b] Concentration of 5,5-diphenylhydantoin in feed given to mice beginning at 8 weeks of age for 2 years
[c] Concentration of 5,5-diphenylhydantoin in feed through breeding, gestation, and lactation until pups were 8 weeks of age

For mice evaluated at 9 months, males and females receiving the highest F_0:F_1 exposure levels had increased relative liver weights. In the 2-year study, the survival of exposed animals was similar to that of the controls; however, body weights were lower for exposed groups, and decreased body weights were most severe in adult females receiving 600 ppm 5,5-diphenylhydantoin. Feed consumption was similar for exposed and control groups.

The incidences of hepatocellular neoplasms were increased in female mice receiving adult-only exposure (0:0 ppm, 5/48; 0:200 ppm, 14/49; 0:600 ppm, 30/50) or combined perinatal and adult exposure (210:200 ppm, 16/50; 210:600 ppm, 34/50). A marginally increased incidence of liver neoplasms (12/49) occurred in females in the perinatal-only (210:0) exposure group. There were no chemical-related increased incidences of liver neoplasms in males receiving adult-only or perinatal-only exposure. However, males receiving the high-exposure combined perinatal and adult exposure regimen (210:300 ppm) had an increased incidence of liver neoplasms (41/50) compared to the 0:0 (29/50), 0:300 (26/49), and 210:0 (33/50) groups. As a result, there was a significant enhancement (interaction) associated with combined perinatal and adult exposure. Such enhancement of neoplasia did not occur in female mice. Decreased incidences of malignant neoplasms in exposed groups were most likely related to the lower body weights.

GENETIC TOXICOLOGY

In general, tests for genotoxic activity of 5,5-diphenylhydantoin were negative. All *in vitro* testing was performed in the presence and the absence of exogenous metabolic activation (S9). 5,5-Diphenylhydantoin did not induce mutations in *Salmonella typhimurium*, in L5178Y mouse lymphoma cells, or in germ cells of male *Drosophila melanogaster*, nor did it induce chromosomal aberrations in cultured Chinese hamster ovary cells. A small but statistically significant increase was obtained in the cultured Chinese hamster ovary cell test for induction of sister chromatid exchanges in the presence of S9; without S9, no increase in sister chromatid exchanges was observed. *In vivo*, 5,5-diphenylhydantoin did not induce micronuclei in polychromatic erythrocytes or chromosomal aberrations in bone marrow cells of male mice; equivocal results were obtained in an *in vivo* test for induction of sister chromatid exchanges in mouse bone marrow cells.

CONCLUSIONS

Adult-Only Exposure

Under the conditions of these 2-year, adult-only, dietary exposure studies, there was *equivocal evidence of carcinogenic activity** of 5,5-diphenylhydantoin in male F344/N rats based on marginally increased incidences of hepatocellular neoplasms. There was *no evidence of carcinogenic activity* of 5,5-diphenylhydantoin in female F344/N rats given 240, 800, or 2,400 ppm. There was *no evidence of carcinogenic activity* of 5,5-diphenylhydantoin in male B6C3F_1 mice given 30, 100, or 300 ppm. There was *clear evidence of carcinogenic activity* of 5,5-diphenylhydantoin in female B6C3F_1 mice based on increased incidences of hepatocellular neoplasms.

Perinatal-Only Exposure

Perinatal exposure alone (through dietary administration of 210 ppm 5,5-diphenylhydantoin during the perinatal period) caused a marginal increase in the incidences of hepatocellular neoplasms in female B6C3F_1 mice evaluated 2 years after cessation of exposure. In male and female F344/N rats, exposure to 630 ppm during the perinatal period did not influence the incidences of hepatocellular or other neoplasms. Similarly, exposure of male B6C3F_1 mice to dietary levels of 210 ppm 5,5-diphenylhydantoin during the perinatal period did not affect neoplasm incidences. No teratologic effects were observed.

Combined Perinatal and Adult Exposure

Combined perinatal and adult dietary exposure to 5,5-diphenylhydantoin confirmed the findings of the increased incidences of hepatocellular neoplasms for adult-only exposures in male F344/N rats and female B6C3F_1 mice, although combined exposure did not enhance these neoplastic effects. However, in male B6C3F_1 mice, combined perinatal and adult exposure resulted in increased incidences of hepatocellular neoplasms (hepatocellular carcinomas and multiple adenomas) that were not seen when dietary exposure was limited to the adult exposure period only.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 11.

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report 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 five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing an 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 neoplasms to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration 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:

- 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 neoplasm incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in neoplasm 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);
- presence or absence of dose relationships;
- statistical significance of the observed neoplasm increase;
- concurrent control neoplasm 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.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on 5,5-diphenylhydantoin on June 23, 1992, 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 in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 23, 1992, the draft Technical Report on the toxicology and carcinogenesis studies of 5,5-diphenylhydantoin received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Dr. R.S. Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of 5,5-diphenylhydantoin by discussing the rationale for incorporating perinatal exposure into the study designs. The study designs included conventional 2-year exposure of adult animals, perinatal exposure only, and perinatal plus adult exposure. The perinatal exposure studies were included to evaluate their potential value in assessing chemical carcinogenicity. Dr. Chhabra described the experimental design, reported on survival and body weight effects, and commented on neoplastic lesions in rats and mice. The proposed conclusions were *some evidence of carcinogenic activity* of 5,5-diphenylhydantoin in adult-only exposure male rats, *no evidence of carcinogenic activity* of 5,5-diphenylhydantoin in adult-only exposure female rats and male mice, and *clear evidence of carcinogenic activity* of 5,5-diphenylhydantoin in adult-only exposure female mice. In the perinatal-only exposure, there was a marginal increase of carcinogenic activity in female mice evaluated 2 years after cessation of exposure. Combined perinatal and adult exposure confirmed the findings of the adult-only exposures.

Dr. Goodman, a principal reviewer, agreed in principle with the proposed conclusions. However, he proposed that the conclusion for male rats under adult-only exposure be changed from *some evidence* to *equivocal evidence of carcinogenic activity* based on decreases in weight exceeding 10 percent in high-exposure animals and the fact that the liver neoplasm incidence was within the historical control range. Dr. Chhabra responded that in the three perinatal studies done in the same laboratory and at the same time, only one liver neoplasm was observed in male rat controls (1/150). Further, one out of four hepatocellular adenomas in 0:2,400 ppm males and four out of five in 630:2,400 males were multiple adenomas, supporting the level of evidence chosen. Dr. Goodman suggested omitting groups of female rats and mice in which the maximum tolerated dose appears to have been exceeded from the carcinogenicity discussion. Dr. Goodman said the speculation about the possible role of arene oxide metabolite binding in the toxicity and carcinogenicity of 5,5-diphenylhydantoin was appropriate in the discussion but mention should be made of the negative genotoxicity results. Dr. Chhabra agreed.

Dr. Hayden, the second principal reviewer, agreed with the proposed conclusions. He noted that because 5,5-diphenylhydantoin is commonly used in combination with other anticonvulsants, such as phenobarbital, it might be of interest to see if such drug combinations enhance or alter the toxicity/carcinogenicity of 5,5-diphenylhydantoin. Dr. Chhabra explained that since the primary rationale for the study was to evaluate the value of perinatal exposure in assessing chemical carcinogenicity and not 5,5-diphenylhydantoin per se, the pure drug itself was preferred. Dr. Hayden asked that the rationale for selecting 5,5-diphenylhydantoin for study be made more specific. Dr. Hayden suggested that the schematic diagram of the experimental design for the chronic studies used by Dr. Chhabra in his opening remarks be in the report. Dr. Chhabra agreed to include more discussion of the rationale and to add the schematic of the design to the final report (Figure 1, p. 23).

Because Dr. McKnight, the third principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read her review into the record. Dr. McKnight agreed with the proposed conclusions. She thought that the experimental design did not make optimum use of the animals, and a better choice would have been to replace the low F_1-low F_0 group with a high F_1-medium F_0 combination. Dr. Chhabra said that the ideal design would have been 16 exposure groups but for practical reasons only 8 were used. Dr. McKnight said the statistical analyses for the combined perinatal and adult exposures should be presented in the Appendixes. Dr. J.K. Haseman, NIEHS, agreed.

Dr. Silbergeld stated that this study failed to detect toxicity of a chemical that is known to be toxic to other systems; i.e., 5,5-diphenylhydantoin is a known teratogen in humans and in rodents within the

exposure range used here. She suggested removing "Toxicology" from the title of the report. Dr. Chhabra replied that exposure levels were chosen that would not have teratogenic effects as this could confound the assessment of carcinogenicity. Dr. J.R. Bucher, NIEHS, added that a complete necropsy was done on perinatally exposed animals at the end of 2 years and any malformations or defects would have been detected. Dr. Chhabra said that for the perinatal-only exposure and combined perinatal and adult exposure it would be noted in the conclusions that no fetal toxicity or teratogenicity was observed under the conditions of these studies. Dr. Zeise inquired as to why the drug was administered in the feed rather than by gavage. Dr. Chhabra said that using feed allowed a maximum systemic exposure of the drug to animals and this mode of oral administration minimized the loss of animals that might have occurred if the gavage route had been chosen.

Dr. Hayden moved that the Technical Report on 5,5-diphenylhydantoin be accepted with the conclusions as written for male and female rats and mice under the three combinations of adult-only exposure, perinatal-only exposure, and combined perinatal and adult exposure. Dr. Davis seconded the motion. Dr. Goodman offered an amendment that for adult-only exposure, the conclusions for male rats be changed from some evidence to equivocal evidence of carcinogenic activity based on a trend test that was only marginally positive, a neoplasm incidence within the historical range, and weight gains less than 90% that of the controls. Dr. Silbergeld seconded the amendment. The amendment was accepted by six yes votes to one no vote (Dr. Zeise) with one abstention (Dr. van Zwieten). Dr. Goodman then offered a second amendment: The maximum tolerated dose was deemed to have been exceeded in female rats in the 0:2,400 ppm and 630:2,400 ppm exposure groups, and in female mice in the 0:600 ppm and 210:600 ppm exposure groups based on an excessive (i.e., 20% to 43%) decrease in body weight gain. The amendment was tabled for lack of a second. Dr. Hayden's original motion as amended by Dr. Goodman was accepted by seven yes votes with one abstention (Dr. van Zwieten).

INTRODUCTION

A series of mishaps with certain therapeutic agents and environmental toxicants has focused attention on the responses of developing organisms to diverse types of biologically active molecules. The occurrence of congenital defects in children resulting from the use of thalidomide by pregnant women, cancer in the daughters of women exposed to diethylstilbestrol during pregnancy, and episodes of congenital methylmercury poisoning have stimulated research in perinatal toxicology (Herbst *et al.*, 1971, 1975; Amin-Zaki *et al.*, 1974). During the perinatal period from conception to birth, and for a short period following birth, some physiologic barriers, such as the blood-brain barrier and some metabolic and excretory systems, such as the liver, kidney, and gut, are not fully developed. Therefore, developing organisms can be more susceptible to the toxic effects of environmental or therapeutic agents (Lewerenz, 1982; Miller, 1983).

Recognition of the heightened sensitivity of developing organisms to chemical toxicity has led to a number of human and laboratory animal studies. Examples of epidemiological studies include evaluations of the relationships between brain neoplasms in children and the occupational exposure of parents to carcinogens (Peters *et al.*, 1981), childhood cancer and parental cigarette smoking (Grufferman *et al.*, 1983; Stjernfeldt *et al.*, 1986; Pershagen, 1989), and childhood leukemia and occupational and home exposure of parents to carcinogens (Lowengart *et al.*, 1987). Arundel and Kinnier-Wilson (1986) have reviewed 14 epidemiology studies that investigate a possible association between childhood cancer and parental occupational exposure to carcinogens. The contradictory observations suggest that more investigations are needed in this field.

Although human data are limited, information on perinatal toxicology and carcinogenesis in laboratory animals began accumulating when Larsen *et al.* (1947) reported a high incidence of lung neoplasms in offspring when pregnant strain A mice were administered urethane 1 day before delivery. This finding of an increased susceptibility of the fetal lung to urethane carcinogenesis was confirmed by Klein (1952). Pietra *et al.* (1959) reported that 12-hour-old mice given a single injection of 9,10-dimethyl-1,2-benzanthracene had a 32% incidence of lymphomas at 15.3 weeks of age, a relatively short period for expression of a tumorigenic effect. Similar decreases in the latency period for expression of tumorigenic effects were obtained with benzo(a)pyrene, 3-methylcholanthrene, and urethane (Pietra *et al.*, 1961). Druckery *et al.* (1966) reported that the teratogen ethylnitrosourea, administered by a single injection to pregnant rats, produced brain neoplasms in offspring at an average age of 160 days, compared to an average age of 360 days for animals exposed to ethylnitrosourea as young adults. The increased sensitivity of fetal nervous tissue to ethylnitrosourea was further studied in Fischer and Sprague-Dawley rats by Swenberg *et al.* (1972), who evaluated the dose-relationship of transplacental brain neoplasm development and concluded that the age at which an animal develops neoplasia following exposure is a function of the exposure levels used. Spontaneous neoplasms of the brain and nerves are rare in mice. However, perinatal exposure of several mouse strains to ethylnitrosourea caused a 6% incidence of neurogenic neoplasms, whereas postnatal ethylnitrosourea exposure resulted in an incidence of only 0.33% (Wechsler *et al.*, 1979). Furthermore, certain types of neoplasms, such as medulloblastomas, astrocytomas, and meningeal neoplasms, were observed only in mice exposed to ethylnitrosourea perinatally.

The carcinogenic response of various tissues following transplacental, neonatal-infant, or adult exposure of mice to a single administration of ethylnitrosourea was studied by Vesselinovitch *et al.* (1979). These studies showed that the age of the animals at the time of exposure to a carcinogen is the most effective modulator of carcinogenesis in the liver, lung, stomach, ovary, and lymphoreticular tissues. Tomatis (1979) reported that exposure of mice to 9,10-dimethyl-1,2-benzanthracene and of rats to ethylnitrosourea or methylnitrosourea during pregnancy resulted in a high incidence of neoplasms in animals of the first generation and in an increased incidence of neoplasms at specific sites in untreated animals of the second and third generations. Germ cell

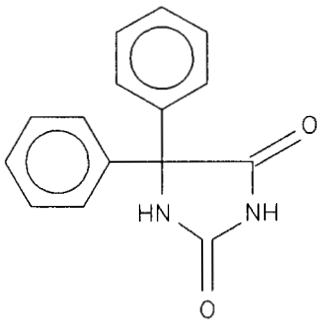
mutation caused by perinatal exposure to a carcinogen was reported by Nomura (1982). The exposure of parent ICR mice to X-rays or urethane resulted in a 90% incidence of lung neoplasms in the offspring; the inheritability of carcinogenic effects in F_1 and F_2 generations was shown. Yamasaki *et al.* (1987) reported that fetal c-Ha-*ras* can be transplacentally activated through a specific point mutation by a carcinogen. Also, when administered to pregnant ICR mice on day 18 of gestation, safrole, 4-aminobiphenyl, and benzo(a)pyrene bind to the DNA of the maternal uterus and placenta and the maternal and fetal liver, lung, kidney, heart, brain, intestine, and skin (Lu *et al.*, 1986).

Toxicology endpoints other than carcinogenicity have also been studied in laboratory animals after perinatal exposure. The toxicity of chemicals to the nervous (Adams and Buelke-Sam, 1981), reproductive (McLachlan *et al.*, 1981), and immune systems (Roberts and Chapman, 1981) is the subject of continuing public health and scientific interest. The field of perinatal toxicology and carcinogenesis has been extensively reviewed (IARC, 1973; NCI, 1979; Alexandrov, 1983; Miller, 1983; Tomatis, 1988). A recent review of environmental, occupational, and therapeutic exposure data by Schardein and Keller (1989) has identified 54 chemicals as potential developmental toxicants in humans.

STUDY RATIONALE

The evaluation of chemicals for carcinogenicity in rodents is usually accomplished by exposing animals to a chemical for 2 years, beginning when the animals are approximately 6 to 8 weeks old (Chhabra *et al.*, 1990). In 1976, a symposium was organized by the National Cancer Institute on perinatal carcinogenesis (NCI, 1979); this group recommended that the perinatal period be incorporated into the period of exposure for conventional carcinogenicity studies (Swenberg, 1979; Vesselinovitch *et al.*, 1979). Therefore, the National Institute of Environmental Health Sciences designed the present studies to incorporate the perinatal period, including exposure of maternal animals prior to breeding, through gestation, lactation, and weaning, followed by conventional exposure of the offspring for 2 years, to compare the sensitivity of the combined perinatal and adult exposure bioassay with the conventional bioassay for detecting carcinogenicity. Three chemicals, ethylene thiourea, 5,5-diphenylhydantoin (phenytoin), and polybrominated biphenyls (Firemaster FF-1®), were selected for these combined perinatal and adult exposure studies. These chemicals can cross the placenta and be secreted in the milk so that developing fetuses and neonates are exposed during the gestation and lactation periods. This report describes the results of the carcinogenicity studies of 5,5-diphenylhydantoin. The studies on ethylene thiourea and polybrominated biphenyls have been previously reported (NTP, 1992, 1993).

5,5-Diphenylhydantoin was selected for study based on its frequent use in the treatment of grand mal and psychomotor seizures in humans and its possible association with increased incidences of lymphomas during long-term treatment of epilepsy and with increased incidences of neuroblastoma seen in children exposed to 5,5-diphenylhydantoin prenatally.



5,5-DIPHENYLHYDANTOIN (PHENYTOIN)

CAS No. 57-41-0

Chemical Formula: $C_{15}H_{12}N_2O_2$ Molecular Weight: 252.27

Synonyms: Diphenylhydantoin; 5,5-diphenyl-2,4-imidazolidinedione;
Trade names: Difhydan; Dihycon; Di-Hydan; Di-Lan; Dilabid; Dilantin; Ekko; Hydantol; Lehydan; Zentropil

Chemical and Physical Properties

5,5-Diphenylhydantoin is a white, odorless, crystalline powder that is virtually insoluble in water, soluble in hot alcohol, and slightly soluble in cold alcohol, chloroform, and ether (*Merck Index*, 1983). 5,5-Diphenylhydantoin is available in the United States as a United States Pharmacopeia grade containing 98.5% to 100.5% active ingredient on a dried basis with a maximum of 0.002% heavy metals.

Use and Human Exposure

5,5-Diphenylhydantoin and its sodium salt are primarily used in the treatment of grand mal and psychomotor seizures, often in combination with other anticonvulsants including phenobarbital. Diphenylhydantoin sodium may be used in the treatment of ventricular tachycardia and paroxysmal atrial tachycardia, particularly in those patients who do not respond to conventional antiarrhythmic agents. In the past, 5,5-diphenylhydantoin has been used in the treatment of acute alcoholism, migraine, polyneuritis, pregnancy disorders, certain psychoses, and trigeminal neuralgia. Diphenylhydantoin sodium is used to control status epilepticus and as a prophylactic for the control of seizures in neurosurgery and has been investigated for use in the treatment of migraine, certain psychoses, and trigeminal neuralgia. In veterinary medicine, 5,5-diphenylhydantoin is used to control epileptiform convulsions in dogs (IARC, 1977; *Merck Index*, 1983).

The primary routes of potential human exposure to 5,5-diphenylhydantoin are oral, injection, inhalation, and dermal contact. The drug is given to a major segment of individuals suffering from epilepsy. The initial oral dosage for adults and children over 6 years of age is 100 mg three times per day; the dosage may be gradually increased by 100 mg every 2 to 4 weeks until the desired therapeutic response is obtained. Maintenance dosages usually range from 300 to 600 mg daily for adults and 3 to 10 mg/kg body weight daily for children under 6 years of age. Exposure of health professionals may occur during the preparation and administration of 5,5-diphenylhydantoin. Occupational exposure may also occur for workers involved in the formulation and packaging of the pharmaceutical (IARC, 1977; NTP, 1989).

5,5-Diphenylhydantoin production was reported to the U.S. International Trade Commission (USITC) by one producer in the years 1984 to 1986, implying that annual production or sales volume was greater than 1,000 pounds (USITC, 1985, 1986, 1987). The National Institute for Occupational Safety and Health (NIOSH) has estimated that 23,400 males and 16,795 females may have been exposed to 5,5-diphenylhydantoin (NIOSH, 1992).

PHARMACOLOGIC EFFECTS

5,5-Diphenylhydantoin exerts antiepileptic activity without causing general depression of the central nervous system. The most easily demonstrated properties of 5,5-diphenylhydantoin are its abilities to limit the development of maximal seizure activity and to reduce the spread of the seizure process from an active focus. The primary site of action appears to be the motor cortex where the spread of seizure activity is inhibited. 5,5-Diphenylhydantoin tends to stabilize the threshold against hyperexcitability caused by excessive stimulation, possibly by promoting sodium efflux from neurons. In addition, 5,5-diphenylhydantoin exhibits antiarrhythmic properties similar to those of quinidine or procainamide. Although the drug has little effect on the electrical excitability of cardiac muscle, it decreases the force of contraction, depresses pacemaker action, and improves atrioventricular conduction, particularly when conduction has been depressed by digitalis glycosides (*Goodman and Gilman's*, 1985; PDR, 1989).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The studies on pharmacokinetics of 5,5-diphenylhydantoin in humans and laboratory animals have been reviewed in the literature (Woodbury and Swinyard, 1972; Richens, 1979). 5,5-Diphenylhydantoin and its sodium salt are usually completely absorbed from the gastrointestinal tract, mainly from the duodenum. The distal portion of the duodenum is also the site of maximum reabsorption of 5,5-diphenylhydantoin after intravenous injection in rats. In humans, peak blood levels are generally reached between 4 and 8 hours after administration of a single oral dose. The time of peak effect appears to be independent of the dose. On entering the circulatory system, 5,5-diphenylhydantoin is rapidly and reversibly bound to plasma proteins. The average plasma protein binding is approximately 90% in humans and 80% in rats. Within 15 minutes after absorption, the drug reaches its maximum volume of distribution, which in humans may range from 1.6 to 2.5 times the free level of 5,5-diphenylhydantoin in plasma. In rats, mice, and cats, 5,5-diphenylhydantoin is present in brain, liver, muscle, and fat at higher concentrations than in plasma. The accumulation of 5,5-diphenylhydantoin in tissues is mainly due to binding, because the concentration of free 5,5-diphenylhydantoin in all tissues of the body is the same as that in plasma (Woodbury and Swinyard, 1972). Placental transfer of 5,5-diphenylhydantoin has been reported to occur in rats, monkeys, goats, mice, and humans (Egger *et al.*, 1978). Recently, Meskin and Lien (1985) have shown that 5,5-diphenylhydantoin is also excreted in human breast milk.

5,5-Diphenylhydantoin is excreted mainly as metabolites in urine and feces. Less than 5% of the total drug is excreted as the nonmetabolized form in the urine of experimental animals and humans; only a very small amount is excreted as this form in the feces. The rate of excretion for 5,5-diphenylhydantoin is slow due to high plasma protein binding (80% to 90%). In rats, about 48 to 60 hours are required for complete excretion of an orally or intravenously administered dose; after oral administration in humans, complete excretion requires 72 to 120 hours (Woodbury and Swinyard, 1972). The major urinary metabolite of 5,5-diphenylhydantoin in humans, rats, and mice is the phenol, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (*p*-HPPH); this metabolite is excreted in the urine as a glucuronic acid conjugate. A number of minor metabolites of 5,5-diphenylhydantoin have been identified. These include dihydrodiol (DHD), a catechol, *m*-HPPH, a methylated catechol, and diphenylhydantoin acid, a product formed by the opening of the hydantoin ring. The constant ratio of *p*-HPPH:DHD excreted in mouse urine suggests that these metabolites have a common precursor, probably an arene oxide (Chow and Fischer, 1982). The pathway of 5,5-diphenylhydantoin metabolism to the major metabolite, *p*-HPPH, is saturable and gives rise to a nonlinear dose-serum concentration relationship. Therefore, the dose range compatible with a therapeutic serum concentration is of particular value in dosage tailoring (Richens, 1979).

5,5-Diphenylhydantoin biotransformation mainly occurs through microsomal P_{450}-mediated metabolic pathways. 5,5-Diphenylhydantoin elimination and metabolism appear to be dependent upon the rate-limiting step involving P_{450}-mediated monooxygenases. A number of drugs that are substrates for P_{450} are competitive inhibitors of 5,5-diphenylhydantoin metabolism, as shown by prolonged half-life, increased steady-state level, or increased signs of toxicity. In turn, 5,5-diphenylhydantoin is known to stimulate or to interfere with the metabolism of many other drugs that are also P_{450} substrates. 5,5-Diphenylhydantoin also appears to be an inducer of its own metabolism in mice (Atlas *et al.*, 1980). The clinical implications of enzyme induction and

inhibition by drugs including 5,5-diphenylhydantoin have been reviewed by Park and Breckenridge (1981).

TOXICITY

5,5-Diphenylhydantoin, the drug of choice in grand mal epilepsy treatment for more than 50 years, is associated with adverse effects that have compromised the success of therapy since its introduction. There are hundreds of research reports on 5,5-diphenylhydantoin in humans and laboratory animals in the literature. A brief description of adverse effects of 5,5-diphenylhydantoin in humans and laboratory animals is given here.

Experimental Animals

The nervous system is a major target of the acute toxicity of 5,5-diphenylhydantoin in experimental animals. Convulsions induced by 5,5-diphenylhydantoin in laboratory animals were first described over 40 years ago (Mareš *et al.*, 1987), who studied the toxic effects of single intraperitoneal injection (200 to 1,000 mg/kg) of 5,5-diphenylhydantoin during ontogenesis in 7-, 12-, 18-, 25-, and 90-day-old male albino rats. Mareš *et al.* found that the exposure level of 5,5-diphenylhydantoin necessary for elicitation of seizures was lowest (75 mg/kg) in 7-day-old rats and increased with age to 200 mg/kg in 18-day-old rats. The 1,000 mg/kg exposure was lethal for 12- and 25-day-old rats, but not for 7-day-old rats. An uncoordinated development of response to the excitatory and inhibitory actions of 5,5-diphenylhydantoin was suggested.

The chronic effects of 5,5-diphenylhydantoin on the peripheral nervous system were studied by Moglia *et al.* (1981). Sixty albino Sprague-Dawley female rats were orally administered 30 mg/kg 5,5-diphenylhydantoin per day. Between days 75 and 90, motor and sensory conduction velocities along the tail were examined in 15 treated and 10 control animals. A slowing of sensory conduction velocity was shown in six treated animals; in two of them, the motor conduction velocity was also slowed. The slowing of sensory and motor conduction velocities was more frequent between days 165 and 180 of 5,5-diphenylhydantoin administration. Out of 25 treated animals, 14 had slowing of sensory conduction velocity and five had slowing of motor conduction velocity. Histological and ultrastructural study of the sciatic nerves revealed changes of the myelinated fibers only in the animals with slowed motor conduction velocity. The nonmyelinated fibers were apparently normal. Decreases in motor activity have also been observed in mice treated with 5,5-diphenylhydantoin (Poncelet *et al.*, 1984). The effects of chronic administration of 5,5-diphenylhydantoin on learning and behavior of offspring of Sprague-Dawley rats were studied by Rowley and Gauron (1977). A group of rats was treated with 5,5-diphenylhydantoin at exposure levels of 5 to 15 mg/kg from days 5 to 55 after birth. At 85 days of age, the treated females were bred to naive males. The F_1 female offspring were bred at maturity to produce an F_2 generation. The F_1 and F_2 generations were not treated. The results showed that in comparison to the control group, the learning ability of both the F_1 and F_2 generations was adversely affected, as measured by avoidance conditioning.

Elmazar and Sullivan (1981) demonstrated that prenatal 5,5-diphenylhydantoin administration to rats, in exposures producing blood levels in the therapeutic range, resulted in delays in motor development and persistent impairment of locomotor function.

5,5-Diphenylhydantoin is teratogenic in the mouse and in the rat. Cleft palate is the major malformation produced in teratologic studies; other anomalies noted in various animal model systems include shortened long bones, fused vertebrae, and other skeletal defects, cardiac abnormalities, internal hydrocephalus, decreased fetal movement, growth retardation, and hydronephrosis (Elmazar and Sullivan, 1981; Lorente *et al.*, 1981). The fetal hydantoin syndrome seen in humans has been reproduced in mice and rat models. The growth deficiencies, as evidenced by low fetal weights and incomplete ossification, and ocular, neural, cardiac, renal, gastrointestinal, and skeletal anomalies found in the mice and rats are similar to those found in the human syndrome (Finnell, 1980; Lorente *et al.*, 1981).

Immunologic disturbances, which are found in many epileptic patients, are commonly ascribed to 5,5-diphenylhydantoin treatment. The various side effects of 5,5-diphenylhydantoin on the immune system in adults include autoimmune diseases and imbalanced gamma-globulinemia, and 5,5-diphenylhydantoin exposure has been linked to increased incidences of pseudolymphomas and malignant lymphomas (Aarli, 1980; Kohler *et al.*, 1987). The effects of 5,5-diphenylhydantoin on cellular immunity in mice were examined by Okamoto *et al.* (1988), who found that 5,5-diphenylhydantoin suppresses the

proliferative response of lymphocytes to mitogens and suppresses natural killer and cytotoxic T lymphocyte activities. The studies in C3H mice have shown that the developing immune system of the fetal mouse is more susceptible to 5,5-diphenylhydantoin than is the immune system of the adult (Kohler *et al.*, 1987).

Humans

Acute overdose by the oral route results in clinical signs of toxicity in the cerebellum and vestibular system. Chronic 5,5-diphenylhydantoin overmedication results in cerebellar and vestibular effects, behavioral changes, increased frequency of seizures, gastrointestinal symptoms, gingival hyperplasia, osteomalacia, and megaloblastic anemia. A broad spectrum of cutaneous and immunologic reactions to 5,5-diphenylhydantoin has been reported. These reactions include tissue proliferative syndromes, drug hypersensitivity syndromes, and possibly lymphoma (Isobe *et al.*, 1983; Silverman *et al.*, 1988). Serious adverse effects, including skin, bone marrow, and liver effects, are possibly secondary to drug allergy (*Goodman and Gilman's*, 1985; PDR, 1989). Hepatotoxicity is a well-documented effect of 5,5-diphenylhydantoin therapy. It is idiosyncratic, not exposure-related, and uncommon. The onset of symptoms occurs early in therapy, usually within the first 6 weeks, and clinically mimics other hepatitis-like syndromes (Smythe and Unstead, 1989).

The teratogenic effects of 5,5-diphenylhydantoin medication in humans have been reported by Dabee *et al.* (1975) and Smith (1980). The fetal hydantoin syndrome, a variable pattern of altered growth and performance which includes unusual facies, distal phalangeal hypoplasia, and other defects, has been reported in some infants exposed *in utero* to hydantoins (Hanson *et al.*, 1976). A prospective study of 35 infants exposed prenatally to this class of anticonvulsants showed that 11% had defects sufficient for classification as having the fetal hydantoin syndrome.

CARCINOGENICITY

Experimental Animals

5,5-Diphenylhydantoin has been studied for carcinogenic potential in various strains of mice and in Fischer 344 rats. In 50 albino mice given daily intraperitoneal injections of 5,5-diphenylhydantoin for 66 days and then observed for 7 months, 4 of 40 survivors developed thymic lymphomas, 2 developed mesenteric lymphomas, and 4 developed leukemia. One animal with thymic lymphoma and one with leukemia were observed among 50 controls. The incidence of lymphoma was only marginally statistically significant, but the short duration of the study may have precluded the development of additional neoplasms (IARC, 1977).

In a 16-month study, groups of female C57BL, C3H/F, or SJL/J mice were fed 60 mg/kg 5,5-diphenylhydantoin sodium in a liquid diet for 168 days. Three of 24 C57BL mice and 3 of 24 C3H/F mice that survived to the end of the study, as well as 6 of 42 SJL/J mice, developed thymic lymphomas. The neoplasm incidences were not statistically significant, but no neoplasms were observed in groups of 48 controls of each strain (Krüger *et al.*, 1972). In another study, groups of 50 male and female $B6C3F_1$ mice were given 0.006% or 0.012% 5,5-diphenylhydantoin in a powdered diet for 78 weeks and were then fed a basal diet for 8 weeks. The incidences of malignant lymphoma, leukemia, or both in exposed groups were similar to those in the control groups, and it was concluded that 5,5-diphenylhydantoin was not carcinogenic in $B6C3F_1$ mice (Maeda *et al.*, 1988).

In male and female F344 rats that received 0.025% or 0.05% exposure levels of 5,5-diphenylhydantoin in the diet for 2 years, the incidences of neoplasms in the treated and control groups were similar, suggesting that, under these conditions, 5,5-diphenylhydantoin was not carcinogenic in F344 rats (Jang *et al.*, 1987).

Humans

In several epidemiological studies, an association has been observed between the incidence of lymphomas and long-term treatment of epilepsy with 5,5-diphenylhydantoin (Anthony, 1970; Li *et al.*, 1975; IARC, 1977). Aguiar *et al.* (1987) suggested that the risk of lymphoma development in patients who took 5,5-diphenylhydantoin was four times higher than in those who did not. There have been several case reports of lymphoma among individuals under 5,5-diphenylhydantoin therapy. However, in two follow-up studies of epilepsy patients, no significantly increased incidences of lymphoma were reported. An increased incidence of neurological neoplasms, including brain neoplasms, was reported among people prescribed 5,5-diphenylhydantoin. This increased incidence is similar to that reported among epileptics and may reflect the underlying diseases rather than use of the drug *per se* (IARC, 1987).

The increased incidences of neoplasms, especially neuroblastoma, seen in children exposed to 5,5-diphenylhydantoin prenatally suggest that 5,5-diphenylhydantoin may be a human transplacental carcinogen (Napalkov, 1986; IARC, 1987).

Based on the results of epidemiology and animal studies, the International Agency for Research on Cancer (IARC) has determined that there is limited evidence of carcinogenicity of 5,5-diphenylhydantoin in humans and animals (IARC, 1977, 1987).

Genetic Toxicity

5,5-Diphenylhydantoin does not appear to induce gene mutations in bacteria or *Drosophila melanogaster*; there are, however, conflicting reports on the ability of 5,5-diphenylhydantoin to induce chromosomal effects *in vitro* and *in vivo*. 5,5-Diphenylhydantoin induces mitotic arrest in human lymphocytes *in vitro* and is believed to act via the inhibition of microtubule polymerization (MacKinney *et al.*, 1978, 1980). It therefore has the potential to induce aneuploidy. Any genetic effects of 5,5-diphenylhydantoin may result from the action of some of its metabolic intermediates such as the arene oxides (Barcellona *et al.*, 1987).

5,5-Diphenylhydantoin was negative for the induction of gene mutations with and without exogenous metabolic activation (S9) in several strains of *Salmonella typhimurium* (Sezzano *et al.*, 1982; Haworth *et al.*, 1983; Léonard *et al.*, 1984) with one exception. Sezzano *et al.* (1982) reported weak induction of gene mutations in the frameshift strain TA1538 in experiments conducted with S9 from 3-methylcholanthrene- or Aroclor 1254-induced rats. Similarly, a significant increase in the number of mutant colonies was seen in strains TA98 and TA1538 after treatment with the hydroxyphenyl derivative, 5,4-hydroxyphenyl-5-phenylhydantoin (HPPH), in the presence of S9 from ß-naphthoflavone-,3-methylcholanthrene-,or Aroclor 1254-induced rats. These experiments with 5,5-diphenylhydantoin and HPPH were repeated by Léonard *et al.* (1984), who obtained negative results. Therefore, the mutagenic activity of 5,5-diphenylhydantoin in *S. typhimurium* must be considered uncertain. 5,5-Diphenylhydantoin did not induce sex-linked recessive lethal mutations in germ cells of male *D. melanogaster* when administered by feeding or injection (Woodruff *et al.*, 1985).

The ability of 5,5-diphenylhydantoin to induce chromosomal aberrations in rodent and human cells *in vitro* has been investigated numerous times and the results, with one exception, were negative (Stenchever and Jarvis, 1971; Bishun *et al.*, 1975; Alving *et al.*, 1976; Léonard *et al.*, 1984; Reidel and Obe, 1984; Galloway *et al.*, 1987). The exception, a positive response in human lymphocytes (Sagredo, 1988), is difficult to evaluate, because the control rate of chromosomal aberrations reported for nonexposed cells (6.88%) was much higher than the 1% to 2% rate that is considered normal for this cell type (Bender *et al.*, 1989). Sister chromatid exchange induction by 5,5-diphenylhydantoin has been reported in human lymphocytes treated without S9 (Maurya and Goyle, 1985) and in cultured Chinese hamster ovary cells treated with S9 (Galloway *et al.*, 1987).

Results of *in vivo* investigations of the clastogenicity of 5,5-diphenylhydantoin in humans are mixed. Chromosomal damage in peripheral lymphocytes of humans treated with 5,5-diphenylhydantoin has been reported (Große *et al.*, 1972; Ayraud *et al.*, 1974; Herha and Obe, 1976), but a number of other investigations found no increases in chromosomal aberrations in patients undergoing 5,5-diphenylhydantoin therapy (Bartsch, 1975; Alving *et al.*, 1976; Knuutila *et al.*, 1977; Eßer *et al.*, 1981; Kulkarni *et al.*, 1984). A major factor in these discordant findings may be the scoring of chromatid and isochromatid gaps, because these are rather subjective lesions to score and are included in some analyses and not in others. Sister chromatid exchange frequencies in 5,5-diphenylhydantoin-treated patients have shown significant increases compared to control subjects (Kulkarni *et al.*, 1984; Schaumann *et al.*, 1985).

No increase in the frequency of chromosomal aberrations was reported in bone marrow cells of rats given three 50 mg/kg doses of 5,5-diphenylhydantoin at 24-hour intervals and sampled after 12 or 24 hours (Alving *et al.*, 1976). Also, de Oliveira *et al.* (1987) found no significant increases in chromosomal aberrations in bone marrow cells of Balb/C female mice treated with 5,5-diphenylhydantoin either in a therapeutic regimen (0.48 mg per animal, 3 days a week for 2 months) or in a regimen designed to produce teratogenic effects on fetuses (three 50 or 100 mg/kg exposures at 24-hour intervals, and sampled after 24 hours). McFee *et al.* (1992) also reported negative results for induction of chromosomal aberrations in bone marrow cells of male

$B6C3F_1$ mice sampled 17 hours (125 to 500 mg/kg) or 36 hours (37.5 to 150 mg/kg) after treatment with 5,5-diphenylhydantoin (Table E7), but slight increases in bone marrow sister chromatid exchanges were observed 23 hours (62.5 to 250 mg/kg) and 42 hours (25 to 100 mg/kg) after treatment (Table E6). Increases in the frequency of micronucleated polychromatic erythrocytes following 5,5-diphenylhydantoin treatment have been reported in the liver tissue of 13-day-old mouse fetuses of dams treated with 100 mg/kg 5,5-diphenylhydantoin on day 12 of gestation (Barcellona *et al.*, 1987). However, no increase in the frequency of micronucleated polychromatic erythrocytes was observed in bone marrow of pregnant Swiss (CD-1®) mice sampled 18 or 30 hours after treatment with 100 mg/kg 5,5-diphenylhydantoin. Also, no increase in micronucleated polychromatic erythrocytes was seen in bone marrow cells of male $B6C3F_1$ mice injected intraperitoneally with 7.5 to 70.0 mg/kg 5,5-diphenylhydantoin three times at 24-hour intervals and sampled 24 hours after the third treatment (Table E8; McFee *et al.*, 1992). Although an increase in micronucleated polychromatic erythrocytes was reported in the bone marrow of male Balb/C mice receiving single injections of 0.5 or 1.0 mg/kg 5,5-diphenylhydantoin (de Oca-Luna *et al.*, 1984), a similar study performed by the NTP yielded negative results (Table E9).

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 5,5-DIPHENYLHYDANTOIN

5,5-Diphenylhydantoin was obtained from Parke-Davis and Company (Detroit, MI) in one lot (H-732008), which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and are discussed in Appendix H. The bulk chemical was identified as 5,5-diphenylhydantoin by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopies.

The purity of 5,5-diphenylhydantoin was approximately 98%, as determined by elemental analyses, Karl Fischer water analysis, titration of the imide group, thin-layer chromatography, and high-performance liquid chromatography. Elemental analysis for carbon was slightly high; elemental analyses for oxygen, hydrogen, and nitrogen were in agreement with the theoretical values. Karl Fischer water analysis indicated 0.28 ± 0.15% water. Titration of the imide group indicated a purity of 99.97 ± 0.74%. Thin-layer chromatography analysis indicated only one trace impurity. High-performance liquid chromatography indicated two impurities with areas of 0.09% and 5.5% of the major peak area. Comparison of lot H-732008 with a United States Pharmacopeia standard indicated the same major areas (± 2%), with the 5.5% impurity eluting in lot H-732008 but not in the standard; this probably indicates that the impurity had a higher absorbance and was present at a much lower concentration than 5.5%.

Stability studies performed by the analytical chemistry laboratory using high-performance liquid chromatography indicated that 5,5-diphenylhydantoin is stable as a bulk chemical for at least 2 weeks at temperatures up to 60° C. Throughout the studies, the bulk chemical was stored in plastic-lined metal containers at room temperature. The stability of the bulk chemical was monitored periodically by the study laboratory using infrared spectroscopy and high-performance liquid chromatography. No significant degradation of the study material was seen throughout the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing 5,5-diphenylhydantoin with feed (Table H1). During the studies, the dose formulations were stored at room temperature for no longer than 2 weeks.

The study laboratory conducted periodic analyses of the dose formulations using high-performance liquid chromatography as described in Appendix H. Dose formulations were analyzed once during the 13-week and maximum neonatal dose determination studies; all dose formulations were within 10% of the target concentrations (Tables H2 and H3). During the 2-year studies, dose formulations were analyzed approximately every 2 months. For rats, 38 of the 39 dose formulations were within 10% of the target concentrations; 62 of 67 dose formulations for mice were within specifications (Table H4).

13-WEEK STUDIES

The 13-week studies were conducted to determine the cumulative toxic effects of repeated exposure to 5,5-diphenylhydantoin and to determine appropriate concentrations for use in the gestational and 2-year studies. Male and female F344 rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Kingston, NY) and observed for 18 to 20 days before the studies began. Rats and mice were 7 to 9 weeks old when the studies began. Groups of 10 male and female rats were exposed to 0, 300, 600, 1,200, 2,400, or 4,800 ppm 5,5-diphenylhydantoin in feed; groups of 10 male and female mice were exposed to 0, 75, 150, 300, 600, or 1,200 ppm 5,5-diphenylhydantoin in feed. All groups were treated for 13 weeks, 7 days a week. Rats and mice were housed five per cage; feed and water were available *ad libitum*. Animals were observed and findings were recorded twice each day. Animals were weighed at the start of the study and weekly

thereafter; feed consumption was measured weekly. Further experimental details are presented in Table 1.

GESTATIONAL STUDIES AND DETERMINATION OF MAXIMUM PERINATAL DOSE

Groups of 10 female F344/N rats and C57BL/6N mice were exposed to 5,5-diphenylhydantoin in feed for 2 weeks before breeding and throughout gestation and lactation. Female rats were exposed to 0, 80, 240, 800, or 2,400 ppm and female mice were exposed to 0, 20, 60, 200, or 600 ppm. Females were bred to previously unexposed male F344/N rats or C3H/HeN mice. Four pregnant rats from each group (no pregnancies occurred in the 2,400 ppm group) were evaluated on prenatal day 18 for numbers of implantations, live fetuses, fetuses per litter, fetal weights, and placental weights. Litter weights of rats and mice were recorded on day 1, and pups were weighed on days 4 and 28. Studies were performed on day 12 postpartum on four rat dams and litters (culled to five pups per litter) from each exposure group to determine absolute and relative liver weights.

After being weaned on day 28 postpartum, selected weanlings (10 per dose group) were continued at the same exposure level for 4 weeks. No more than one male and one female rat from the same litter were placed in the postweaning dose groups; due to a low number of litters, all mouse weanlings were used. Following the 28-day period of exposure, all animals were killed with CO_2 and a complete necropsy examination was performed. A histopathologic examination was performed on all F_1 animals. Tissues examined are listed in Table 1.

2-YEAR STUDIES

Study Design

Groups of 60 male and 60 female rats and mice received perinatal exposure (F_0), adult exposure (F_1), or both to various concentrations of 5,5-diphenylhydantoin (Table 1).

Female F344/N rats were exposed to 0, 63, 210, or 630 ppm in feed for 1 week before breeding. Female C57BL/6N mice were exposed to 0, 21, 70, or 210 ppm in feed for 1 week before breeding. After breeding to previously unexposed males (F344/N rats, C3H/HeN mice), all females were housed singly and were continued on their previous diet. Exposure continued throughout pregnancy and lactation. Weaning occurred on day 28 postpartum, and dietary exposure at these same concentrations continued until the pups were approximately 8 weeks of age (Figure 1).

On postpartum day 4 (rats) or day 7 (mice), litters were culled to a maximum of eight pups, and the number, sex, and body weight of pups were recorded. After weaning, pups were weighed and separated by sex, and litter mates were cohoused. At approximately 8 weeks of age, groups of 60 male and 60 female pups began receiving the adult (F_1) dietary concentrations and were continued on these diets for up to 2 years. Rats received F_1 concentrations of 0, 240, 800, or 2,400 ppm. Male mice received 0, 30, 100, or 300 ppm, and female mice received 0, 60, 200, or 600 ppm. After 9 months of 5,5-diphenylhydantoin administration, 10 animals from each group were evaluated.

Source and Specification of Breeder Animals

Male and female F344 rats and male C3H/HeN and female C57BL/6N mice were obtained from Charles River Breeding Laboratories (rats, Portage, MI; mice, Kingston, NY). Rats were observed for 6 to 7 weeks and mice for 6 to 8 weeks. Rats were 10 to 12 weeks old and mice were 10 to 14 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix J).

Animal Maintenance

Animals were housed five per cage. Cages were rotated within racks and racks were rotated within rooms monthly. Feed and water were available *ad libitum*. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

Clinical observations were made twice daily, and findings were recorded weekly. F_1 animals were weighed at study initiation, weekly for 13 weeks, and monthly thereafter. Necropsies were performed on all animals.

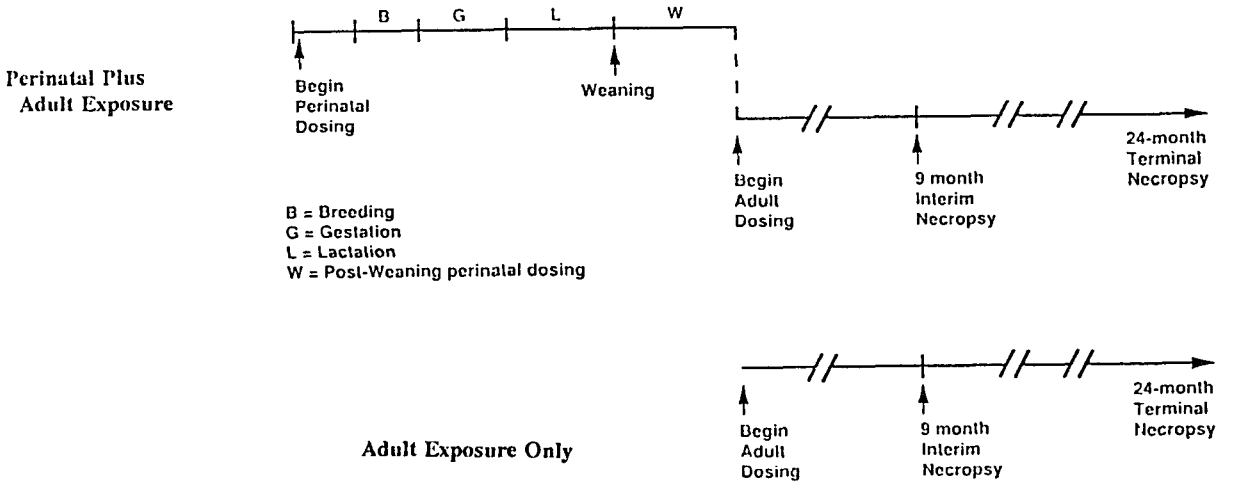


FIGURE 1
2-Year Study Design

The adrenal gland, brain, heart, right kidney, liver, lung, ovary, pituitary gland, prostate gland, right testis, thymus, thyroid gland, and uterus of each animal were weighed at necropsy. Further details of the interim evaluations are presented in Table 1.

Hematologic and biochemical analyses were performed at the 9-month interim evaluations. Analyses methods are provided in Appendix G.

Animals found in a moribund state, selected for the 9-month interim evaluations, or surviving to the end of the 2-year studies were killed with CO_2. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Complete histopathologic examinations were performed on all animals that died or were killed moribund and all animals in the control (0:0 ppm) and high-dose (0:2,400 and 630:2,400 ppm for rats; 0:300, 0:600, 210:300, and 210:600 ppm for mice) groups. Tissues examined from all low-dose animals are listed in Table 1.

Upon completion of the microscopic evaluation by the study laboratory pathologist, the pathology data were entered into the Toxicology Data Management System. The microscope 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 pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slide and tissue counts were verified, and histotechnique was evaluated by the quality assessment laboratory. The adrenal medulla of male rats and the liver of male and female rats and mice were reviewed microscopically by the quality assessment pathologist for both neoplastic and nonneoplastic lesions. All neoplastic diagnoses in all tissues from all rats and mice and all tissues from a randomly selected 10% of the control and high-dose rats and mice were reevaluated microscopically by a quality assessment pathologist.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnosis between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chair to the PWG for review. These included examples of lesions of the liver, adrenal medulla, and uterus of rats and hemangiosarcomas and lesions of the liver, thyroid gland, ovary, and forestomach of mice. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed to reflect the PWG consensus. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of pathology data, the diagnosed lesions for each tissue type are evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

The experimental design of these studies was complex (a 4 × 4 matrix with missing cells), and both perinatal and postnatal effects were evaluated. The effect of adult-only exposure to 5,5-diphenylhydantoin (i.e., the standard 2-year study design) was analyzed by comparison of F_0:F_1 groups 0:0, 0:800, and 0:2,400 (rats), 0:0, 0:100, and 0:300 (male mice), and 0:0, 0:200, and 0:600 (female mice). To determine perinatal effects, supplemental analyses were carried out in addition to the usual comparison of exposed groups to controls. Specifically, for a fixed adult (F_1) exposure concentration, the effect of varying perinatal (F_0) exposure was evaluated. For example, in rats, comparisons were made between groups 0:0 and 630:0, among groups 0:800, 210:800, and 630:800, and between groups 0:2,400 and 630:2,400. Comparisons were also made between groups with varying perinatal and adult exposure concentrations and the 0:0 ppm control group. It is recognized that these multiple comparisons are not all strictly independent, but taken collectively, they should provide a reasonable evaluation of the overall effects of perinatal and adult exposure to 5,5-diphenylhydantoin.

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. Statistical analyses for a possible dose-related effect on survival were performed using the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 are given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are also given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Neoplasm Incidences

The majority of neoplasms in these studies 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 neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm 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 if it did not significantly enhance the fit of the model. The exposed 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 neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm 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 neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals. The possible enhancing effects of F_0 and F_1 exposure were assessed by the test for interaction developed by Piegorsch *et al.* (1986).

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

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in these studies were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluations, the Fisher exact test, a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which have typically skewed distributions, were analyzed using the multiple comparison methods of Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response (Dunnett's or Dunn's test).

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 neoplasm incidence. Consequently, control neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

Quality Assurance Methods

As study records for the 2-year studies were submitted to the NTP archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and board review draft of the NTP Technical Report were conducted. Audit procedures and findings are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so that all discrepancies had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of 5,5-diphenylhydantoin was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, trifluorothymidine resistance in L5178Y mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells and mouse bone marrow cells, sex-linked recessive lethal mutations in *Drosophila melanogaster*, and micronucleated erythrocytes in mouse bone marrow cells. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of 5,5-diphenylhydantoin are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure of the chemical and its responses in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemical-induced DNA damage and to predict carcinogenicity in animals based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 5,5-Diphenylhydantoin

13-Week Studies	Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Study Laboratory		
Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species		
Rats: F344/N Mice: $B6C3F_1$	F_0 and F_1 rats: F344/N F_0 mice: C3H/HeN males and C57BL/6N females F_1 mice: $B6C3F_1$	F_0 and F_1 rats: F344/N F_0 mice: C3H/HeN males and C57BL/6N females F_1 mice: $B6C3F_1$
Animal Source		
Charles River Breeding Laboratories (Kingston, NY)	F_0: Male rats, Harlan Industries (Indianapolis, IN); female rats, Charles River Breeding Laboratories (Portage, MI); mice, Charles River Breeding Laboratories (Kingston, NY) F_1: bred at the study laboratory from F_0 animals	F_0: Rats, Charles River Breeding Laboratories (Portage, MI); mice, Charles River Breeding Laboratories (Kingston, NY) F_1: bred at the study laboratory from F_0 animals
Size of Study Groups		
10 males and 10 females	Same as 13-week studies	60 males and 60 females
Time Held Before Study		
Rats: 18 days Mice: 19-20 days	F_0 females: 24-26 days	F_0 females: 6-7 weeks (rats); 6-8 weeks (mice)
Average Age When Placed on Study		
7-9 weeks	F_0 females: 8-10 weeks	F_0 females: 10-12 weeks (rats); 10-14 weeks (mice) F_1: 8 weeks (age when adult dosing began)
Date of First Dose		
Rats: 30 October 1979 Mice: 1-2 November 1979	F_0 females: 1 December 1980	F_0 females: 29 June 1982 (rats); 23 April 1982 (mice) F_1: 1 October 1982 (male rats); 4 October 1982 (female rats); 28 July 1982 (male mice); 26 July 1982 (female mice)
Duration of Dosing		
13 weeks	F_0 females: from 2 weeks before breeding through weaning F_1: 7 days a week for up to 8 weeks (4 weeks post weaning)	F_0 females: from 1 week before breeding through weaning F_1: F_0 doses through gestation, lactation, and 4 weeks post weaning; F_1 doses 7 days/week for 105-106 weeks (rats and male mice) or 106-107 weeks (female mice)

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 5,5-Diphenylhydantoin
(continued)

13-Week Studies	Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Method of Sacrifice		
CO_2	CO_2	CO_2
Necropsy Dates		
Rats: 29 January 1980 Mice: 29-30 January 1980	Rats: 9 March 1981 Mice: 5 March 1981	Interim: 5-8 July 1983 (rats), 25-28 April 1983 (mice) Terminal: 1-11 October 1984 (rats), 30 July 1984 - 10 August 1984 (mice)
Average Age at Necropsy		
20-21 weeks	F_0: 18-19 weeks F_1: 8 weeks	F_0: 18-19 weeks F_1: 11 or 26 months
Method of Animal Distribution		
Animals were randomized by weight with a computer randomization program.	F_0 females: Randomized by weight with a computer randomization program F_1: Random among littermates of same sex. Groups included no more than one male rat and one female rat from a single litter; all mouse weanlings were used.	F_0 females: Randomized by weight with a computer randomization program F_1: Random among littermates of same sex; groups included no more than two males and two females from a single litter
Animals per Cage		
5	F_0: 1 female and 1 male at night during breeding; females housed singly after becoming pregnant F_1: 1	F_0: 1 male and 2 (rats) or 3 (mice) females during breeding; females housed singly after becoming pregnant F_1: 5 after weaning
Method of Animal Identification		
Ear tag	Ear tag	Ear tag and toe clip
Diet		
Purina Certified Rodent Chow® meal (No. 5002), available *ad libitum*	Purina Certified Rodent Chow® meal (No. 5002); available *ad libitum* except at night during breeding	Same as 13-week studies

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 5,5-Diphenylhydantoin
(continued)

13-Week Studies	Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Water		
Tap water (City of Columbus) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Tap water (City of Columbus) via plastic disposable water bottles, available *ad libitum*	Tap water (City of Columbus) via plastic disposable water bottles through weaning, then automatic watering system (Edstrom Industries, Inc., Waterford, WI); available *ad libitum*
Cages		
Polycarbonate (Lab Products, Inc., Garfield, NJ), changed twice weekly	Same as 13-week studies	Polycarbonate (Lab Products, Inc., Garfield, NJ), changed twice weekly except during week 1 postpartum
Bedding		
Absorb-Dri® hardwood chips (Absorb-Dri, Inc., Maywood, NJ), changed twice weekly	Same as 13-week studies	Absorb-Dri® hardwood chips (Absorb-Dri, Inc., Maywood, NJ), changed twice weekly except during week 1 postpartum
Cage Filters		
Spun-bonded polyester (DuPont 2024)	Same as 13-week studies	Same as 13-week studies
Racks		
Stainless steel (Lab Products, Inc., Garfield, NY), changed once monthly	Same as 13-week studies	Same as 13-week studies
Nesting Material		
None	None	Nestlets (Ancare Corp., Manhasset, Long Island, NY)
Animal Room Environment		
Temperature: 21°-23° C Relative humidity: 40%-60% Fluorescent light: 12 hours/day Room air changes: 15 changes/hour	Temperature: 21°-23° C Relative humidity: 40%-60% Fluorescent light: 12 hours/day Room air changes: 15 changes/hour	Temperature: 21°-23° C Relative humidity: 40%-60% Fluorescent light: 12 hours/day Room air changes: 15 changes/hour

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 5,5-Diphenylhydantoin
(continued)

13-Week Studies	Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Doses Rats: 0, 300, 600, 1,200, 2,400, or 4,800 ppm 5,5-diphenylhydantoin in feed Mice: 0, 75, 150, 300, 600, or 1,200 ppm 5,5-diphenylhydantoin in feed	Rats: 0, 80, 240, 800, or 2,400 ppm 5,5-diphenylhydantoin in feed Mice: 0, 20, 60, 200, or 600 ppm 5,5-diphenylhydantoin in feed	F_0 females administered perinatal (F_0) doses in feed from 1 week before breeding through the weaning of the F_1 generation; pups administered same diet as dams from weaning at week 4 until 8 weeks of age, then administered adult (F_1) doses. The following concentrations (ppm) of 5,5-diphenylhydantoin were administered in feed:
Type and Frequency of Observation Observed and clinical observations recorded twice/day; weighed initially and once/week; feed consumption measured weekly	F_0: Observed twice/day; weighed initially and once/week; clinical observations recorded twice/day F_1: Observed twice/day; litter weights recorded on day 1; weighed on days 4 and 28 and once/week thereafter; clinical observations recorded twice/day; feed consumption measured weekly	F_0: Observed twice/day; weighed once/week except during immediate postnatal period; clinical observations recorded weekly F_1: Observed twice/day; weighed on day 4 (rats) or 7 (mice), on day 28, once/week through week 12 (mice) or 13 (rats) of adult dosing, once/month thereafter; clinical observations recorded once/week for 13 weeks, once/month thereafter; feed consumption measured weekly

Rats		Mice		
F_0	F_1	F_0	F_1	
			M	F
0	0	0	0	0
0	800	0	100	200
0	2,400	0	300	600
63	240	21	30	60
210	800	70	100	200
630	0	210	0	0
630	800	210	100	200
630	2,400	210	300	600

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 5,5-Diphenylhydantoin
(continued)

13-Week Studies	Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Necropsy		
Necropsy was performed on all animals.	F_0: None F_1: Necropsy performed on all animals.	F_0: None F_1: Necropsy performed on all animals. The following organs were weighed at 9 months: adrenal gland, brain, heart, right kidney, liver, lung, ovary, pituitary gland, prostate gland, right testis, thymus, thyroid gland, and uterus
Clinical Pathology		
None	None	Clinical pathology studies on 10 rats from each dose group at 9 months. ***Hematology:*** hematocrit, hemoglobin, erythrocyte count, mean erythrocyte volume, platelets, reticulocytes, and leukocyte count and differential ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, total bilirubin, cholesterol, triglycerides, alkaline phosphatase, alanine aminotransferase, and sorbitol dehydrogenase ***Urinalysis:*** specific gravity and pH

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 5,5-Diphenylhydantoin
(continued)

13-Week Studies	Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Histopathology Complete histopathology was performed on all control animals, all rats receiving 4,800 ppm, all mice receiving 600 or 1,200 ppm, and all mice dying before the end of the study. Tissues examined included: adrenal gland, brain, colon, epididymis, esophagus, femur and marrow, heart, jejunum, kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The liver from all mice in lower exposure groups was also examined microscopically.	Complete histopathology performed on all F_1 animals. Tissues examined included: adrenal gland, brain, colon, epididymis, esophagus, femur and marrow, heart, jejunum, kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.	F_0: None F_1: Complete histopathology performed on all animals that died or were killed moribund and all control (0:0) and 0:2,400 and 630:2,400 ppm (rats) and 0:300, 0:600, 210:300, and 210:600 ppm (mice) animals from the 9-month evaluations and 2-year studies. Tissues examined included: adrenal glands (cortex and medulla), femur and marrow, brain, cecum, colon, duodenum, epididymis, esophagus, gallbladder (mice), gross lesions, heart, ileum, jejunum, kidney, liver, lung, mammary gland (females), mandibular or mesenteric lymph node, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, rectum, salivary gland, skin, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. At the 9-month interim evaluations, the clitoral or preputial glands of control and high-dose animals and the liver from rats in the 0:800, 210:800, and 630:800 ppm groups and mice in the 0:100, 0:200, 70:100, 70:200, 210:100, and 210:200 ppm groups were examined. At study termination, the adrenal gland of male rats and the liver of all rats and mice were examined from all other exposure groups.

RESULTS

RATS

13-WEEK STUDY

All rats survived to the end of the study (Table 2). The final mean body weights of males and females exposed to 2,400 or 4,800 ppm 5,5-diphenylhydantoin were significantly lower than those of the controls. Mean body weight gains of males and females exposed to 2,400 or 4,800 ppm were significantly lower than those of the controls. The significantly lower body weight gains in the other exposure groups of females may be a reflection of poor randomization of animals rather than a chemical-related effect. For males and females exposed to 4,800 ppm, feed consumption was lower through week 7 and was similar to that of control groups thereafter. There were no clinical findings that could be clearly attributed to chemical exposure.

There were no chemical-related gross lesions in male or female rats. Chemical-related microscopic lesions were limited to the liver of rats in the 4,800 ppm groups and consisted of centrilobular hypertrophy of hepatocytes (Plates 1 and 2). This was a minimal to

TABLE 2
Survival, Body Weights, and Feed and Compound Consumption of Rats in the 13-Week Feed Study of 5,5-Diphenylhydantoin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Consumption	
		Initial	Final	Change		Feed[c]	Compound[d]
Male							
0	10/10	138 ± 2	320 ± 4	182 ± 3		11.9	
300	10/10	138 ± 2	321 ± 6	183 ± 4	100	12.0	15.7
600	10/10	138 ± 2	315 ± 6	177 ± 5	98	11.9	31.5
1,200	10/10	137 ± 3	314 ± 4	177 ± 4	98	11.4	60.7
2,400	10/10	136 ± 3	292 ± 5**	156 ± 4**	91	10.9	122.2
4,800	10/10	138 ± 2	251 ± 4**	113 ± 4**	78	9.5	234.4
Female							
0	10/10	100 ± 3	180 ± 3	80 ± 2		9.2	
300	10/10	109 ± 1**	180 ± 3	71 ± 2**	100	8.7	18.1
600	10/10	109 ± 2**	178 ± 2	69 ± 2**	99	8.4	35.1
1,200	10/10	108 ± 1**	178 ± 2	70 ± 2**	99	8.6	72.2
2,400	10/10	107 ± 2**	167 ± 3**	60 ± 2**	92	8.2	143.6
4,800	10/10	109 ± 1**	149 ± 2**	40 ± 2**	83	6.9	256.7

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day for 13 weeks.
[d] Compound consumption is expressed as mg/kg body weight per day for 13 weeks.

mild effect characterized by enlargement of hepatocytes in the central one-third to one-half of the hepatic lobules. These enlarged cells had a more homogeneous, slightly less eosinophilic-staining cytoplasm than was present in the centrilobular hepatocytes from control rats. Hypertrophy was slightly more prominent in males than in females.

Dose Selection Rationale for Adult Exposure

In the 13-week study, all groups showed a net weight gain over the study period, although the weight gain of the females exposed to 4,800 ppm was only one-half that of the control group. Feed consumption also decreased with increasing exposure and was most apparent at the 4,800 ppm level. No chemical-related gross or histomorphologic lesions occurred in the tissues of rats receiving 2,400 ppm or less. Based primarily on the reduced body weight gains, 2,400 ppm was selected as the highest exposure level for the adult exposure portion of the 2-year study.

GESTATIONAL STUDY: DETERMINATION OF MAXIMUM PERINATAL DOSE

The gestational study was conducted to determine the dietary concentrations for perinatal exposure to be used in the 2-year study. Selected dams from each exposure group were evaluated at gestation day 18 for reproductive effects. The numbers of litters, implantations, live fetuses, and fetuses per litter in the 80, 240, and 800 ppm groups were similar to those of the controls (Table 3); no pregnancies occurred among females exposed to 2,400 ppm. All rat dams not designated for evaluation at gestation day 18 survived to the end of the study.

The number of pups was greater in the 80 and 240 ppm groups than in the controls through day 28; however, the number of pups in the 800 ppm group surviving to day 28 was less than the number of control pups (Table 4). Mean pup weight on day 28 was significantly increased in the 80 ppm group.

Selected pups from the various exposure groups were weaned onto feed containing 5,5-diphenylhydantoin. All weanling rats exposed to 5,5-diphenylhydantoin in feed survived until the end of the study (Table 5). The final body weights of males exposed to 240 or 800 ppm and females exposed to 800 ppm were significantly lower than those of the controls. There were no chemical-related gross or microscopic lesions in male or female rats.

TABLE 3
Prenatal Day 18 Litter Data for Rats in the Maximum Perinatal Dose Determination Feed Study of 5,5-Diphenylhydantoin

	0 ppm	80 ppm	240 ppm	800 ppm	2,400 ppm[a]
Litters	4	4	4	4	
Implantations	40	40	37	42	
Live fetuses	40	38	36	41	
Fetuses/litter	10.0	9.5	9.0	10.5	
Fetal weight[b]	1.43 ± 0.07	1.42 ± 0.15	1.43 ± 0.09	1.41 ± 0.09	
Placental weight[b]	0.36 ± 0.03	0.35 ± 0.04	0.36 ± 0.03	0.34 ± 0.04	

[a] No pregnancies occurred among females exposed to 2,400 ppm.
[b] Mean ± standard deviation. Fetal body and placental weights are given in grams. Differences from the control group are not significant by Dunnett's test.

TABLE 4
Survival, Sex Ratios, and Mean Body Weights of Rat Pups in the Maximum Perinatal Dose Determination Feed Study of 5,5-Diphenylhydantoin

	0 ppm	80 ppm	240 ppm	800 ppm	2,400 ppm[a]
Precull					
Litters on day 0[b]	15	18	15	15	
Pups on day 0	127	170	146	129	
Number of males on day 0	67	86	81	69	
Number of females on day 0	60	84	65	60	
Male/female ratio on day 0	1.12	1.02	1.25	1.15	
Litters on days 1-28[c]	10	14	11	9	
Pups on day 1	92	128	105	79	
Pup weight on day 1	5.27	5.20	5.53	5.06	
Pups dead days 1-4	4	4	1	10	
Pups on day 4	88	124	104	69	
Pup weight on day 4[d]	7.07 ± 1.39	7.12 ± 0.71	7.52 ± 0.70**	6.81 ± 1.05	
Postcull					
Pups on day 4	73	104	85	64	
Pup weight on day 4[d]	7.05 ± 1.53	7.19 ± 0.69	7.55 ± 0.74**	6.93 ± 0.95	
Pups dead days 4-28	1	0	0	3	
Pups on day 28	72	104	85	61	
Pup weight on day 28[d]	51.2 ± 12.1	56.0 ± 4.35**	53.8 ± 11.3	50.2 ± 3.17	

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test
[a] No pregnancies occurred among females exposed to 2,400 ppm.
[b] Does not include four litters per group evaluated on gestation day 18
[c] Includes only those litters for which body weight data were available on days 1, 4, and 28; does not include litters evaluated on day 12 postpartum
[d] Mean ± standard deviation. Pup weights are given in grams.

TABLE 5
Survival and Body Weights of Rat Weanlings in the Maximum Perinatal Dose Determination Feed Study of 5,5-Diphenylhydantoin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Mean Body Weight[b] (g) Final	Final Weight Relative to Controls (%)
Male				
0	10/10	81 ± 2	187 ± 2	
80	10/10	82 ± 2	181 ± 4	97
240	10/10	70 ± 4**	165 ± 5**	89
800	10/10	74 ± 2	170 ± 3**	91
Female				
0	10/10	71 ± 1	135 ± 1	
80	10/10	74 ± 1	135 ± 1	100
240	10/10	65 ± 3*	130 ± 3	96
800	10/10	66 ± 1	122 ± 2**	91

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving/number initially in group
[b] Weights are given as mean ± standard error.

Dose Selection Rationale for Perinatal Exposure

The exposure level of 2,400 ppm 5,5-diphenylhydantoin had reproductive and embryotoxic effects, as none of the sperm-positive females delivered litters. A greater number of pups died between postnatal day 1 and day 28 in the 800 ppm group than in the control group. No gross external malformations were observed among fetuses or pups surviving to term in any exposure group, and no gross or histopathologic lesions were observed in rats exposed to 800 ppm for 4 weeks following weaning. Based on these results, 630 ppm was selected as the highest exposure level for the perinatal exposure period of the 2-year study.

2-YEAR STUDY

9-Month Interim Evaluation

Three females designated for evaluation at 9 months died; one death was due to a malfunctioning water system (Table 6). All male rats survived. Final mean body weights of females exposed to 800 or 2,400 ppm as adults were significantly lower than those of controls; mean body weight gains of females exposed as adults were significantly lower than controls. The final mean body weight of males in the 630:2,400 ppm group was significantly lower than that of controls. No clinical findings related to chemical exposure were observed.

In male rats receiving adult-only exposure to 2,400 ppm, absolute liver weights were slightly greater than that of the controls (Table F1). Relative liver weights of males and females exposed to 2,400 ppm as adults were significantly greater than those of controls. In females exposed to F_0:F_1 concentrations of 630:2,400 ppm, absolute and relative uterus weights were significantly lower than those of the controls.

There were no chemical-related gross lesions in males or females. Chemical-related microscopic lesions were present in the liver of males and females from the 0:2,400 and 630:2,400 ppm groups. Microscopic lesions in the liver consisted of a minimal to mild centrilobular hypertrophy of hepatocytes similar to that seen in rats in the 4,800 ppm group in the 13-week study. Hypertrophy was slightly more prominent in males than in females. There was no difference in the average severity of hepatocellular hypertrophy between the 0:2,400 and 630:2,400 ppm groups.

In male rats, mild but significant increases in platelet and erythrocyte counts occurred in the 63:240 (erythrocyte counts only), 210:800, 0:2,400, and 630:2,400 ppm groups (Table G1). Hematocrit was also mildly increased in the 63:240 and 210:800 ppm groups. Differences in serum biochemical variables were minimal to mild and included decreases in triglyceride concentrations in the 63:240, 210:800, 630:800, 0:2,400, and 630:2,400 ppm groups and in alanine aminotransferase activity in the 630:800 and 630:2,400 ppm groups.

Exposed female rats had effects similar to those in males. Platelet counts were significantly increased in all exposure groups and erythrocyte counts were increased in the 63:240, 210:800, and 630:2,400 ppm groups (Table G1). Minimal but significant decreases in mean cell volume occurred in all exposed females. Serum biochemical differences included decreases in triglyceride concentrations and alkaline phosphatase activities in all exposure groups and in alanine aminotransferase activities in the 0:800, 630:800, and 0:2,400 ppm groups.

TABLE 6
Survival and Body Weights of Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0:0	10/10	183 ± 7	428 ± 11	245 ± 9	
630:0	10/10	169 ± 7	416 ± 8	247 ± 6	97
63:240	10/10	191 ± 11	440 ± 8	249 ± 16	103
0:800	10/10	189 ± 5	422 ± 7	232 ± 6	99
210:800	10/10	166 ± 5	427 ± 10	262 ± 9	100
630:800	10/10	159 ± 7	411 ± 11	252 ± 11	96
0:2,400	10/10	184 ± 6	410 ± 6	226 ± 5	96
630:2,400	10/10	167 ± 3	392 ± 7*	225 ± 5	92
Female					
0:0	10/10	145 ± 2	259 ± 3	114 ± 3	
630:0	10/10	134 ± 3*	251 ± 4	118 ± 5	97
63:240	9/10[c]	147 ± 3	247 ± 4	96 ± 5**	95
0:800	10/10	147 ± 2	234 ± 2**	86 ± 3**	90
210:800	9/10[d]	134 ± 5*	227 ± 4**	89 ± 2**	87
630:800	9/10[e]	139 ± 3	227 ± 3**	92 ± 5**	88
0:2,400	10/10	145 ± 3	204 ± 3**	59 ± 4**	79
630:2,400	10/10	135 ± 2	202 ± 4**	67 ± 4**	78

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies.
[c] Week of death: 37
[d] Week of death: 21 (malfunction in automatic watering system)
[e] Week of death: 10

Survival

Two-year survival rates of exposed groups were similar to those of the controls (Table 7 and Figure 2).

Body Weights, Feed Consumption, and Clinical Findings

The final mean body weights of male rats exposed to 0:2,400 or 630:2,400 ppm and females exposed to 0:800, 210:800, 630:800, 0:2,400, or 630:2,400 ppm were more than 10% less than those of the controls (Tables 8 and 9 and Figures 3a,b,c, and d). Females exposed to 2,400 ppm as adults had final mean body weights 35% less than that of the controls. Feed consumption was similar among exposed and control groups (Tables I1 and I2). Adult exposure levels of 800 and 2,400 ppm 5,5-diphenylhydantoin resulted in compound consumption levels of 35 or 105 mg/kg body weight (males) and 40 or 125 mg/kg (females). There were no clinical findings that could be clearly attributed to chemical exposure.

TABLE 7
Survival of Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin

	F_0:F_1 Concentration (ppm)							
	0:0	630:0	63:240	0:800	210:800	630:800	0:2,400	630:2,400
Male								
Animals initially in study	60	60	60	60	60	60	60	60
9-Month interim evaluation[a]	10	10	10	10	10	10	10	10
Moribund	22	21	13	21	24	21	25	17
Natural deaths	2	5	4	3	4	6	3	2
Animals surviving to study termination	26[b]	24[b]	33	26	22[b]	23[b]	22[b]	31[b]
Percent probability of survival at end of study[c]	52	48	66	52	44	46	44	62
Mean survival (days)[d]	687	682	690	697	685	663	665	691
Survival analysis[e]		P=0.830	P=0.238N	P=0.895N	P=0.656	P=0.607	P=0.550	P=0.441N
Female								
Animals initially in study	60	60	60	60	60	60	60	60
9-Month interim evaluation[a]	10	10	10	10	10	10	10	10
Moribund	17	19	19	9	19	15	11	13
Natural deaths	2	2	4	4	2	2	1	1
Accidental deaths[a]	0	0	0	0	0	0	0	5
Animals surviving to study termination	31	29	27	37	29[b]	33	38[b]	31
Percent probability of survival at end of study[c]	62	58	54	74	58	66	76	69
Mean survival (days)[d]	669	690	685	684	690	696	709	636
Survival analysis[e]		P=0.970	P=0.676	P=0.288N	P=0.990	P=0.693N	P=0.140N	P=0.564N

[a] Censored from survival analyses
[b] Includes one animal that died or was killed moribund during the last week of the study (two males in the 630:800 ppm group died during the last week of the study)
[c] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice)
[e] The results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposure columns. A lower mortality in an exposure group is indicated by N.

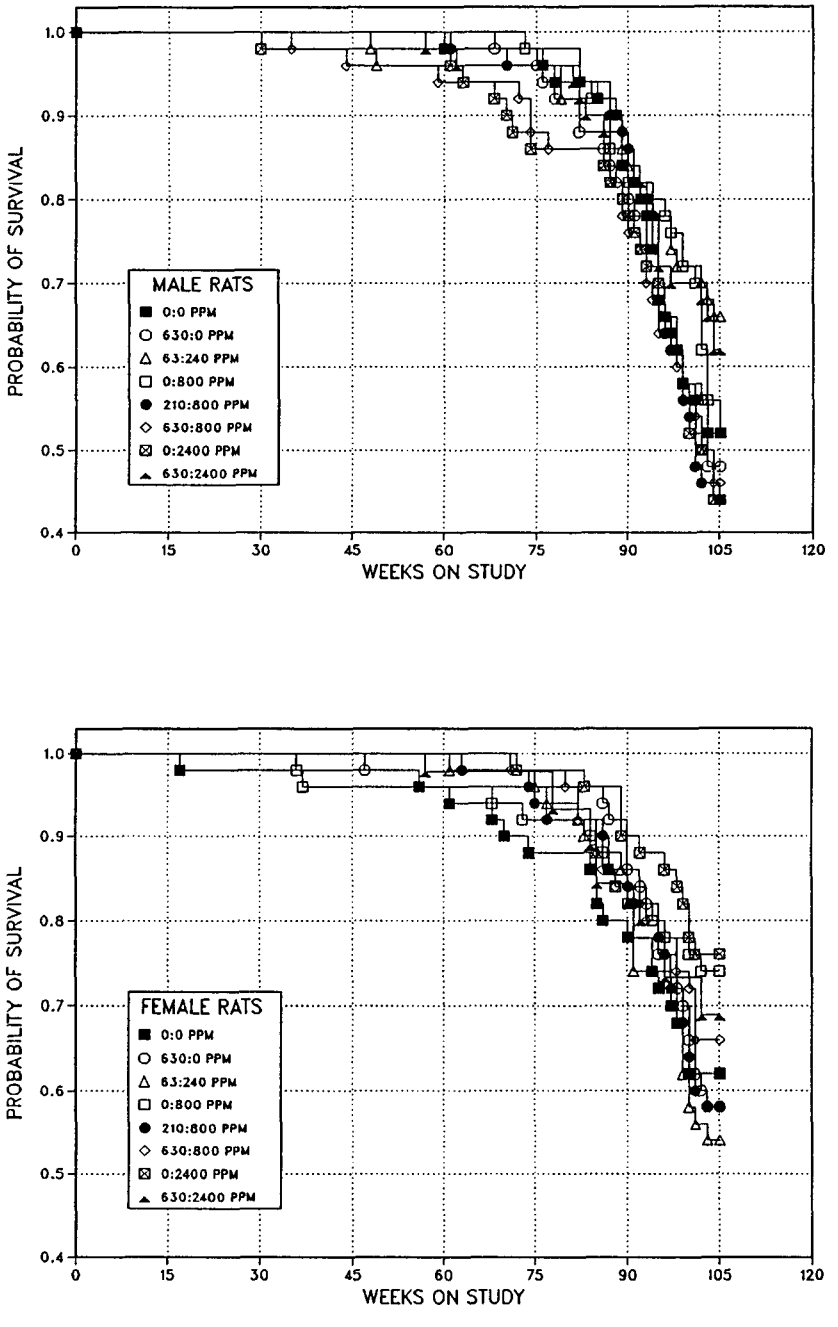


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered 5,5-Diphenylhydantoin in Feed for 2 Years

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin

Week on Study	0:0 ppm Av. Wt. (g)	0:0 ppm No. of Survivors	630:0 ppm Av. Wt. (g)	630:0 ppm Wt. (% of controls)	630:0 ppm No. of Survivors	63:240 ppm Av. Wt. (g)	63:240 ppm Wt. (% of controls)	63:240 ppm No. of Survivors	0:800 ppm Av. Wt. (g)	0:800 ppm Wt. (% of controls)	0:800 ppm No. of Survivors
1	204	50	192	94	50	186	91	50	199	98	50
2	229	50	215	94	50	215	94	50	219	96	50
3	256	50	245	96	50	247	96	50	249	97	50
4	268	50	256	96	50	265	99	50	263	98	50
5	278	50	268	96	50	277	100	50	277	100	50
6	283	50	276	98	50	289	102	50	287	101	50
7	299	50	291	97	50	301	101	50	297	99	50
8	309	50	298	96	50	309	100	50	306	99	50
9	312	50	304	97	50	313	100	50	311	100	50
10	326	50	319	98	50	327	100	50	319	98	50
11	340	50	332	98	50	339	100	50	324	95	50
12	347	50	341	98	50	348	100	50	332	96	50
13	352	50	344	98	50	351	100	50	341	97	50
17	366	50	360	98	50	359	98	50	356	97	50
21	390	50	384	98	50	388	99	50	378	97	50
25	407	50	396	97	50	403	99	50	400	98	50
29	424	50	418	99	50	419	99	50	416	98	50
33	438	50	428	98	50	428	98	50	426	97	50
38	445	50	430	97	50	435	98	50	431	97	50
41	449	50	439	98	50	443	99	50	442	98	50
46	456	50	447	98	50	450	99	50	451	99	50
50	456	50	449	98	50	453	99	48	450	99	50
54	470	50	462	98	50	468	100	48	462	98	50
60	473	50	465	98	50	471	100	48	463	98	50
65	469	49	463	99	50	470	100	48	464	99	50
70	473	49	466	99	49	472	100	48	461	97	50
74	471	49	467	99	49	469	100	48	456	97	49
77	469	48	465	99	47	467	100	48	453	97	49
82	470	47	461	98	46	474	101	46	450	96	49
87	467	46	463	99	43	469	100	46	449	96	46
91	466	42	466	100	40	486	104	41	448	96	41
96	466	34	468	100	34	472	101	39	437	94	40
100	458	29	468	102	29	465	102	36	429	94	36
104	453	26	466	103	24	458	101	35	420	93	28
Mean for weeks											
1-13	293		283	97		290	99		286	98	
14-52	426		417	98		420	99		417	98	
53-104	467		465	100		470	101		449	96	

(continued)

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin
(continued)

Week on Study	210:800 ppm			630:800 ppm			0:2,400 ppm			630:2,400 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	179	88	50	176	86	50	195	96	50	177	87	50
2	214	93	50	209	91	50	220	96	50	195	85	50
3	244	95	50	232	91	50	232	91	50	213	83	50
4	261	97	50	254	95	50	256	96	50	235	88	50
5	273	98	50	264	95	50	265	95	50	247	89	50
6	287	101	50	279	99	50	273	96	50	246	87	50
7	296	99	50	283	95	50	279	93	50	256	86	50
8	304	98	50	292	94	50	284	92	50	263	85	50
9	308	99	50	301	96	50	291	93	50	272	87	50
10	318	98	50	308	94	50	303	93	50	286	88	50
11	330	97	50	317	93	50	315	93	50	297	87	50
12	338	97	50	327	94	50	323	93	50	306	88	50
13	343	97	50	333	95	50	327	93	50	311	88	50
17	358	98	50	348	95	50	338	92	50	324	89	50
21	388	99	50	384	98	50	363	93	50	348	89	50
25	406	100	50	395	97	50	377	93	50	362	89	50
29	421	99	50	412	97	50	396	93	50	381	90	50
33	429	98	50	420	96	50	400	91	49	386	88	50
38	435	98	50	428	96	50	410	92	49	392	88	50
41	442	98	50	433	96	50	415	92	49	398	89	50
46	452	99	50	442	97	48	425	93	49	406	89	50
50	452	99	50	443	97	48	422	93	49	403	88	50
54	469	100	50	459	98	48	436	93	49	417	89	50
60	471	100	50	463	98	47	438	93	49	419	89	49
65	474	101	49	463	99	47	436	93	47	421	90	48
70	474	100	49	460	97	47	432	91	46	419	89	48
74	470	100	48	457	97	46	429	91	44	413	88	48
77	464	99	48	455	97	43	428	91	43	408	87	48
82	461	98	48	444	94	43	425	90	43	407	87	47
87	456	98	47	453	97	42	418	90	42	403	86	44
91	456	98	43	447	96	38	408	88	38	398	85	42
96	443	95	34	440	94	32	389	83	35	394	85	36
100	438	96	28	437	95	28	384	84	29	390	85	35
104	431	95	23	427	94	25	381	84	25	377	83	33
Mean for weeks												
1-13	284	97		275	94		274	94		254	87	
14-52	420	99		412	97		394	92		378	89	
53-104	459	98		450	96		417	89		406	87	

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin

Week on Study	0:0 ppm Av. Wt. (g)	0:0 ppm No. of Survivors	630:0 ppm Av. Wt. (g)	630:0 ppm Wt. (% of controls)	630:0 ppm No. of Survivors	63:240 ppm Av. Wt. (g)	63:240 ppm Wt. (% of controls)	63:240 ppm No. of Survivors	0:800 ppm Av. Wt. (g)	0:800 ppm Wt. (% of controls)	0:800 ppm No. of Survivors
1	148	50	140	95	50	151	102	50	149	101	50
2	162	50	150	93	50	159	98	50	157	97	50
3	163	50	154	94	50	166	102	50	165	101	50
4	176	50	168	95	50	175	99	50	168	95	50
5	180	50	173	96	50	181	101	50	174	97	50
6	189	50	181	96	50	186	98	50	180	95	50
7	189	50	188	99	50	190	101	50	182	96	50
8	197	50	191	97	50	194	98	50	185	94	50
9	198	50	193	97	50	198	100	50	187	94	50
10	206	50	199	97	50	202	98	50	192	93	50
11	210	50	201	96	50	202	96	50	196	93	50
12	213	50	203	95	50	207	97	50	197	92	50
13	214	50	205	96	50	207	97	50	195	91	50
17	220	50	216	98	50	213	97	50	205	93	50
21	232	50	224	97	50	220	95	50	210	91	50
25	240	49	235	98	50	230	96	50	217	90	50
29	245	49	237	97	50	229	93	50	220	90	50
33	250	49	242	97	50	236	94	50	219	88	50
38	259	49	247	95	50	244	94	50	227	88	48
41	266	49	257	97	50	253	95	50	232	87	48
46	267	49	256	96	50	256	96	50	232	87	48
50	270	49	260	96	49	259	96	50	238	88	48
54	279	49	270	97	49	270	97	50	247	89	48
60	288	48	283	98	48	277	96	50	250	87	48
65	300	47	297	99	48	286	95	49	261	87	48
70	312	46	301	96	48	293	94	49	262	84	47
74	319	45	308	97	48	296	93	49	265	83	47
77	324	44	312	96	48	300	93	48	271	84	46
82	336	44	324	96	48	313	93	47	282	84	46
87	342	41	326	95	48	325	95	44	285	83	45
91	354	40	336	95	46	327	92	43	287	81	41
96	355	36	338	95	38	326	92	37	292	82	40
100	358	34	338	94	36	323	90	34	296	83	39
104	357	31	334	94	29	333	93	27	300	84	37
Mean for weeks											
1-13	188		180	96		186	99		179	95	
14-52	250		242	97		238	95		222	89	
53-104	327		314	96		306	94		275	84	

(continued)

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin
(continued)

Week on Study	210:800 ppm			630:800 ppm			0:2,400 ppm			630:2,400 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	143	97	50	138	93	50	140	95	50	133	90	50
2	150	93	50	147	91	50	146	90	50	140	86	50
3	161	99	50	154	94	50	152	93	50	147	90	50
4	164	93	50	160	91	50	158	90	50	154	88	50
5	172	96	50	168	93	50	159	88	50	158	88	50
6	177	94	50	173	92	50	164	87	50	163	86	50
7	179	95	50	174	92	50	169	89	50	166	88	50
8	183	93	50	178	90	50	172	87	50	169	86	50
9	183	92	50	182	92	50	175	88	50	171	86	50
10	191	93	50	188	91	50	178	86	50	171	83	50
11	195	93	50	191	91	50	182	87	50	178	85	50
12	196	92	50	192	90	50	182	85	50	178	84	50
13	195	91	50	187	87	50	181	85	50	177	83	50
17	205	93	50	198	90	50	186	85	50	184	84	50
21	210	91	50	207	89	50	192	83	50	189	81	45
25	215	90	50	212	88	50	198	83	50	194	81	45
29	218	89	50	215	88	50	196	80	50	193	79	45
33	219	88	50	214	86	50	198	79	50	194	78	45
38	226	87	50	220	85	50	202	78	50	199	77	45
41	231	87	50	225	85	50	204	77	50	197	74	45
46	235	88	50	231	87	50	205	77	50	203	76	45
50	238	88	50	238	88	50	206	76	50	205	76	45
54	247	89	50	240	86	50	209	75	50	207	74	45
60	245	85	50	249	86	50	210	73	50	208	72	44
65	261	87	49	259	86	50	214	71	50	213	71	44
70	263	84	49	261	84	50	213	68	50	212	68	44
74	265	83	49	265	83	49	213	67	49	212	66	44
77	267	82	48	268	83	49	214	66	49	212	65	44
82	283	84	46	281	84	48	219	65	49	219	65	42
87	289	85	46	284	83	44	218	64	48	222	65	38
91	296	84	43	292	82	42	225	64	45	226	64	38
96	296	83	40	291	82	39	227	64	44	225	63	36
100	299	84	35	291	81	37	230	54	42	231	65	33
104	280	78	30	282	79	33	232	65	38	232	65	31
Mean for weeks												
1-13	176	94		172	91		166	88		162	86	
14-52	222	89		218	87		199	80		195	78	
53-104	274	84		272	83		219	67		218	67	

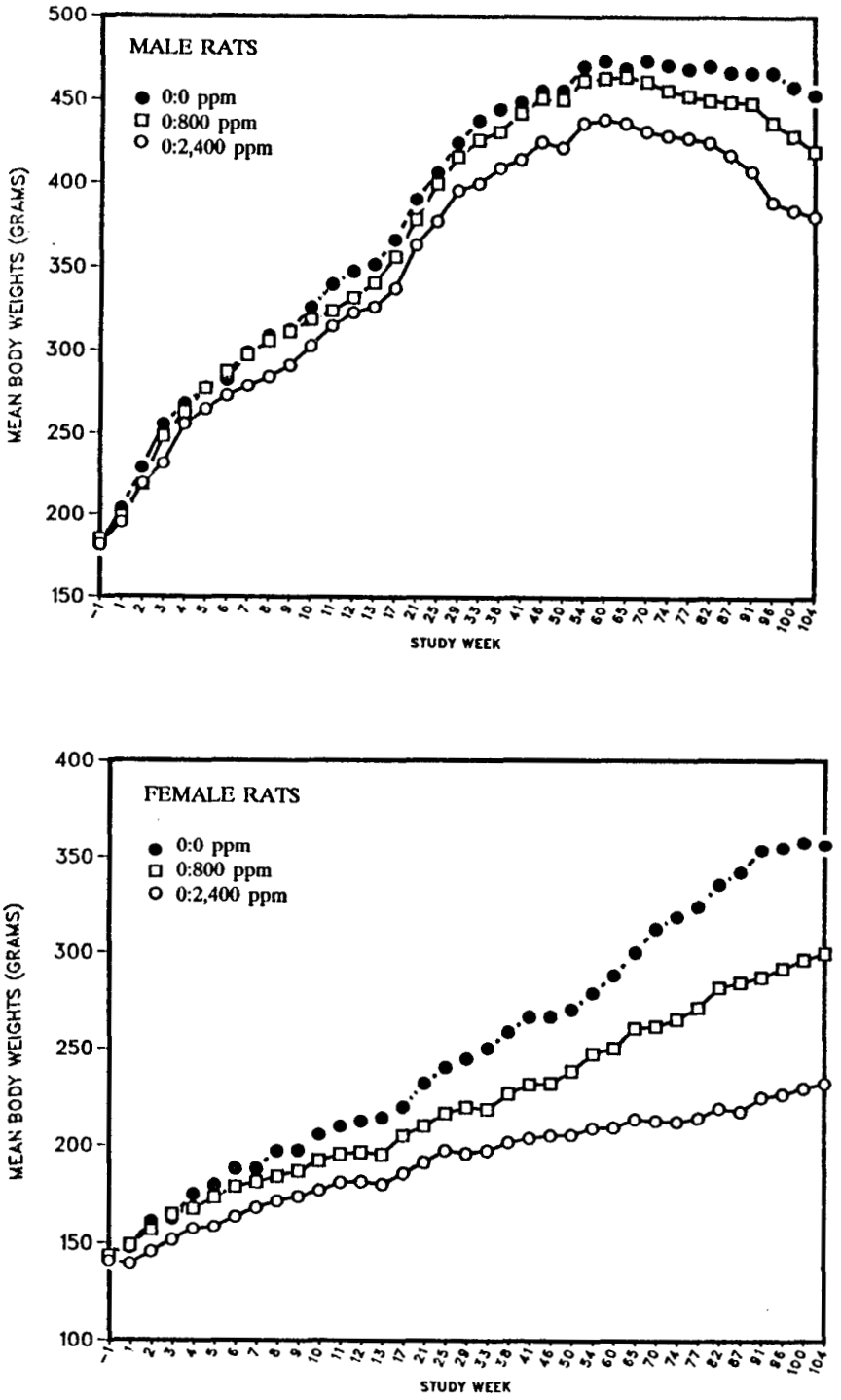


FIGURE 3a
Growth Curves for Rats Administered 5,5-Diphenylhydantoin in Feed for 2 Years: 0:0, 0:800, and 0:2,400 ppm Groups

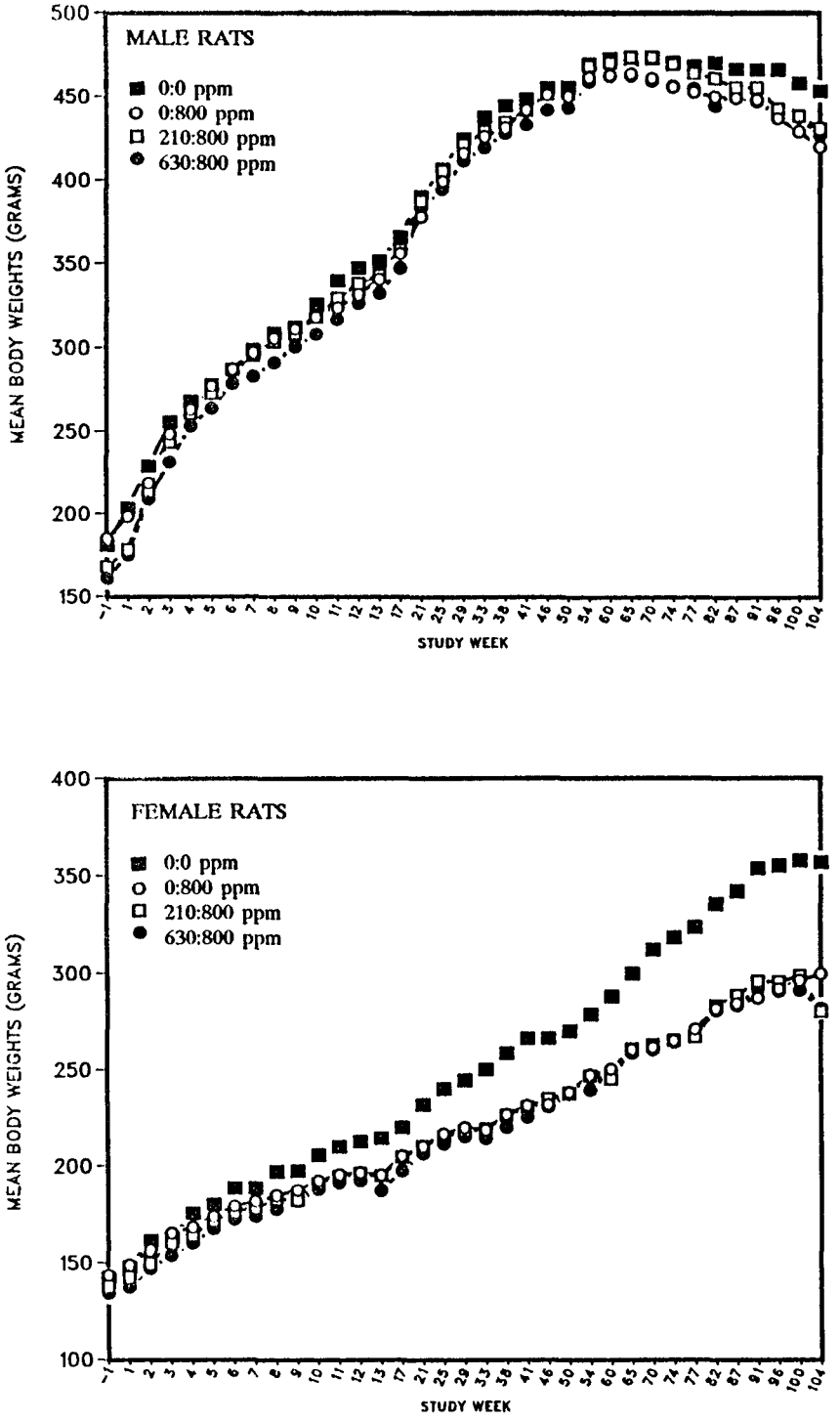


FIGURE 3b
Growth Curves for Rats Administered 5,5-Diphenylhydantoin in Feed for 2 Years: 0:0, 0:800, 210:800, and 630:800 ppm Groups

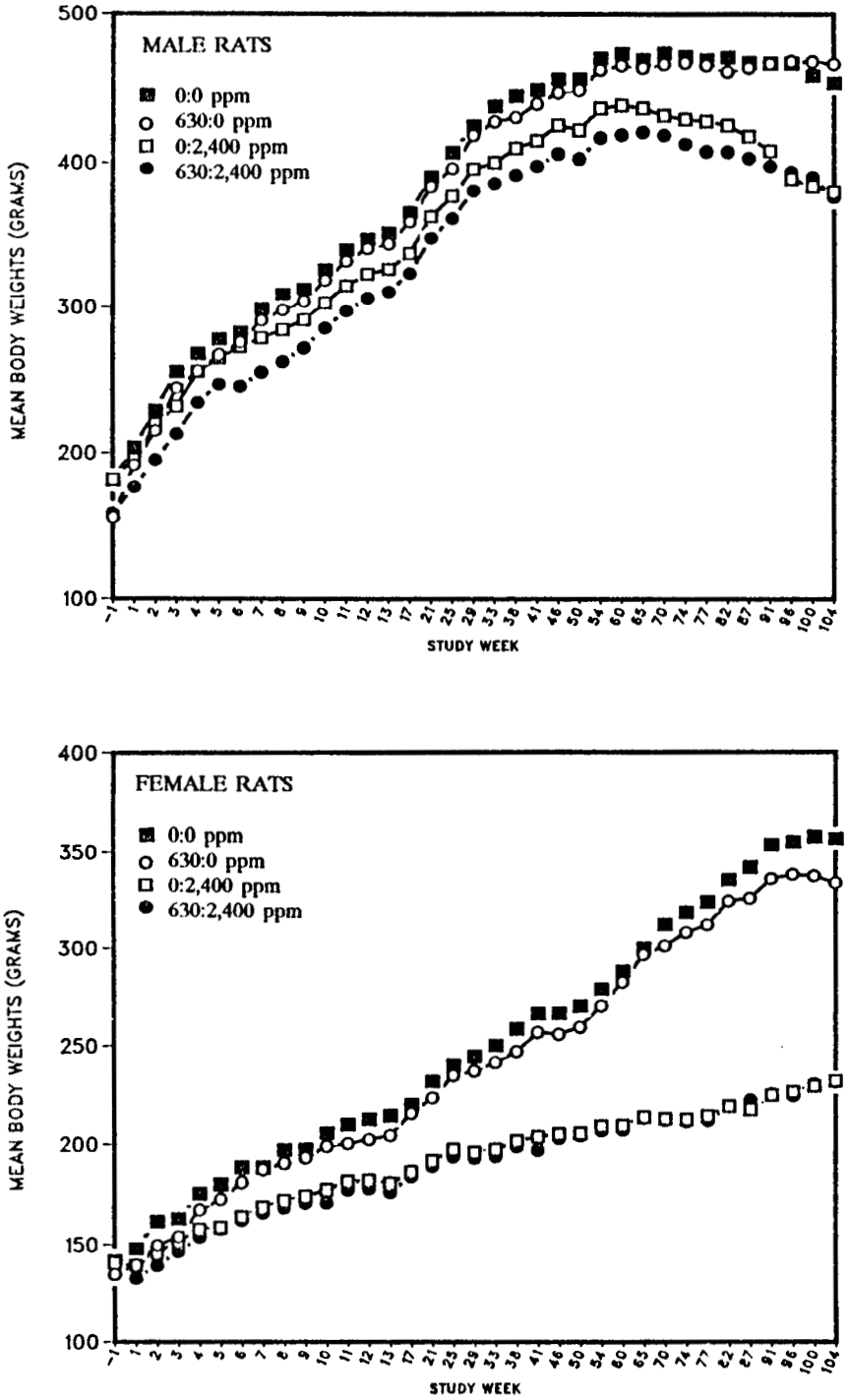


FIGURE 3c
Growth Curves for Rats Administered 5,5-Diphenylhydantoin in Feed for 2 Years: 0:0, 630:0, 0:2,400, and 630:2,400 ppm Groups

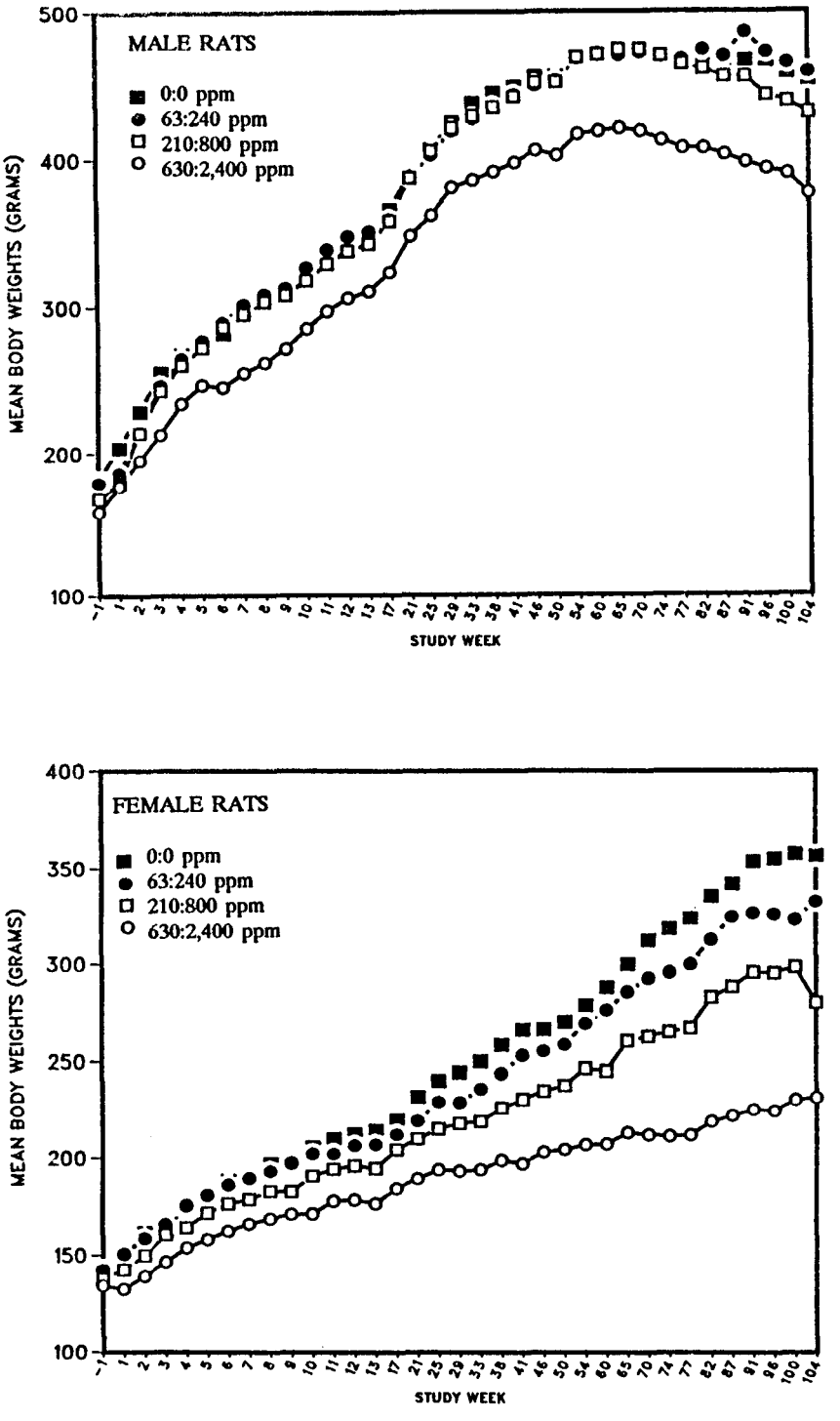


FIGURE 3d
Growth Curves for Rats Administered 5,5-Diphenylhydantoin in Feed for 2 Years: 0:0, 63:240, 210:800, and 630:2,400 ppm Groups

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms or nonneoplastic lesions of the liver, mammary gland, pituitary gland, and thyroid gland in rats. Chemical-related histopathologic effects associated with chronic administration of 5,5-diphenylhydantoin were seen in the liver of rats from the adult-only and the combined perinatal and adult exposure groups.

Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats. Historical incidences of hepatocellular neoplasms in control rats are given in Table A3 for males and Table B3 for females.

Effects of Adult-Only Exposure of Rats to 5,5-Diphenylhydantoin

The neoplastic and nonneoplastic effects of adult-only exposure were determined by comparison of the incidences of lesions in the 0:0, 0:800, and 0:2,400 ppm groups, which correspond to a standard carcinogenicity study.

Liver: The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were increased in exposed male rats; multiple adenomas were present in one high-dose male (Table 10). A single hepatocellular carcinoma occurred in a low-dose male. Hepatocellular adenomas were not increased in females (Table 10). The incidences of centrilobular hypertrophy of hepatocytes were significantly increased in exposed male and female rats. This difference was minimal to mild in males and females and consisted of slight enlargement of hepatocytes around the central veins of hepatic lobules. The cytoplasm of affected hepatocytes appeared more homogeneous and stained slightly less eosinophilic than centrilobular hepatocytes in the liver of control rats. The incidence of basophilic foci of cellular alteration were significantly decreased in exposed male and female rats. There were also slightly decreased incidences of fatty change and slightly increased incidences of eosinophilic foci of cellular alteration in exposed females (Table 10).

Decreasing Incidences of Neoplasms: Exposed males had significantly decreased incidences of mammary gland fibroadenoma or carcinoma (4/50, 0/50, 0/50; Table A2); the incidences of mammary gland fibroadenoma (17/50, 6/50, 4/50) and fibroadenoma, adenoma, or carcinoma (21/50, 8/50, 4/50) were decreased in exposed females (Table B2). The incidences of pituitary gland adenoma (25/50, 19/32, 13/50) and thyroid gland C-cell adenoma or carcinoma (15/48, 1/9, 5/50) were decreased in exposed females.

TABLE 10
Liver Lesions in Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 0:800, and 0:2,400 ppm Groups

	0:0 ppm	0:800 ppm	0:2,400 ppm
Male			
Basophilic Focus[a]	17/50 (34%)	15/50 (30%)	4/50 (8%)**
Clear Cell Focus	2/50 (4%)	8/50 (16%)*	1/50 (2%)
Eosinophilic Focus	5/50 (10%)	6/50 (12%)	8/50 (16%)
Mixed Cell Focus	2/50 (4%)	0/50 (0%)	3/50 (6%)
Fatty Change	3/50 (6%)	1/50 (2%)	2/50 (4%)
Centrilobular Hypertrophy[b]	0/50 (0%)	13/50 (26%)**	27/50 (54%)**
Mean severity[c]		1.2	1.1
Hepatocellular Adenoma[d]			
Overall rate	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rate[e]	0.0%	3.8%	15.9%
Terminal rate[f]	0/26 (0%)	1/26 (4%)	2/22 (9%)
First incidence (days)	–[h]	732 (T)	695
Logistic regression test[g]	P=0.018	P=0.500	P=0.054
Hepatocellular Adenoma, Multiple	0/50 (0%)	0/50 (0%)	1/50 (2%)
Hepatocellular Carcinoma	0/50 (0%)	1/50 (2%)	0/50 (0%)
Hepatocellular Adenoma or Carcinoma[d,i]			
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	7.7%	15.9%
Terminal rate	0/26 (0%)	2/26 (8%)	2/22 (9%)
First incidence (days)	–	732 (T)	695
Logistic regression test	P=0.033	P=0.238	P=0.054

(continued)

TABLE 10
Liver Lesions in Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 0:800, and 0:2,400 ppm Groups (continued)

	0:0 ppm	0:800 ppm	0:2,400 ppm
Female			
Basophilic Focus	40/50 (80%)	33/50 (66%)*	6/50 (12%)**
Clear Cell Focus	1/50 (2%)	0/50 (0%)	2/50 (4%)
Eosinophilic Focus	0/50 (0%)	3/50 (6%)	5/50 (10%)*
Mixed Cell Focus	1/50 (2%)	1/50 (2%)	1/50 (2%)
Fatty Change	5/50 (10%)	0/50 (0%)*	0/50 (0%)*
Centrilobular Hypertrophy	0/50 (0%)	22/50 (44%)**	42/50 (84%)**
Mean severity		1.0	1.1
Hepatocellular Adenoma[j]	0/50 (0%)	1/50 (2%)	1/50 (2%)
Hepatocellular Carcinoma	0/50 (0%)	0/50 (0%)	0/50 (0%)

* Significantly different (P≤0.05) from the 0:0 ppm group by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report, because it is more widely used and understood.
[c] Average severity grade of lesions in affected rats (1 = minimal; 2 = mild; 3 = moderate; 4 = marked)
[d] Includes multiple adenomas
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms as nonfatal.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 32/1,001 (3.2% ± 3.6%); range 0%-10%
[j] Historical incidence: 5/1,001 (0.5% ± 1.4%); range 0%-6%

Effects of Perinatal-Only Exposure of Rats to 5,5-Diphenylhydantoin

The neoplastic and nonneoplastic effects of perinatal-only exposure were determined by comparison of the incidences of lesions in the 0:0 and 630:0 ppm groups.

Liver: Perinatal exposure to 5,5-diphenylhydantoin did not increase the incidences of liver neoplasms or of neoplasms at other sites in male or female rats (Table 11).

TABLE 11
Liver Lesions in Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 ppm Groups

	0:0 ppm	630:0 ppm
Male		
Basophilic Focus[a]	17/50 (34%)	17/50 (34%)
Clear Cell Focus	2/50 (4%)	5/50 (10%)
Eosinophilic Focus	5/50 (10%)	2/50 (4%)
Mixed Cell Focus	2/50 (4%)	0/50 (0%)
Fatty Change	3/50 (6%)	1/50 (2%)
Hepatocellular Adenoma	0/50 (0%)	1/50 (2%)
Female		
Basophilic Focus	40/50 (80%)	35/49 (71%)
Clear Cell Focus	1/50 (2%)	2/49 (4%)
Fatty Change	5/50 (10%)	3/49 (6%)
Mixed Cell Focus	1/50 (2%)	0/49 (0%)
Hepatocellular Adenoma	0/50 (0%)	0/49 (0%)

[a] Number of lesion-bearing animals/number of animals examined microscopically

Effects of Combined Perinatal and Adult Exposure of Rats to 5,5-Diphenylhydantoin

The effects of combined perinatal and adult exposure were determined by comparison of the incidences of lesions in rats in the 0:800, 210:800, and 630:800 ppm groups and in the 0:2,400 and 630:2,400 ppm groups.

Liver: Chemical-related increases in the incidences of hepatocellular adenoma similar to those seen in males receiving adult-only exposure were seen in males receiving the highest exposure (630:2,400 ppm). Five of 49 males had hepatocellular adenomas; four of these had multiple adenomas (Table 12). In female rats exposed to varying F_0 concentrations and a constant F_1 concentration of 800 or 2,400 ppm and male rats exposed to varying F_0 concentrations and a constant F_1 concentration of 800 ppm, the incidences of hepatocellular adenoma and carcinoma were not affected by F_0 exposure (Tables 12 and 13). Combined exposure at the highest concentrations of 5,5-diphenylhydantoin resulted in slight but statistically significant increases in the incidences of centrilobular hypertrophy of hepatocytes in male and female rats and in basophilic foci of cellular alteration in males compared to the equivalent adult-only exposure to 800 ppm.

TABLE 12
Liver Lesions in Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:2,400 and 630:2,400 ppm Groups

	0:2,400 ppm	630:2,400 ppm
Male		
Basophilic Focus[a]	4/50 (8%)	7/49 (14%)
Clear Cell Focus	1/50 (2%)	7/49 (14%)
Eosinophilic Focus	8/50 (16%)	7/49 (14%)
Mixed Cell Focus	3/50 (6%)	6/49 (12%)
Fatty Change	2/50 (4%)	3/49 (6%)
Centrilobular Hypertrophy[b]	27/50 (54%)	31/49 (63%)
Mean severity[c]	1.1	1.1
Hepatocellular Adenoma[d]	4/50 (8%)	5/49 (10%)
Hepatocellular Adenoma, Multiple	1/50 (2%)	4/49 (8%)
Female		
Basophilic Focus	6/50 (12%)	3/50 (6%)
Clear Cell Focus	2/50 (4%)	3/50 (6%)
Eosinophilic Focus	5/50 (10%)	2/50 (4%)
Mixed Cell Focus	1/50 (2%)	4/50 (8%)
Centrilobular Hypertrophy	42/50 (84%)	38/50 (76%)
Mean severity	1.1	1.1
Hepatocellular Adenoma	1/50 (2%)	0/50 (0%)

[a] Number of lesion-bearing animals/number of animals examined microscopically at site
[b] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report, because it is more widely used and understood.
[c] Average severity grade of lesions in affected rats (1 = minimal; 2 = mild; 3 = moderate; 4 = marked)
[d] Includes multiple adenomas

TABLE 13
Liver Lesions in Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:800, 210:800, and 630:800 ppm Groups

	0:800 ppm	210:800 ppm	630:800 ppm
Male			
Basophilic Focus[a]	15/50 (30%)	12/49 (24%)	26/49 (53%)**
Clear Cell Focus	8/50 (16%)	6/49 (12%)	3/49 (6%)
Eosinophilic Focus	6/50 (12%)	8/49 (16%)	11/49 (22%)
Mixed Cell Focus	0/50 (0%)	1/49 (2%)	5/49 (10%)*
Fatty Change	1/50 (2%)	3/49 (6%)	3/49 (6%)
Centrilobular Hypertrophy[b]	13/50 (26%)	14/49 (29%)	29/49 (59%)**
Mean severity[c]	1.2	1.1	1.0
Hepatocellular Adenoma	1/50 (2%)	2/49 (4%)	1/49 (2%)
Hepatocellular Carcinoma	1/50 (2%)	0/49 (0%)	0/49 (0%)
Hepatocellular Adenoma or Carcinoma	2/50 (4%)	2/49 (4%)	1/49 (2%)
Female			
Basophilic Focus	33/50 (66%)	35/50 (70%)	31/50 (62%)
Clear Cell Focus	0/50 (0%)	1/50 (2%)	1/50 (2%)
Eosinophilic Focus	3/50 (6%)	1/50 (2%)	1/50 (2%)
Mixed Cell Focus	1/50 (2%)	0/50 (0%)	2/50 (4%)
Fatty Change	0/50 (0%)	1/50 (2%)	2/50 (4%)
Centrilobular Hypertrophy	22/50 (44%)	24/50 (48%)	29/50 (58%)*
Mean severity	1.0	1.1	1.0
Hepatocellular Adenoma	1/50 (2%)	1/50 (2%)	0/50 (0%)

* Significantly different ($P \leq 0.05$) from the 0:800 ppm group by the logistic regression test
** $P \leq 0.01$
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report, because it is more widely used and understood.
[c] Average severity grade of lesions in affected rats (1 = minimal; 2 = mild; 3 = moderate; 4 = marked)

The combined incidences of hepatocellular adenoma and carcinoma for all exposure groups are shown in Table 14. A single logistic regression analysis applied to all eight experimental groups of male rats indicates that significant ($P \leq 0.05$) increases in the incidences of hepatocellular neoplasms are associated with increasing F_1 concentration levels of 5,5-diphenylhydantoin, confirming the effects observed in the adult-only exposure groups. No significant exposure-related trend was associated with F_0 exposure, nor was there a significant $F_0 \times F_1$ interaction. The lack of interaction implies that the effect of F_1 exposure is similar, regardless of the level of F_0 exposure. For female rats, the low occurrence of hepatocellular neoplasms was not considered to be chemical related.

Decreasing Incidences of Neoplasms: For males exposed to an F_1 concentration of 800 ppm, the incidence of mononuclear cell leukemia was significantly decreased (24/50, 22/50, 9/50; Table A2e).

TABLE 14
Hepatocellular Adenomas and Carcinomas in Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

	F_0 Concentration (ppm)			
F_1 Concentration (ppm)	0	63	210	630
Male				
0	0/50	–[b]	–	1/50
240	–	3/49	–	–
800	2/50	–	2/49	1/49
2,400	4/50	–	–	5/49*
Female				
0	0/50	–	–	0/49
240	–	0/50	–	–
800	1/50	–	1/50	0/50
2,400	1/50	–	–	0/50

* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by the logistic regression tests
[a] Incidences are given as the number of neoplasm-bearing animals/number of animals necropsied.
[b] Animals were not exposed at these concentrations.

MICE

13-WEEK STUDY

Nine males and all females exposed to 1,200 ppm died before the end of the study; one 75 ppm male and one control female also died (Table 15). Final mean body weights and mean body weight gains of all exposed groups of males were significantly lower than controls. Feed consumption by exposed and control groups was generally similar. No clinical findings were clearly attributable to chemical exposure.

There were no chemical-related gross lesions in mice. Chemical-related microscopic lesions were limited to the liver and consisted of centrilobular hypertrophy of hepatocytes (Plates 3 and 4). Severity of hypertrophy was minimal to mild in females and mild to moderate in males in the 600 ppm groups; minimal hypertrophy was also present in 300 ppm males. Hypertrophy also occurred in a few 1,200 ppm males that survived until near the end of the study; this lesion was not present in female mice in the 1,200 ppm group, all of which died before day 7 of the study. No microscopic lesions were seen in males exposed to 150 ppm or females exposed to 300 ppm. Hypertrophy was characterized by enlargement of hepatocytes in the centrilobular area; in livers where hypertrophy was of moderate severity,

TABLE 15
Survival, Body Weights, and Feed and Compound Consumption of Mice in the 13-Week Feed Study of 5,5-Diphenylhydantoin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Consumption	
		Initial	Final	Change		Feed[c]	Compound[d]
Male							
0	10/10	24.6 ± 0.8	31.5 ± 0.5	6.9 ± 0.7		6.5	
75	9/10[e]	25.4 ± 0.5	29.4 ± 0.9*	4.3 ± 0.8**	93	7.5	20.5
150	10/10	25.2 ± 0.6	29.3 ± 0.7*	4.1 ± 0.4**	93	6.4	35.2
300	10/10	25.3 ± 0.9	29.3 ± 0.6*	4.0 ± 0.8**	93	6.2	68.1
600	10/10	24.8 ± 0.7	28.8 ± 0.7**	4.0 ± 0.6**	91	5.8	129.9
1,200	1/10[f]	25.0 ± 0.5	22.7	−0.2	72	–	–
Female							
0	9/10[g]	19.1 ± 0.5	23.8 ± 0.5	4.6 ± 0.3		7.7	
75	10/10	19.0 ± 0.4	24.4 ± 0.5	5.5 ± 0.5	103	6.8	23.5
150	10/10	19.4 ± 0.6	25.3 ± 0.6	5.9 ± 0.3	106	6.7	45.0
300	10/10	19.0 ± 0.5	22.8 ± 0.6	3.8 ± 0.4	96	6.3	90.4
600	10/10	18.6 ± 0.5	22.6 ± 0.5	4.1 ± 0.3	95	6.2	180.6
1,200	0/10[h]	18.7 ± 0.4	–	–	–	–	–

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies. No final mean body weights were calculated for groups with 100% mortality. No standard errors were calculated for groups with high mortality.
[c] Feed consumption is expressed as grams per animal per day for 13 weeks.
[d] Compound consumption is expressed as mg/kg body weight per day for 13 weeks.
[e] Week of death: 11
[f] Week of death: 1, 1, 1, 1, 1, 3, 7, 7, 11
[g] Week of death: 10
[h] Week of death: 1, 1, 1, 1, 2, 2, 2, 2, 2, 2

most cells throughout the lobules were enlarged (Plate 5). Cytoplasm of hypertrophic hepatocytes had an eosinophilic, granular staining appearance; nuclei of some cells were also enlarged and stained more intensely basophilic than hepatocyte nuclei in the liver of control mice. In some mice with hepatocellular hypertrophy, a few markedly enlarged hepatocytes contained multiple (six or more) nuclei.

Dose Selection Rationale for Adult Exposure

In the 13-week study, all but one mouse exposed to 1,200 ppm died before the end of the study. All groups except the 1,200 ppm groups gained weight over the 13-week period, although an exposure-related depression in body weight gain was seen in males and females and feed consumption by exposed females was lower than that by controls. Chemical-related histopathologic lesions were present in the liver of exposed mice. Females appeared to be less sensitive to the effects of 5,5-diphenylhydantoin on growth and histomorphologic effects in the liver than males. Based on these results, 300 ppm (males) and 600 ppm (females) were selected as the highest exposure levels for the adult exposure portion of the 2-year carcinogenicity study.

GESTATIONAL STUDY: DETERMINATION OF MAXIMUM PERINATAL DOSE

The gestational study was conducted to determine the dietary concentrations for perinatal exposure to be used in the 2-year study. There were no litters in the 600 ppm group. No pups in the 20 ppm group survived past day 0 (Table 16). The numbers of pups in the 60 and 200 ppm groups surviving to day 28 were similar to the number of survivors in the controls. On postpartum day 4, litters were culled to a maximum of eight.

Because reproductive performance and maternal care were poor in all groups, all available pups were allocated for direct exposure to 5,5-diphenylhydantoin in feed for 4 weeks after weaning. Two of four males and two of nine females died before the end of the study (Table 17). Mean body weights and mean body weight gains of exposed animals were similar to those of the controls. There were no chemical-related gross or microscopic lesions in male or female mice.

Dose Selection Rationale for Perinatal Exposure

In general, reproductive performance and maternal care were poor in all groups, including the controls; this restricted the sample size and sensitivity of this evaluation. Nevertheless, fertility and maternal weight gain were affected in the 600 ppm group. There were no gross external malformations among pups surviving to term, and no gross or histopathologic lesions were observed in any mice exposed for 4 weeks following weaning. Based on these results, 210 ppm was selected as the highest exposure level for the perinatal exposure period of the 2-year carcinogenicity study.

TABLE 16
Survival, Sex Ratios, and Mean Body Weights of Mouse Pups in the Maximum Perinatal Dose Determination Feed Study of 5,5-Diphenylhydantoin

	0 ppm	20 ppm	60 ppm	200 ppm	600 ppm[a]
Precull					
Litters on day 0	6	2	8	8	
Pups on day 0	45	18	69	57	
Number of males on day 0	27	7	28	29	
Number of females on day 0	18	11	41	28	
Male/female ratio on day 0	1.5	0.64	0.68	1.04	
Litters on days 1-28[b]	2	0	2	3	
Pups on day 1	18	–	18	26	
Pup weight on day 1	1.26	–	1.29	1.34	
Pups dead days 1-4	5	–	0	9	
Pups on day 4	13	–	18	17	
Pup weight on day 4[c]	1.22 ± 0.3	–	2.11 ± 0.40**	1.92 ± 0.42**	
Postcull					
Pups on day 4	12	–	16	17	
Pup weight on day 4[c]	1.27 ± 0.3	–	2.19 ± 0.33**	1.92 ± 0.42**	
Pups dead days 4-28	1	–	1	4	
Pups on day 28	11	–	15	13	
Pup weight on day 28[c]	14.6 ± 1.15	–	14.5 ± 1.84	14.2 ± 1.42	

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test
[a] There were no litters in the 600 ppm group.
[b] Includes only those litters for which body weight data were available on days 1, 4, and 28
[c] Mean ± standard deviation. Pup weights are given in grams.

TABLE 17
Survival and Body Weights of Mouse Weanlings in the Maximum Perinatal Dose Determination Feed Study of 5,5-Diphenylhydantoin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	4/4	15.9 ± 0.3	24.2 ± 0.2	8.3 ± 0.1	
60	5/5	16.2 ± 0.9	24.6 ± 0.7	8.3 ± 0.4	101
200	2/4	15.6 ± 0.7	24.6 ± 0.1	8.2 ± 0.9	101
Female					
0	7/7	13.9 ± 0.2	19.8 ± 0.2	5.9 ± 0.4	
60	10/10	13.6 ± 0.3	19.8 ± 0.3	6.2 ± 0.2	100
200	7/9	13.6 ± 0.3	19.6 ± 0.2	5.9 ± 0.4	99

[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies. Differences from the control group were not significant by Dunnett's test.

2-YEAR STUDY

9-Month Interim Evaluation

Of the mice scheduled for evaluation at 9 months, four of 10 males in the 70:100 ppm group died during the first week because of fight injuries and dehydration, and one male in the 0:300 ppm group died during week 36 of the study; all females survived until the interim evaluation (Table 18). The final mean body weights of males exposed to 300 ppm as adults were more that of than 10% lower than controls; the mean body weights and mean body weight gains of females in the 0:200, 70:200, 0:600, and 210:600 ppm groups were also significantly lower than those of controls.

Absolute liver weights in females exposed to 210:600 ppm and relative liver weights in males receiving adult exposure to 300 ppm and females receiving adult exposure to 200 or 600 ppm were significantly greater than those of controls (Table F2). Relative kidney weights in males exposed to 210:100 or 210:300 ppm and females exposed to 210:200 ppm were significantly lower than those of controls.

There were no chemical-related gross lesions in male or female mice. Microscopic lesions were present in the liver of most males exposed to 300 ppm as adults, most females exposed to 600 ppm as adults, and most males in the 210:100 ppm group. Liver lesions consisted of centrilobular hypertrophy that was characterized by enlargement of hepatocytes in the central portion of the hepatic lobules. Cytoplasm of hypertrophic cells often had an eosinophilic granular appearance, and some hepatocyte nuclei were slightly enlarged compared to those in the liver of control mice. In groups receiving adult-only exposure at the highest levels, hypertrophy was more severe in males (minimal to moderate) than in females (minimal to mild); in the groups receiving combined perinatal and adult exposure at the highest levels, the severity of hypertrophy was generally moderate. In exposed groups of males and females, there were multinucleated hepatocytes similar to those seen in mice in the 13-week study. In the 210:100 (male) and 210:200 ppm (female) groups, minimal hepatocellular hypertrophy occurred in mice.

TABLE 18
Survival and Body Weights of Mice at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0:0	10/10	23.8 ± 0.7	38.9 ± 1.4	15.2 ± 1.4	
210:0	10/10	22.1 ± 0.4	36.1 ± 1.3	14.0 ± 1.1	93
21:30	10/10	23.0 ± 0.5	36.5 ± 0.9	13.5 ± 0.5	94
0:100	10/10	23.0 ± 0.5	35.2 ± 1.4	12.2 ± 1.3	90
70:100	6/10[c]	22.5 ± 0.6	33.6 ± 1.9	11.3 ± 1.0	86
210:100	10/10	21.1 ± 0.4**	35.7 ± 1.6	14.6 ± 1.5	92
0:300	9/10[d]	23.3 ± 0.6	33.0 ± 0.8*	9.8 ± 0.6**	85
210:300	10/10	20.9 ± 0.4**	32.3 ± 0.8**	11.4 ± 0.9	83
Female					
0:0	10/10	19.0 ± 0.4	30.5 ± 1.0	11.5 ± 0.8	
210:0	10/10	17.1 ± 0.4*	28.5 ± 1.2	11.4 ± 0.9	94
21:60	10/10	19.1 ± 0.2	29.6 ± 1.1	10.5 ± 0.9	97
0:200	10/10	19.2 ± 0.2	27.1 ± 0.8*	7.8 ± 0.8**	89
70:200	10/10	18.2 ± 0.3	26.9 ± 0.8*	8.7 ± 0.6*	88
210:200	10/10	17.9 ± 0.4	28.0 ± 1.0	10.1 ± 0.8	92
0:600	10/10	18.5 ± 0.6	23.2 ± 0.6**	4.7 ± 0.4**	76
210:600	10/10	17.9 ± 0.6	23.9 ± 0.6**	5.9 ± 0.2**	78

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies.
[c] Week of death: all deaths occurred during week 1 (cage fighting and dehydration)
[d] Week of death: 36

Survival

Two-year survival rates of exposed groups were similar to those of the controls (Table 19 and Figure 4).

Body Weights, Feed Consumption, and Clinical Findings

The final mean body weights of male mice exposed to 210:100, 0:300, or 210:300 ppm and females exposed to 210:200, 0:600, or 210:600 ppm were more than 10% lower than those of the controls (Tables 20 and 21 and Figures 5a,b,c, and d). Females exposed to 600 ppm as adults had final mean body weights at least 40% less than that of the controls. Feed consumption was similar among exposed and control mice (Tables I3 and I4). Adult exposure of males to 100 or 300 ppm 5,5-diphenylhydantoin resulted in dietary exposure levels of 20 or 60 mg/kg body weight. Adult exposure of females to 200 or 600 ppm resulted in dietary exposures of 50 or 160 mg/kg. There were no clinical findings that could be clearly attributed to chemical exposure.

TABLE 19
Survival of Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin

	F_0:F_1 Concentration (ppm)							
Male	**0:0**	**210:0**	**21:30**	**0:100**	**70:100**	**210:100**	**0:300**	**210:300**
Animals initially in study	60	60	60	60	60	60	60	60
9-Month interim evaluation[a]	10	10	10	10	10	10	10	10
Moribund	6	8	2	3	10	7	9	4
Natural deaths	5	5	9	6	7	6	7	2
Animals surviving to study termination	39[b]	37[b]	39[b]	40[b]	33	36	34	44
Missing[a]	0	0	0	1	0	1	0	0
Percent probability of survival at end of study[c]	78	74	78	82	66	74	68	88
Mean survival (days)[d]	712	708	703	695	692	707	612	726
Survival analysis[e]		P=0.765	P=0.902	P=0.897N	P=0.245	P=0.764	P=0.282	P=0.251N

	F_0:F_1 Concentration (ppm)							
Female	**0:0**	**210:0**	**21:60**	**0:200**	**70:200**	**210:200**	**0:600**	**210:600**
Animals initially in study	60	60	60	60	60	60	60	60
9-Month interim evaluation[a]	10	10	10	10	10	10	10	10
Moribund	8	11	9	8	6	6	8	6
Natural deaths	6	5	6	4	6	7	5	4
Animals surviving to study termination	36	34	35	38	38	37	37	40
Percent probability of survival at end of study[c]	72	68	70	76	76	74	74	80
Mean survival (days)[d]	696	689	692	709	709	709	712	719
Survival analysis[e]		P=0.724	P=0.893	P=0.743N	P=0.746N	P=0.919N	P=0.910N	P=0.430N

[a] Censored from survival analyses
[b] Includes one animal that died or was killed moribund during the last week of the study
[c] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice)
[e] The results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposure columns. A lower mortality in an exposure group is indicated by N.

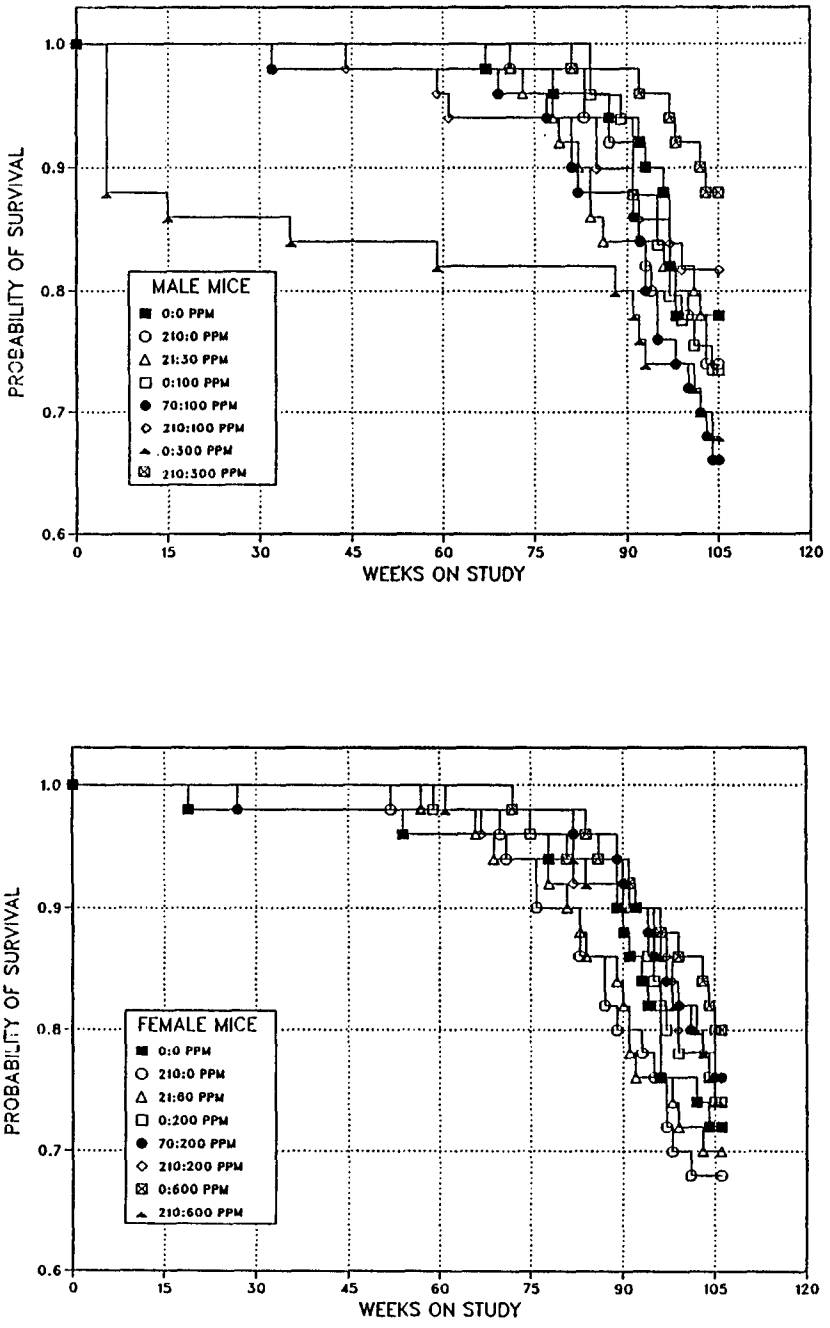


FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered 5,5-Diphenylhydantoin in Feed for 2 Years

TABLE 20
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin

Week on Study	0:0 ppm Av. Wt. (g)	0:0 ppm No. of Survivors	210:0 ppm Av. Wt. (g)	210:0 ppm Wt. (% of controls)	210:0 ppm No. of Survivors	21:30 ppm Av. Wt. (g)	21:30 ppm Wt. (% of controls)	21:30 ppm No. of Survivors	0:100 ppm Av. Wt. (g)	0:100 ppm Wt. (% of controls)	0:100 ppm No. of Survivors
1	25.5	50	23.3	91	50	24.9	98	50	24.4	96	50
2	27.2	50	25.1	92	50	26.0	96	50	25.8	95	50
3	27.9	50	26.0	93	50	27.0	97	50	26.5	95	50
4	28.1	50	26.2	93	50	27.5	98	50	26.6	95	50
5	29.0	50	27.1	93	50	28.2	97	50	27.5	95	50
6	29.1	50	27.3	94	50	28.3	97	50	28.0	96	50
7	30.0	50	28.0	93	50	29.3	98	50	28.4	95	50
8	30.3	50	27.9	92	50	29.9	99	50	28.5	94	50
9	31.3	50	29.1	93	50	30.5	97	50	29.5	94	50
10	32.3	50	30.2	93	50	30.7	95	50	29.8	92	50
11	32.4	50	30.9	95	50	31.1	96	50	30.4	94	50
12	33.2	50	31.2	94	50	31.8	96	50	30.8	93	50
16	35.6	50	33.2	93	50	34.3	96	50	32.8	92	50
20	36.9	50	34.9	95	50	36.2	98	50	33.7	91	50
25	39.1	50	36.8	94	50	37.8	97	50	35.1	90	50
29	40.6	50	37.9	93	50	39.1	96	50	37.0	91	50
33	41.1	50	37.9	92	50	38.5	94	50	37.1	90	50
37	41.5	50	39.1	94	50	40.5	98	50	38.2	92	50
42	41.5	50	38.7	93	50	40.1	97	50	38.5	93	50
47	41.1	50	39.1	95	50	40.0	97	50	39.3	96	49
51	42.2	50	40.2	95	50	41.5	98	50	40.0	95	49
56	41.9	50	39.8	95	50	39.9	95	50	38.8	93	49
59	41.4	50	38.9	94	50	40.3	97	50	38.4	93	48
62	41.7	50	40.0	96	50	40.0	96	50	38.6	93	46
67	42.9	49	40.7	95	50	42.1	98	50	39.9	93	46
72	42.1	49	41.4	98	49	42.9	102	49	39.5	94	46
77	40.8	49	40.3	99	49	41.6	102	48	39.8	98	46
81	39.9	48	39.5	99	49	41.6	104	46	38.5	96	46
85	41.0	48	40.7	99	47	41.7	102	43	38.8	95	44
90	40.2	47	39.5	98	46	40.4	100	42	38.4	96	44
94	39.2	45	38.2	97	40	39.4	101	42	36.4	93	42
98	40.9	39	39.1	96	40	39.4	96	41	36.6	89	41
103	40.3	39	38.7	96	37	38.3	95	39	36.9	92	40
Mean for weeks											
1-13	29.7		27.7	93		28.8	97		28.0	94	
14-52	40.0		37.5	94		38.7	97		36.9	92	
53-103	41.0		39.7	97		40.6	99		38.4	94	

(continued)

TABLE 20
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin
(continued)

Week on Study	70:100 ppm			210:100 ppm			0:300 ppm			210:300 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.3	91	50	23.3	91	50	25.1	98	50	23.1	91	50
2	24.6	90	50	24.3	89	50	25.8	95	50	24.2	89	50
3	25.5	91	50	25.2	90	50	26.3	94	50	25.0	90	50
4	25.9	92	50	25.4	90	50	24.6	88	50	24.9	89	50
5	26.6	92	50	26.0	90	50	26.9	93	44	25.6	88	50
6	27.0	93	50	26.7	92	50	26.9	92	44	25.7	88	50
7	27.6	92	50	27.0	90	50	26.9	90	44	26.1	87	50
8	28.0	92	50	27.4	90	50	27.6	91	44	25.8	85	50
9	28.9	92	50	28.3	90	50	28.2	90	44	27.2	87	50
10	29.4	91	50	28.8	89	50	28.8	89	44	27.8	86	50
11	29.2	90	50	28.6	88	50	29.2	90	44	27.1	84	50
12	30.1	91	50	29.9	90	50	29.6	89	44	28.4	86	50
16	31.7	89	50	31.2	88	50	30.8	87	43	29.8	84	50
20	33.1	90	50	32.8	89	50	32.0	87	43	30.8	83	50
25	34.5	88	50	34.4	88	50	32.8	84	43	32.3	83	50
29	35.4	87	50	35.9	88	50	34.3	84	43	33.4	82	50
33	36.0	88	50	36.8	90	50	34.8	85	43	34.1	83	50
37	37.2	90	50	37.6	91	50	35.1	85	42	34.8	84	50
42	37.7	91	49	37.5	90	50	35.7	86	42	36.0	87	50
47	37.3	91	49	37.1	90	50	35.4	86	42	35.9	87	50
51	38.1	90	49	38.3	91	50	36.5	86	42	37.1	88	50
56	37.2	89	49	37.2	89	50	36.4	87	41	36.3	87	50
59	37.3	90	49	37.3	90	49	35.6	86	41	36.8	89	50
62	37.9	91	49	38.0	91	49	37.4	90	41	36.5	88	50
67	38.6	90	49	39.0	91	49	38.3	89	41	37.5	87	50
72	39.7	94	48	39.2	93	49	37.3	89	41	37.4	89	50
77	39.9	98	47	38.6	95	49	38.1	93	41	36.7	90	50
81	38.6	97	45	37.5	94	49	36.6	92	41	35.6	89	49
85	38.6	94	44	37.0	90	47	37.4	91	41	35.8	87	49
90	38.4	96	44	37.3	93	46	37.0	92	40	35.6	89	49
94	36.2	92	40	35.5	91	43	35.8	91	37	34.4	88	48
98	36.2	89	37	35.7	87	39	35.9	88	37	34.1	83	46
103	36.3	90	34	35.3	88	37	35.8	89	35	34.0	84	44
Mean for weeks												
1-13	27.2	92		26.7	90		27.2	92		25.9	87	
14-52	35.7	89		35.7	89		34.2	86		33.8	85	
53-103	37.9	92		37.3	91		36.8	90		35.9	88	

TABLE 21
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin

Week on Study	0:0 ppm Av. Wt. (g)	0:0 ppm No. of Survivors	210:0 ppm Av. Wt. (g)	210:0 ppm Wt. (% of controls)	210:0 ppm No. of Survivors	21:60 ppm Av. Wt. (g)	21:60 ppm Wt. (% of controls)	21:60 ppm No. of Survivors	0:200 ppm Av. Wt. (g)	0:200 ppm Wt. (% of controls)	0:200 ppm No. of Survivors
1	20.0	50	18.3	92	50	20.2	101	50	20.7	104	50
2	20.5	50	19.4	95	50	20.2	99	50	20.9	102	50
3	19.1	50	20.8	109	50	21.4	112	50	21.4	112	50
4	21.8	50	20.9	96	50	21.8	100	50	21.9	100	50
5	22.6	50	21.7	96	50	22.2	98	50	22.3	99	50
6	23.0	50	22.0	96	50	22.9	100	50	22.8	99	50
7	23.1	50	22.6	98	50	23.1	100	50	22.7	98	50
8	24.1	50	23.3	97	50	23.6	98	50	24.0	100	50
9	23.7	50	23.8	100	50	23.5	99	50	24.1	102	50
10	23.9	50	24.2	101	50	24.0	100	50	24.2	101	50
11	24.7	50	24.5	99	50	24.5	99	50	24.5	99	50
12	25.0	50	24.7	99	50	25.1	100	50	24.5	98	50
16	26.9	50	26.8	100	50	26.7	99	50	25.9	96	50
20	28.3	49	28.9	102	50	28.1	99	50	27.4	97	50
25	29.0	49	29.6	102	50	29.0	100	50	27.9	96	50
29	30.5	49	31.2	102	50	29.8	98	50	29.2	96	50
33	31.0	49	31.6	102	50	30.3	98	50	29.8	96	50
37	32.1	49	33.0	103	50	31.6	98	50	30.8	96	50
42	32.8	49	32.8	100	50	32.1	98	50	31.8	97	50
47	33.3	49	34.9	105	50	33.5	101	50	32.3	97	50
51	34.7	49	35.9	103	50	33.8	97	50	33.3	96	50
56	33.2	48	34.5	104	49	33.2	100	50	32.6	98	49
59	34.7	48	36.0	104	49	34.2	99	49	33.4	96	49
62	36.4	48	36.6	101	49	35.6	98	49	33.5	92	49
67	37.8	48	38.4	102	49	37.5	99	48	35.7	94	48
72	38.9	48	39.4	101	47	38.8	100	47	36.3	93	48
77	38.4	48	38.8	101	45	38.7	101	47	36.2	94	48
81	36.1	47	37.7	104	45	38.3	106	45	35.2	98	47
85	39.8	47	38.7	97	43	39.5	99	43	36.4	91	46
90	39.7	43	38.2	96	40	40.2	101	41	36.8	93	46
94	40.4	41	37.9	94	39	40.2	100	38	37.0	92	45
98	41.4	38	39.2	95	35	40.7	98	37	37.4	90	42
103	41.1	37	38.9	95	34	41.4	101	35	39.0	95	39
Mean for weeks											
1-13	22.6		22.2	98		22.7	100		22.8	101	
14-52	31.0		31.6	102		30.5	98		29.8	96	
53-103	38.2		37.9	99		38.2	100		35.8	94	

(continued)

TABLE 21
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin
(continued)

Week on Study	70:200 ppm			210:200 ppm			0:600 ppm			210:600 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.1	96	50	19.1	96	50	19.3	97	50	18.4	92	50
2	19.2	94	50	18.9	92	50	20.0	98	50	18.8	92	50
3	19.8	104	50	20.0	105	50	20.5	107	50	19.1	100	50
4	20.4	94	50	20.5	94	50	20.7	95	50	19.4	89	50
5	20.6	91	50	20.5	91	50	21.5	95	50	20.2	89	50
6	20.9	91	50	21.2	92	50	20.9	91	50	20.1	87	50
7	21.3	92	50	21.5	93	50	21.2	92	50	19.9	86	50
8	21.8	90	50	22.6	94	50	22.2	92	50	21.3	88	50
9	22.8	96	50	22.6	95	50	22.6	95	50	21.3	90	50
10	23.1	97	50	22.9	96	50	22.3	93	50	21.5	90	50
11	22.2	90	50	23.1	94	50	23.1	94	50	21.8	88	50
12	23.1	92	50	23.5	94	50	23.4	94	50	22.2	89	50
16	24.1	90	50	24.8	92	50	24.1	90	50	23.2	86	50
20	25.7	91	50	25.9	92	50	24.5	87	50	23.4	83	50
25	26.2	90	50	26.2	90	50	24.5	84	50	23.3	80	50
29	27.1	89	50	27.5	90	50	24.8	81	50	24.1	79	50
33	27.1	87	50	28.4	92	50	25.0	81	50	23.4	75	50
37	28.2	88	50	29.1	91	50	25.3	79	50	24.0	75	50
42	29.6	90	50	29.7	91	50	25.5	78	50	24.6	75	50
47	30.3	91	49	30.9	93	50	25.7	77	50	24.9	75	50
51	31.2	90	49	32.0	92	50	26.1	75	50	25.4	73	50
56	30.9	93	49	31.6	95	50	25.4	77	50	25.0	75	50
59	31.2	90	49	31.5	91	49	25.5	73	50	25.2	73	50
62	32.5	89	49	32.3	89	49	25.7	71	49	24.8	68	50
67	33.9	90	49	34.0	90	49	25.8	68	49	25.0	66	50
72	34.9	90	49	34.6	89	49	25.6	66	49	24.5	63	49
77	34.9	91	49	34.8	91	48	25.5	66	49	24.6	64	49
81	33.8	94	49	33.6	93	47	24.4	68	49	23.9	66	49
85	35.2	88	48	34.7	87	47	25.6	64	46	24.8	62	47
90	35.9	90	46	35.1	88	47	24.9	63	45	24.2	61	47
94	35.3	87	44	35.2	87	42	25.1	62	45	24.1	60	45
98	36.5	88	41	36.3	88	40	24.1	58	41	23.4	57	43
103	37.1	90	40	36.0	88	39	24.6	60	39	23.8	58	42
Mean for weeks												
1-13	21.2	94		21.4	95		21.5	95		20.3	90	
14-52	27.7	89		28.3	91		25.1	81		24.0	77	
53-103	34.3	90		34.1	89		25.2	66		24.4	64	

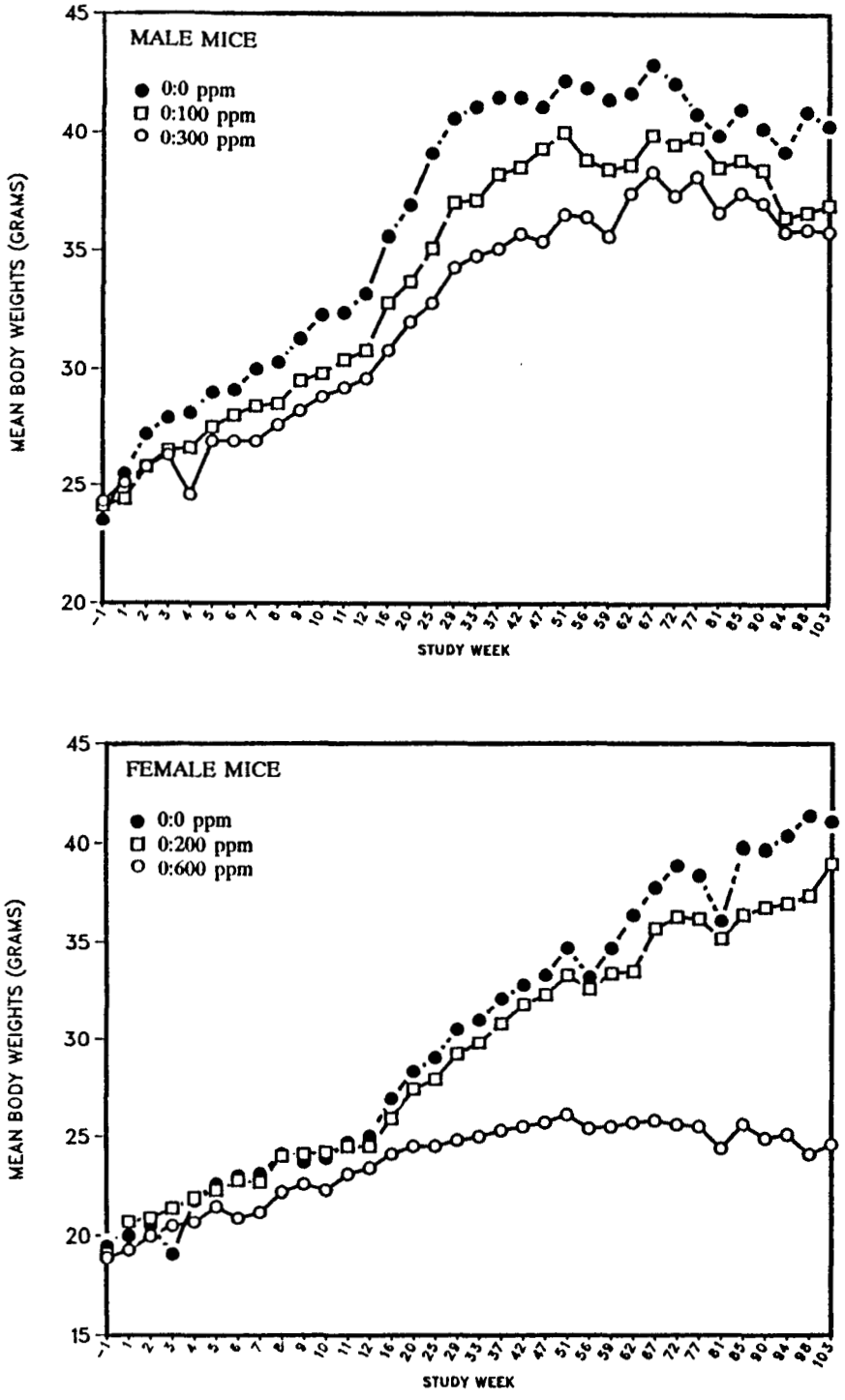


FIGURE 5a
Growth Curves for Mice Administered 5,5-Diphenylhydantoin in Feed for 2 Years: 0:0, 0:100, and 0:300 ppm (Male) and 0:0, 0:200, and 0:600 ppm (Female) Groups

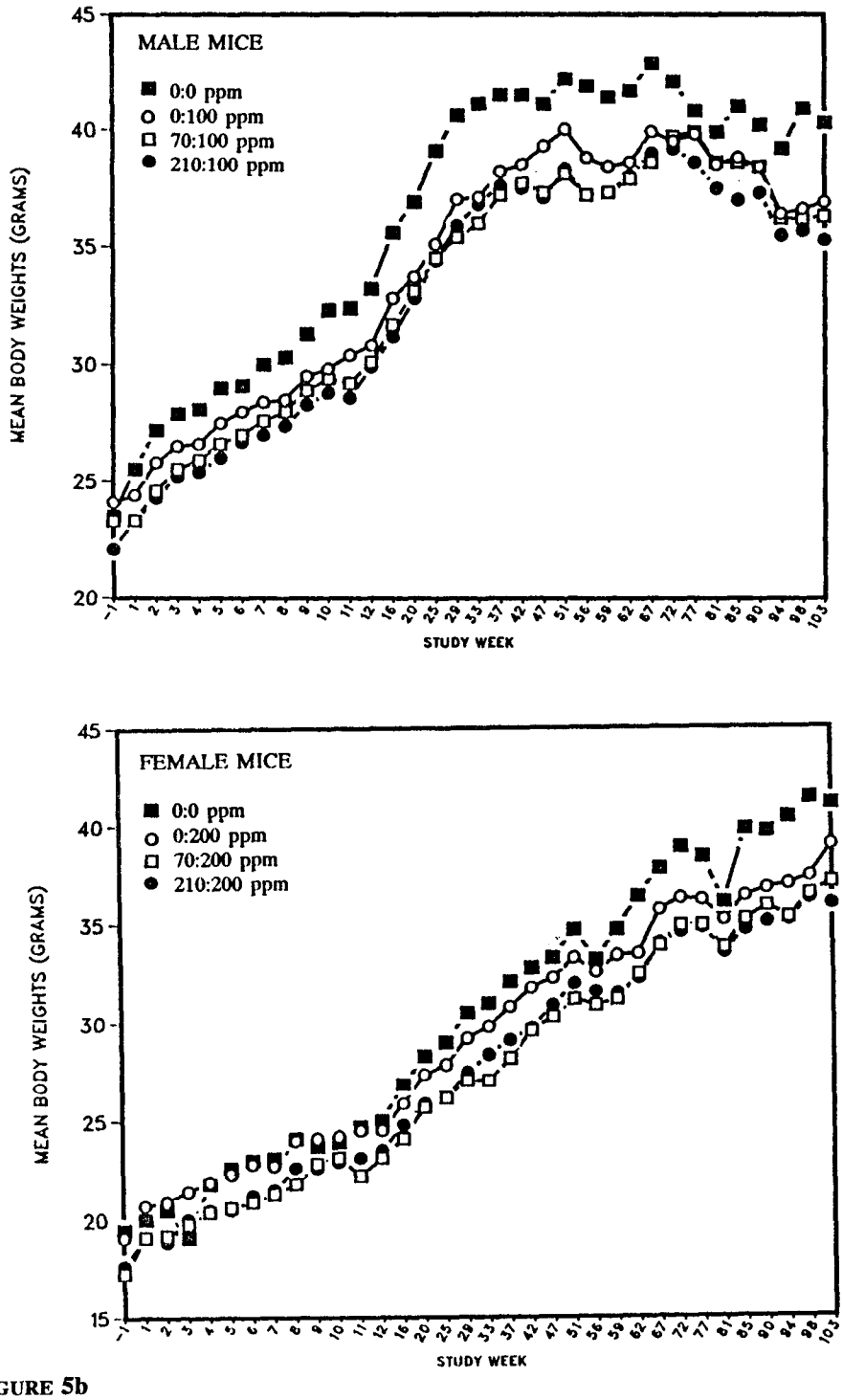


FIGURE 5b
Growth Curves for Mice Administered 5,5-Diphenylhydantoin in Feed for 2 Years: 0:0, 0:100, 70:100, and 210:100 ppm (Male) and 0:0, 0:200, 70:200, and 210:200 ppm (Female) Groups

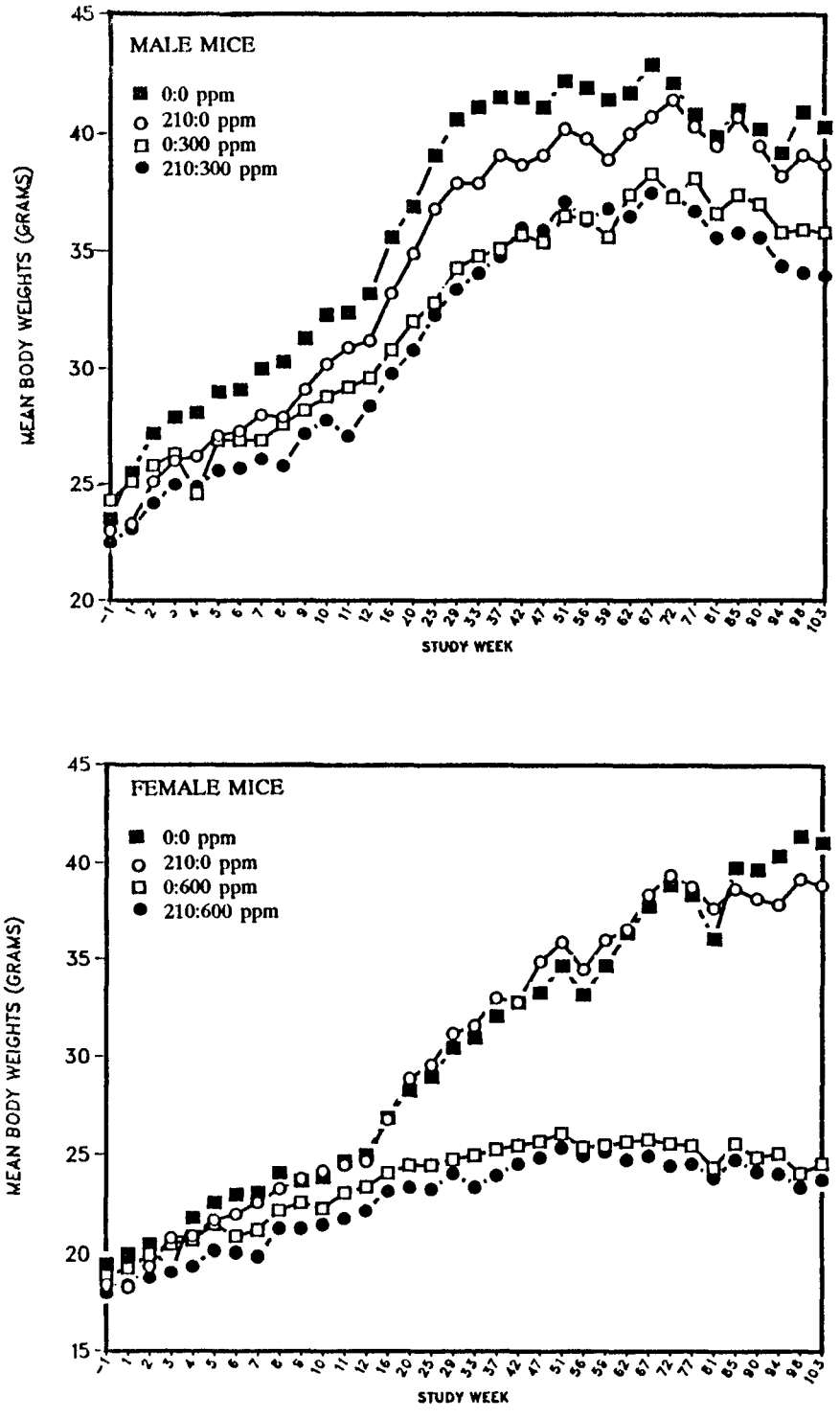


FIGURE 5c
Growth Curves for Mice Administered 5,5-Diphenylhydantoin in Feed for 2 Years: 0:0, 210:0, 0:300, and 210:300 ppm (Male) and 0:0, 210:0, 0:600, and 210:600 ppm (Female) Groups

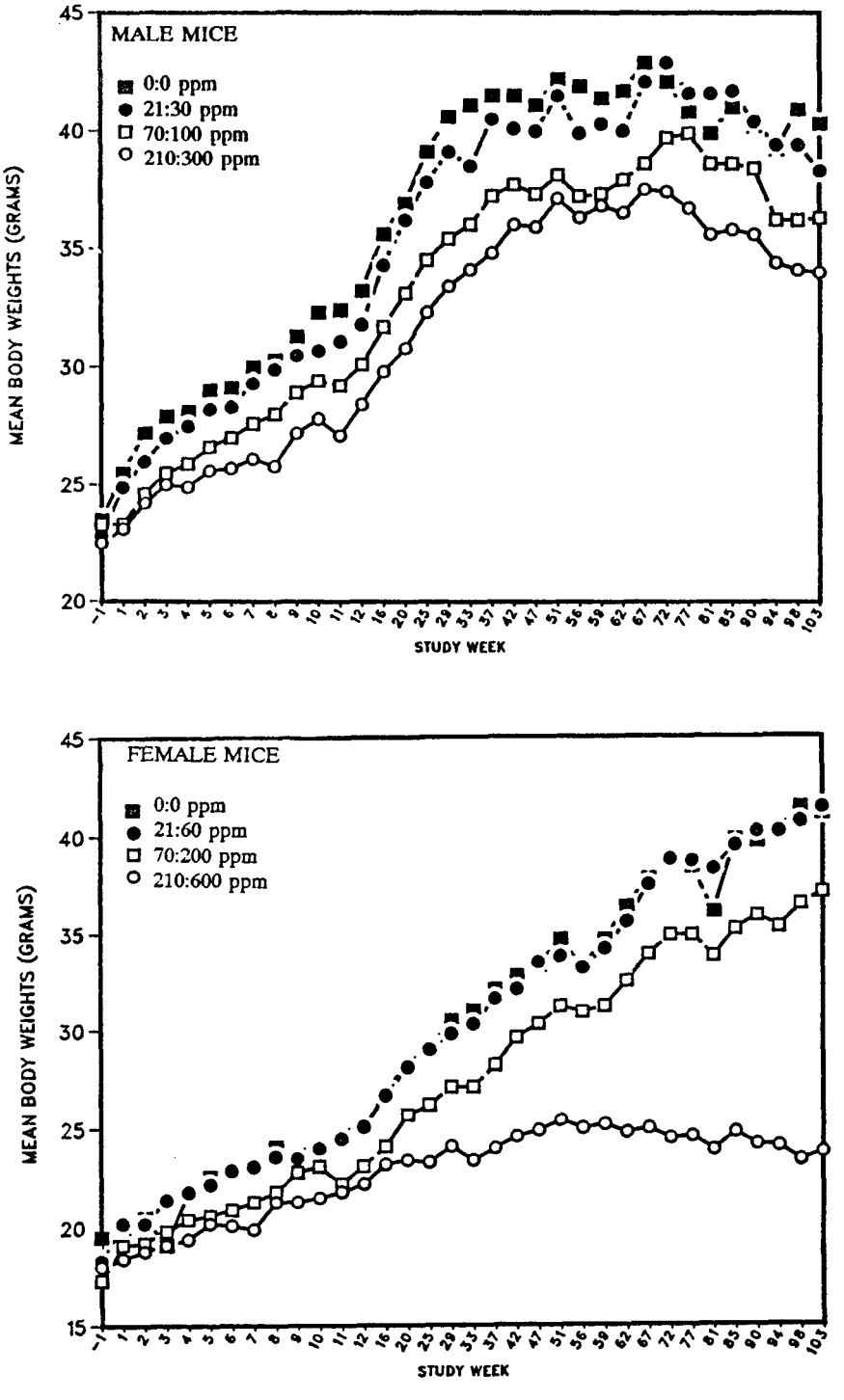


FIGURE 5d
Growth Curves for Mice Administered 5,5-Diphenylhydantoin in Feed for 2 Years: 0:0, 21:30, 70:100, and 210:300 ppm (Male) and 0:0, 21:60, 70:200, and 210:600 ppm (Female) Groups

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant lymphoma and neoplasms or nonneoplastic lesions of the liver in mice.

Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for male mice and Appendix D for female mice. Historical incidences of neoplasms in control mice are given in Table C3 for males and Table D3 for females.

Effects of Adult-Only Exposure of Mice to 5,5-Diphenylhydantoin

The neoplastic and nonneoplastic effects of adult-only exposure were determined by comparison of the incidences of lesions in the 0:0, 0:100, and 0:300 ppm groups of males and in the 0:0, 0:200, and 0:600 ppm groups of females, which correspond to a standard carcinogenicity study.

Liver: The incidences of hepatocellular adenoma, carcinoma (Plates 6, 7, and 8), and adenoma or carcinoma (combined) were significantly increased in exposed female mice (Table 22). There was no chemical-related effect on the incidence of hepatocellular neoplasms in exposed males. The incidences of nonneoplastic lesions were increased in male and female mice. In both sexes, centrilobular hypertrophy of hepatocytes occurred in all exposed groups. This lesion was characterized by enlargement of hepatocytes around central veins of the hepatic lobules (Plate 9). These cells contained a granular, eosinophilic cytoplasm that sometimes had a finely vacuolated appearance. Nuclei of hypertrophic hepatocytes were sometimes enlarged and a few hepatocytes contained multiple nuclei similar to those seen in the earlier studies. The incidence of clear cell foci of cellular alteration was increased in 0:300 ppm males; hepatocellular cytoplasm of clear cell foci had minimal or no eosinophilic staining characteristics. Cystic degeneration of the liver also occurred in exposed mice, particularly in 0:300 ppm males and consisted of single, large vacuoles or cystic spaces that were generally peripheral to the centrilobular area of hypertrophic hepatocytes (Plate 10). The specific location of these cysts could not be identified with certainty. In the areas of cystic degeneration, there were hepatocytes containing one or more cytoplasmic vacuoles. Larger spaces characteristic of cystic degeneration contained erythrocytes and an eosinophilic proteinaceous fluid or granular debris. Some of the larger cysts were partially lined by endothelium. Cystic degeneration was present in a few exposed females, but a more frequently occurring lesion, diagnosed as fatty change, was seen in females exposed to 0:200 ppm. Fatty change consisted of single, or sometimes several, large vacuolar spaces in the cytoplasm of hepatocytes in the midzonal to periportal areas peripheral to the centrilobular areas of hypertrophy (Plates 11 and 12). In some mice with fatty change, hepatocytes with multiple small cytoplasmic vacuoles were adjacent to cells with single, large, intracytoplasmic vacuoles.

TABLE 22
Liver Lesions in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 0:100, and 0:300 ppm (Male) and the 0:0, 0:200, and 0:600 ppm (Female) Groups

Male	0:0 ppm	0:100 ppm	0:300 ppm
Basophilic Focus[a]	4/50 (8%)	6/49 (12%)	3/49 (6%)
Clear Cell Focus	7/50 (14%)	6/49 (12%)	20/49 (41%)**
Eosinophilic Focus	3/50 (6%)	5/49 (10%)	2/49 (4%)
Mixed Cell Focus	3/50 (6%)	1/49 (2%)	0/49 (0%)
Fatty Change	5/50 (10%)	2/49 (4%)	0/49 (0%)*
Mean severity[b]	1.2	1.5	
Cystic Degeneration	0/50 (0%)	9/49 (18%)**	29/49 (59%)**
Mean severity		1.7	2.1
Centrilobular Hypertrophy[c]	0/50 (0%)	19/49 (39%)**	37/49 (76%)**
Mean severity		1.9	2.3
Hepatocellular Adenoma[d]			
Overall rate	19/50 (38%)	19/49 (39%)	22/49 (45%)
Adjusted rate[e]	43.7%	45.1%	59.3%
Terminal rate[f]	15/39 (38%)	17/40 (43%)	19/34 (56%)
First incidence (days)	540	589	612
Logistic regression test[g]	P=0.110	P=0.522	P=0.139
Hepatocellular Adenoma, Multiple	5/50 (10%)	8/49 (16%)	11/49 (22%)
Hepatoblastoma or Hepatocellular Carcinoma[d]			
Overall rate	13/50 (26%)	15/49 (31%)	7/49 (14%)
Adjusted rate	28.8%	35.6%	19.1%
Terminal rate	8/39 (21%)	13/40 (33%)	5/34 (15%)
First incidence (days)	463	590	612
Logistic regression test	P=0.118N	P=0.388	P=0.154N
Hepatocellular Carcinoma, Multiple	2/50 (4%)	3/49 (6%)	0/49 (0%)
Hepatoblastoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma[d,h]			
Overall rate	29/50 (58%)	29/49 (59%)	26/49 (53%)
Adjusted rate	60.1%	65.8%	68.3%
Terminal rate	20/39 (51%)	25/40 (63%)	22/34 (65%)
First incidence (days)	463	589	612
Logistic regression test	P=0.477	P=0.517	P=0.508
Hepatocellular Adenoma or Carcinoma, Multiple	6/50 (12%)	13/49 (27%)	13/49 (27%)*

(continued)

TABLE 22
Liver Lesions in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 0:100, and 0:300 ppm (Male) and the 0:0, 0:200, and 0:600 ppm (Female) Groups (continued)

Female	0:0 ppm	0:200 ppm	0:600 ppm
Basophilic Focus	1/48 (2%)	1/49 (2%)	4/50 (8%)
Clear Cell Focus	1/48 (2%)	1/49 (2%)	1/50 (2%)
Eosinophilic Focus	0/48 (0%)	3/49 (6%)	8/50 (16%)**
Fatty Change	2/48 (4%)	23/49 (47%)**	0/50 (0%)
Mean severity	2.5	1.3	
Cystic Degeneration	0/48 (0%)	1/49 (2%)	5/50 (10%)*
Mean severity		1.0	1.0
Centrilobular Hypertrophy	0/48 (0%)	25/49 (51%)**	31/50 (62%)**
Mean severity		1.8	1.3
Hepatocellular Adenoma[d]			
Overall rate	5/48 (10%)	13/49 (27%)	22/50 (44%)
Adjusted rate	13.3%	32.3%	50.9%
Terminal rate	4/36 (11%)	11/38 (29%)	16/37 (43%)
First incidence (days)	670	675	664
Logistic regression test	P<0.001	P=0.042	P<0.001
Hepatocellular Adenoma, Multiple	0/48 (0%)	5/49 (10%)*	6/50 (12%)*
Hepatoblastoma or Hepatocellular Carcinoma			
Overall rate	0/48 (0%)	1/49 (2%)	12/50 (24%)
Adjusted rate	0.0%	2.6%	29.1%
Terminal rate	0/36 (0%)	1/38 (3%)	9/37 (24%)
First incidence (days)	–[i]	736 (T)	570
Logistic regression test	P<0.001	P=0.511	P<0.001
Hepatocellular Carcinoma, Multiple	0/48 (0%)	0/49 (0%)	2/50 (4%)
Hepatoblastoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma[d,j]			
Overall rate	5/48 (10%)	14/49 (29%)	30/50 (60%)
Adjusted rate	13.3%	34.8%	66.4%
Terminal rate	4/36 (11%)	12/38 (32%)	22/37 (59%)
First incidence (days)	670	675	570
Logistic regression test	P<0.001	P=0.026	P<0.001
Hepatocellular Adenoma or Carcinoma, Multiple	0/48 (0%)	5/49 (10%)*	9/50 (18%)**

* Significantly different (P≤0.05) from the 0:0 ppm group by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Average severity grade of lesions in affected mice (1 = minimal; 2 = mild; 3 = moderate; 4 = marked)
[c] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report, because it is more widely used and understood.
[d] Includes multiple neoplasms
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.
[h] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 363/1,114 (32.6% ± 13.6%); range 10%-68%
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence: 153/1,113 (13.7% ± 8.6%); range 3%-34%

Effects of Perinatal-Only Exposure of Mice to 5,5-Diphenylhydantoin

The neoplastic and nonneoplastic effects of perinatal-only exposure were determined by comparison of the incidences of lesions in the 0:0 and 210:0 ppm groups.

Liver: The incidences of hepatocellular adenoma and adenoma or carcinoma (combined) were slightly increased in exposed females; although no significantly increased incidences of liver neoplasms occurred in exposed males, the number of exposed males with multiple liver neoplasms was slightly increased (Table 23). Centrilobular hepatocyte hypertrophy, the only nonneoplastic lesion with significantly increased incidence, occurred in males with perinatal exposure. This lesion was of minimal severity and was characterized by the presence of enlarged hepatocytes located around central veins of hepatic lobules. These cells had a granular cytoplasm that stained less intensely eosinophilic than did the centrilobular hepatocytes in control males.

TABLE 23
Liver Lesions in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 210:0 ppm Groups

	0:0 ppm	210:0 ppm
Male		
Basophilic Focus[a]	4/50 (8%)	3/50 (6%)
Clear Cell Focus	7/50 (14%)	6/50 (12%)
Eosinophilic Focus	3/50 (6%)	2/50 (4%)
Mixed Cell Focus	3/50 (6%)	1/50 (2%)
Fatty Change	5/50 (10%)	2/50 (4%)
Mean severity[b]	1.2	1.5
Cystic Degeneration	0/50 (0%)	3/50 (6%)
Mean severity		1.7
Centrilobular Hypertrophy[c]	0/50 (0%)	16/50 (32%)**
Mean severity		1.3
Hepatocellular Adenoma[d]		
Overall rate	19/50 (38%)	23/50 (46%)
Adjusted rate[e]	43.7%	55.8%
Terminal rate[f]	15/39 (38%)	19/37 (51%)
First incidence (days)	540	633
Logistic regression test[g]		P=0.253
Hepatocellular Adenoma, Multiple	5/50 (10%)	12/50 (24%)
Hepatocellular Carcinoma[d]		
Overall rate	13/50 (26%)	14/50 (28%)
Adjusted rate	28.8%	32.6%
Terminal rate	8/39 (21%)	9/37 (24%)
First incidence (days)	463	575
Logistic regression test		P=0.526
Hepatocellular Carcinoma, Multiple	2/50 (4%)	4/50 (8%)
Hepatocellular Adenoma or Carcinoma[d]		
Overall rate	29/50 (58%)	33/50 (66%)
Adjusted rate	60.1%	71.6%
Terminal rate	20/39 (51%)	24/37 (65%)
First incidence (days)	463	575
Logistic regression test		P=0.281
Hepatocellular Adenoma or Carcinoma, Multiple	6/50 (12%)	16/50** (32%)

(continued)

TABLE 23
Liver Lesions in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 210:0 ppm Groups (continued)

	0:0 ppm	210:0 ppm
Female		
Basophilic Focus	1/48 (2%)	2/49 (4%)
Clear Cell Focus	1/48 (2%)	0/49 (0%)
Eosinophilic Focus	0/48 (0%)	1/49 (2%)
Mixed Cell Focus	0/48 (0%)	1/49 (2%)
Fatty Change	2/48 (4%)	0/49 (0%)
Mean severity	2.5	
Centrilobular Hypertrophy	0/48	0/49
Hepatocellular Adenoma[d]		
Overall rate	5/48 (10%)	11/49 (22%)
Adjusted rate	13.3%	29.9%
Terminal rate	4/36 (11%)	8/33 (24%)
First incidence (days)	670	607
Logistic regression test		P=0.070
Hepatocellular Adenoma, Multiple	0/48 (0%)	3/49 (6%)
Hepatocellular Carcinoma		
Overall rate	0/48 (0%)	1/49 (2%)
Adjusted rate	0.0%	2.0%
Terminal rate	0/36 (0%)	0/33 (0%)
First incidence (days)	-[h]	486
Logistic regression test		P=0.441
Hepatocellular Adenoma or Carcinoma[d]		
Overall rate	5/48 (10%)	12/49 (24%)
Adjusted rate	13.3%	31.3%
Terminal rate	4/36 (11%)	8/33 (24%)
First incidence (days)	670	486
Logistic regression test		P=0.055

** Significantly different (P≤0.01) from the 0:0 ppm group by the logistic regression test
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Average severity grade of lesions in affected mice (1 = minimal; 2 = mild; 3 = moderate; 4 = marked)
[c] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report, because it is more widely used and understood.
[d] Includes multiple neoplasms
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal.
[h] Not applicable; no neoplasms in animal group

Decreasing Incidences of Neoplasms: The incidence of malignant lymphoma was slightly decreased in exposed perinatal-only females (22/50, 12/50; Table D2b).

Effects of Combined Perinatal and Adult Exposure of Mice to 5,5-Diphenylhydantoin

The effects of combined perinatal and adult exposure were determined by comparison of the incidences of lesions in mice in the 0:100, 70:100, and 210:100 ppm (male) and 0:200, 70:200, and 210:200 ppm (female) exposure groups and in the 0:300 and 210:300 ppm (male) and 0:600 and 210:600 ppm (female) exposure groups.

Liver: In male mice exposed to various F_0 concentrations and an F_1 concentration of 100 ppm, the incidences of hepatocellular adenoma and carcinoma did not increase with increasing F_0 concentration (Table 24); however, in male mice exposed to an F_1 concentration of 300 ppm, the incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma were significantly increased with increased F_0 exposure (Table 25). The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in female mice receiving 70 ppm perinatal exposure and an F_1 concentration of 200 ppm (Table 24); however, the increase was not exposure related, and no similar increase was observed in females receiving an F_1 exposure to 600 ppm (Table 25). Perinatal exposure combined with adult exposure did not affect the incidences or severity of nonneoplastic lesions (centrilobular hypertrophy, cystic degeneration, fatty change, and foci of cellular alteration) of the liver.

TABLE 24
Liver Lesions in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:100, 70:100, and 210:100 ppm (Male) and the 0:200, 70:200, and 210:200 ppm (Female) Groups

Male	0:100 ppm	70:100 ppm	210:100 ppm
Basophilic Focus[a]	6/49 (12%)	1/50 (2%)	0/49 (0%)*
Clear Cell Focus	6/49 (12%)	4/50 (8%)	3/49 (6%)
Eosinophilic Focus	5/49 (10%)	3/50 (6%)	3/49 (6%)
Fatty Change	2/49 (4%)	4/50 (8%)	0/49 (0%)
Mean severity[b]	1.5	1.3	
Cystic Degeneration	9/49 (18%)	7/50 (14%)	9/49 (18%)
Mean severity	1.7	1.9	1.6
Centrilobular Hypertrophy[c]	19/49 (39%)	20/50 (40%)	33/49 (67%)**
Mean severity	1.9	2.0	1.8
Hepatocellular Adenoma[d]			
Overall rate	19/49 (39%)	20/50 (40%)	23/49 (47%)
Adjusted rate[e]	45.1%	50.1%	58.5%
Terminal rate[f]	17/40 (43%)	14/33 (42%)	20/36 (56%)
First incidence (days)	589	535	585
Logistic regression test[g]	P=0.268	P=0.502	P=0.291
Hepatocellular Adenoma, Multiple	8/49 (16%)	5/50 (10%)	13/49 (27%)
Hepatocellular Carcinoma[d]			
Overall rate	14/49 (29%)	18/50 (36%)	18/49 (37%)
Adjusted rate	33.2%	41.8%	41.0%
Terminal rate	12/40 (30%)	9/33 (27%)	11/36 (31%)
First incidence (days)	590	481	585
Logistic regression test	P=0.266	P=0.280	P=0.264
Hepatocellular Carcinoma, Multiple	3/49 (6%)	4/50 (8%)	7/49 (14%)
Hepatocellular Adenoma or Carcinoma[d]			
Overall rate	29/49 (59%)	31/50 (62%)	35/49 (71%)
Adjusted rate	65.8%	68.3%	79.2%
Terminal rate	25/40 (63%)	19/33 (58%)	27/36 (75%)
First incidence (days)	589	481	585
Logistic regression test	P=0.141	P=0.446	P=0.178
Hepatocellular Adenoma or Carcinoma, Multiple	13/49 (27%)	12/50 (24%)	22/49 (45%)*

(continued)

TABLE 24
Liver Lesions in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:100, 70:100, and 210:100 ppm (Male) and the 0:200, 70:200, and 210:200 ppm (Female) Groups (continued)

Female	0:200 ppm	70:200 ppm	210:200 ppm
Basophilic Focus	1/49 (2%)	2/50 (4%)	3/50 (6%)
Clear Cell Focus	1/49 (2%)	0/50 (0%)	0/50 (0%)
Eosinophilic Focus	3/49 (6%)	4/50 (8%)	5/50 (10%)
Fatty Change	23/49 (47%)	27/50 (54%)	26/50 (52%)
Mean severity	1.3	1.5	1.6
Cystic Degeneration	1/49 (2%)	0/50 (0%)	1/50 (2%)
Mean severity	1.0		2.0
Centrilobular Hypertrophy	25/49 (51%)	25/50 (50%)	26/50 (52%)
Mean severity	1.8	1.2	1.7
Hepatocellular Adenoma[d]			
Overall rate	13/49 (27%)	25/50 (50%)	12/50 (24%)
Adjusted rate	32.3%	60.9%	31.4%
Terminal rate	11/38 (29%)	22/38 (58%)	11/37 (30%)
First incidence (days)	675	660	670
Logistic regression test	P=0.240N	P=0.010	P=0.516N
Hepatocellular Adenoma, Multiple	5/49 (10%)	5/50 (10%)	5/50 (10%)
Hepatocellular Carcinoma			
Overall rate	1/49 (2%)	3/50 (6%)	4/50 (8%)
Adjusted rate	2.6%	7.9%	10.1%
Terminal rate	1/38 (3%)	3/38 (8%)	2/37 (5%)
First incidence (days)	736 (T)	736 (T)	675
Logistic regression test	P=0.170	P=0.305	P=0.180
Hepatocellular Adenoma or Carcinoma[d]			
Overall rate	14/49 (29%)	26/50 (52%)	16/50 (32%)
Adjusted rate	34.8%	63.4%	39.8%
Terminal rate	12/38 (32%)	23/38 (61%)	13/37 (35%)
First incidence (days)	675	660	670
Logistic regression test	P=0.510N	P=0.010	P=0.399

* Significantly different (P≤0.05) from the 0:200 ppm group by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Average severity grade of lesions in affected mice (1 = minimal; 2 = mild; 3 = moderate; 4 = marked)
[c] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report, because it is more widely used and understood.
[d] Includes multiple neoplasms
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.

TABLE 25
Liver Lesions in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:300 and 210:300 ppm (Male) and the 0:600 and 210:600 ppm (Female) Groups

Male	0:300 ppm	210:300 ppm
Basophilic Focus[a]	3/49 (6%)	1/50 (2%)
Clear Cell Focus	20/49 (41%)	26/50 (52%)
Eosinophilic Focus	2/49 (4%)	5/50 (10%)
Mixed Cell Focus	0/49 (0%)	3/50 (6%)
Mean severity[b]		3.0
Cystic Degeneration	29/49 (59%)	39/50 (78%)
Mean severity	2.1	2.2
Centrilobular Hypertrophy[c]	37/49 (76%)	46/50 (92%)
Mean severity	2.3	2.2
Hepatocellular Adenoma[d]		
Overall rate	22/49 (45%)	31/50 (62%)
Adjusted rate[e]	59.3%	65.9%
Terminal rate[f]	19/34 (56%)	28/44 (64%)
First incidence (days)	612	678
Logistic regression test[g]		P=0.294
Hepatocellular Adenoma, Multiple	11/49 (22%)	19/50 (38%)
Hepatocellular Carcinoma[d]		
Overall rate	7/49 (14%)	20/50 (40%)
Adjusted rate	19.1%	41.5%
Terminal rate	5/34 (15%)	16/44 (36%)
First incidence (days)	612	564
Logistic regression test		P=0.012
Hepatocellular Carcinoma, Multiple	0/49 (0%)	2/50 (4%)
Hepatocellular Adenoma or Carcinoma[d]		
Overall rate	26/49 (53%)	41/50 (82%)
Adjusted rate	68.3%	82.0%
Terminal rate	22/34 (65%)	35/44 (80%)
First incidence (days)	612	564
Logistic regression test		P=0.035
Hepatocellular Adenoma or Carcinoma, Multiple	13/49 (27%)	22/50 (44%)

(continued)

TABLE 25
Liver Lesions in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:300 and 210:300 ppm (Male) and the 0:600 and 210:600 ppm (Female) Groups (continued)

Female	0:600 ppm	210:600 ppm
Basophilic Focus	4/50 (8%)	3/50 (6%)
Clear Cell Focus	1/50 (2%)	5/50 (10%)
Eosinophilic Focus	8/50 (16%)	8/50 (16%)
Mixed Cell Focus	0/50 (0%)	2/50 (4%)
Cystic Degeneration	5/50 (10%)	0/50 (0%)*
Mean severity	1.0	
Centrilobular Hypertrophy	31/50 (62%)	37/50 (74%)
Mean severity	1.3	1.5
Hepatocellular Adenoma[d]		
Overall rate	22/50 (44%)	26/50 (52%)
Adjusted rate	50.9%	54.0%
Terminal rate	16/37 (43%)	18/40 (45%)
First incidence (days)	664	503
Logistic regression test		P=0.276
Hepatocellular Adenoma, Multiple	6/50 (12%)	7/50 (14%)
Hepatoblastoma or Hepatocellular Carcinoma[d]		
Overall rate	12/50 (22%)	10/50 (20%)
Adjusted rate	29.1%	24.2%
Terminal rate	9/37 (24%)	9/40 (23%)
First incidence (days)	570	670
Logistic regression test		P=0.404N
Hepatocellular Carcinoma, Multiple	2/50 (4%)	0/50 (0%)
Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma[d]		
Overall rate	30/50 (60%)	34/50 (68%)
Adjusted rate	66.4%	69.3%
Terminal rate	22/37 (59%)	25/40 (63%)
First incidence (days)	570	503
Logistic regression test		P=0.264
Hepatocellular Adenoma or Carcinoma, Multiple	9/50 (18%)	9/50 (18%)

* Significantly different (P≤0.05) from the 0:600 ppm group by the logistic regression test
[b] Average severity grade of lesions in affected mice (1 = minimal; 2 = mild; 3 = moderate; 4 = marked)
[a] Number of lesion-bearing animals/number of animals examined microscopically at site
[c] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report, because it is more widely used and understood.
[d] Includes multiple neoplasms
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposure group is indicated by N.

The combined incidences of hepatocellular adenoma and carcinoma for all exposure groups of male and female mice are shown in Table 26. A single logistic regression analysis applied to all eight experimental groups indicates that significant ($P \leq 0.001$) increases in the incidence of hepatocellular neoplasms are associated with increasing F_1 concentration levels of 5,5-diphenylhydantoin in females, but not in males, confirming the effects observed in the adult-only exposure groups. In male mice, there were significant ($P \leq 0.01$) increases in the incidences of hepatocellular neoplasms associated with F_0 exposure, and, importantly, these groups had a significant ($P \leq 0.001$) $F_0 \times F_1$ interaction. This interaction reflected the enhancing effects of combined 210 ppm perinatal exposure and 300 ppm adult exposure on liver neoplasm incidences (Table 26). For female mice, there was no significant interaction, implying that the effect of F_1 exposure was similar, regardless of the level of F_0 exposure.

TABLE 26
Hepatocellular Adenomas and Carcinomas in Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_1 Concentration (ppm)	F_0 Concentration (ppm)			
	0	21	70	210
Male				
0	29/50	–[b]	–	33/50
30	–	25/50	–	–
100	29/49	–	31/50	35/49
300	26/49	–	–	41/50**
Female				
0	5/48	–	–	12/49
60	–	13/50*	–	–
200	14/49*	–	26/50**	16/50**
600	30/50**	–	–	34/50**▲▲

* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by the logistic regression test
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by the logistic regression test
▲▲ Significantly different ($P \leq 0.01$) from the 210:0 ppm group by the logistic regression test
[a] Incidences are given as the number of neoplasm-bearing animals/number of animals necropsied.
[b] Animals were not exposed at these concentrations.

GENETIC TOXICOLOGY

5,5-Diphenylhydantoin (100 to 10,000 μg/plate) was not mutagenic in any of four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) when tested in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 in each of two laboratories (Table E1; Haworth *et al.*, 1983). No induction of trifluorothymidine resistance was observed in L5178Y mouse lymphoma cells tested with and without S9 from Aroclor 1254-induced male F344/N rat liver (Table E2; Myhr *et al.*, 1985). Concentrations of 5,5-diphenylhydantoin tested in this assay ranged from 15 to 500 μg/mL in the absence of S9, and 18.75 to 350 μg/mL in the presence of S9; relative total growth at the highest concentrations was less than 30%. In cytogenetic tests with cultured Chinese hamster ovary cells, high doses of 5,5-diphenylhydantoin (1.6 and 5.0 mg/mL) induced a small, but statistically significant, increase in sister chromatid exchanges in the trial conducted in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Table E3; Galloway *et al.*, 1987); no increase in sister chromatid exchanges was observed in cultured Chinese hamster ovary cells treated in the absence of S9, nor were chromosomal aberrations induced in cultured Chinese hamster ovary cells treated with 5,5-diphenylhydantoin with or without S9 (Table E4; Galloway *et al.*, 1987). Doses of 5,5-diphenylhydantoin used in the chromosomal aberration test equaled or exceeded those used in the sister chromatid exchange test. No induction of sex-linked recessive lethal mutations was observed in the germ cells of adult male *Drosophila melanogaster* administered 5,5-diphenylhydantoin by feeding (5,000 ppm) or by injection (100 ppm) (Table E5; Woodruff *et al.*, 1985).

5,5-Diphenylhydantoin was also tested *in vivo* following a single intraperitoneal injection for induction of cytogenetic effects in mouse bone marrow cells (McFee *et al.*, 1992). Weakly positive responses were observed in the sister chromatid exchange test at both the standard (23-hour) and the extended (42-hour) post-treatment sample times (Table E6), but no increase in chromosomal aberrations was observed in samples taken 17, 36, or 42 hours after treatment (Table E7). In the 23-hour exposure, the middle dose of 125 mg/kg produced a significant increase in sister chromatid exchanges, and this was sufficient for the trial to be considered positive. The data from the 42-hour harvest time showed a small, exposure-related increase in sister chromatid exchanges, but no individual exposures were judged positive. The results of this second trial were considered to be questionable, and the assay was concluded to be equivocal. No significant increase in the frequency of micronucleated polychromatic erythrocytes was observed in bone marrow of male B6C3F$_1$ mice treated with three intraperitoneal injections of 5,5-diphenylhydantoin (17.5, 35, and 70 mg/kg) dissolved in corn oil (Table E8). Also, no increase was observed in the frequency of micronucleated polychromatic erythrocytes in bone marrow of male Balb/C mice administered a single caudal vein injection of 0.1 to 20.0 mg/kg 5,5-diphenylhydantoin dissolved in 0.1 N NaOH (Table E9).

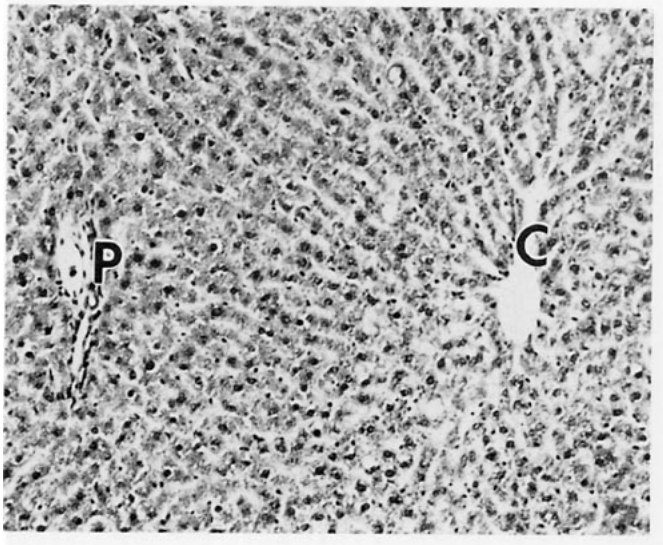


PLATE 1
Liver from control male F344/N rat in the 13-week feed study shows normal size relationship of hepatocytes in periportal (P) and centrilobular (C) areas of the liver lobule. H&E, 100X

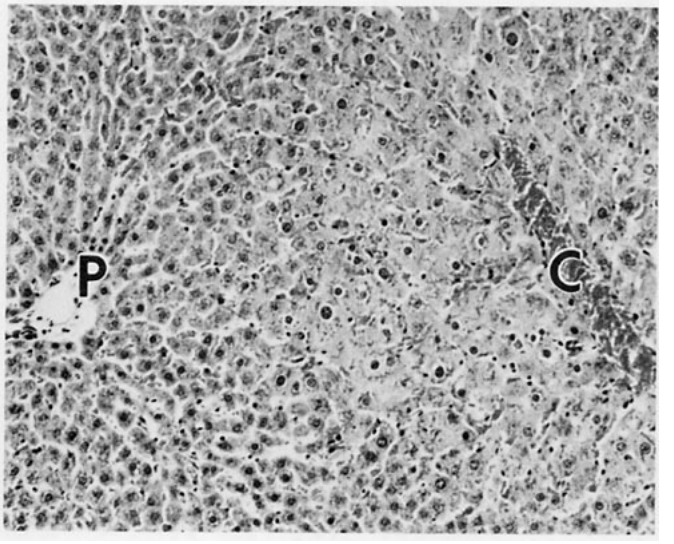


PLATE 2
Liver from male F344/N rat exposed to 4,800 ppm diphenylhydantoin in the 13-week feed study shows the periportal (P) and centrilobular (C) areas. Note mild centrilobular hypertrophy of hepatocytes around central vein compared to same area shown in Plate 1. H&E, 100X

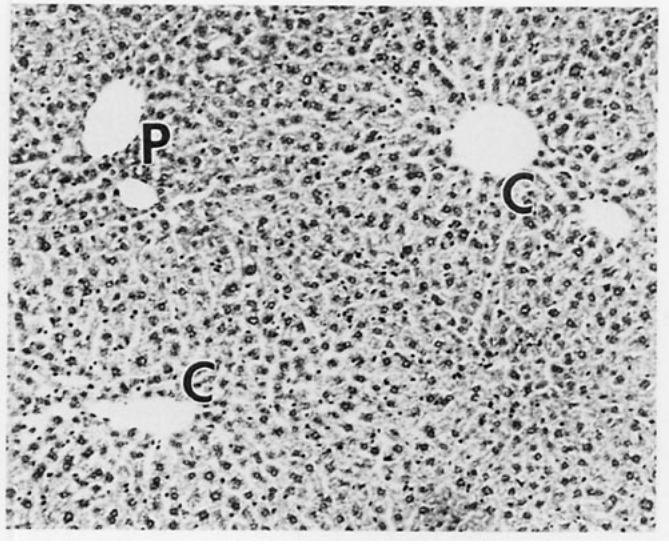


PLATE 3
Liver from control male $B6C3F_1$ mouse in the 13-week feed study shows normal size relationship of hepatocytes in periportal (P) and centrilobular (C) areas of the liver lobule. H&E, 100X

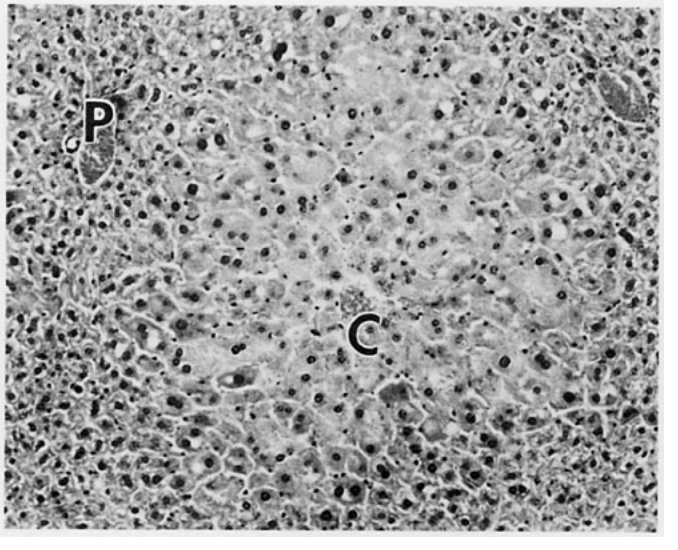


PLATE 4
Liver from male $B6C3F_1$ mouse exposed to 600 ppm diphenylhydantoin in the 13-week feed study shows the periportal (P) and centrilobular (C) areas with mild hypertrophy of hepatocytes around central vein. H&E, 100X

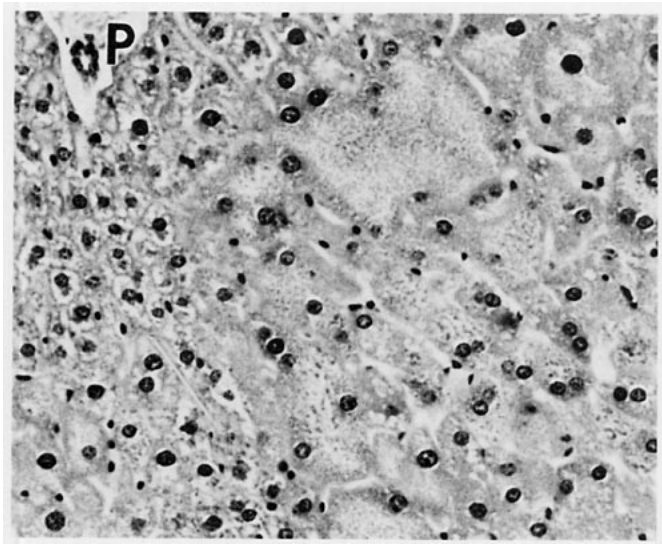


PLATE 5
Higher magnification of liver from male B6C3F$_1$ mouse exposed to 600 ppm diphenylhydantoin in the 13-week feed study shows a moderate hypertrophy of hepatocytes that extends nearly to the periportal (P) area of the lobule. H&E, 200X

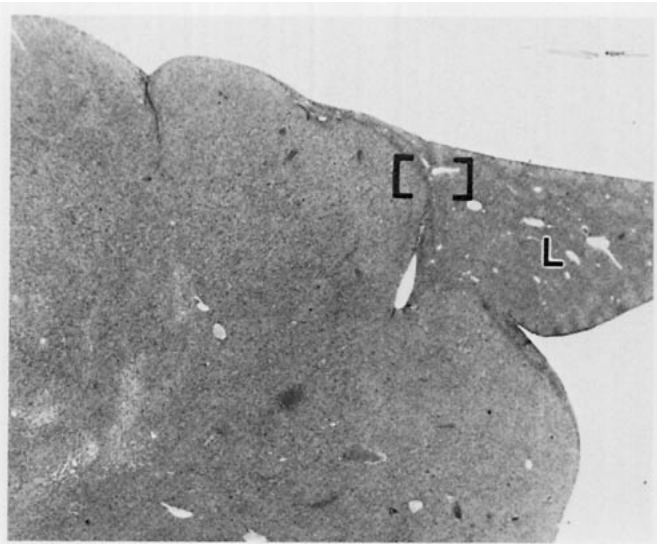


PLATE 6
Hepatocellular adenoma in male B6C3F$_1$ mouse exposed to 0:300 ppm diphenylhydantoin in the 2-year feed study. Multinodular adenoma extends from normal portion of hepatic lobe (L). Detail of area within brackets is shown in Plate 7. H&E, 10X

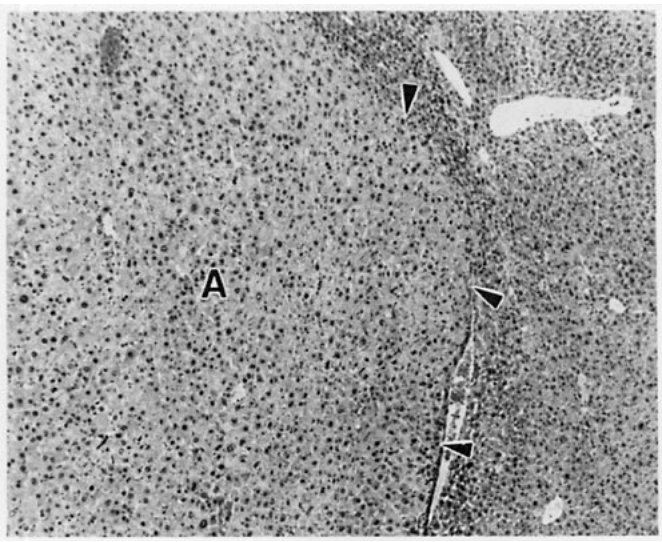


PLATE 7
Higher magnification of hepatocellular adenoma in male B6C3F$_1$ mouse exposed to 0:300 ppm diphenylhydantoin in the 2-year feed study shows distinct demarcation (arrows) between hepatocellular adenoma (A) and liver lobe on right. H&E, 40X

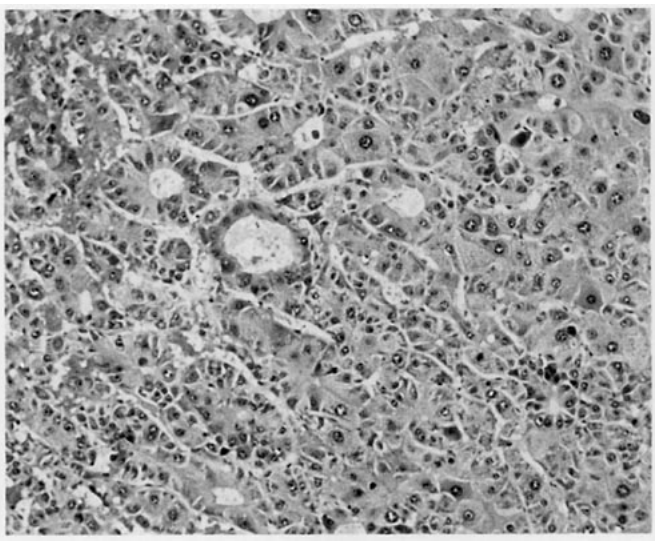


PLATE 8
Hepatocellular carcinoma from female B6C3F$_1$ mouse exposed to 0:600 ppm diphenylhydantoin in the 2-year feed study. Note solid, trabecular and acinar/glandular patterns within the carcinoma. H&E, 100X

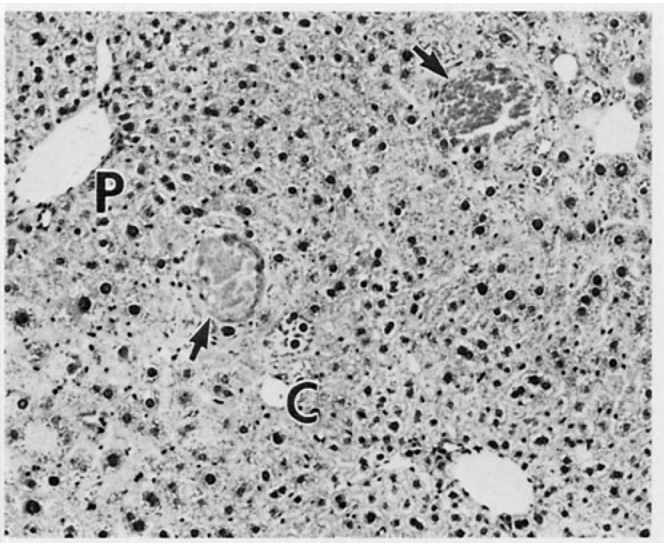


PLATE 9
Liver from male B6C3F$_1$ mouse exposed to 0:300 ppm diphenylhydantoin in the 2-year feed study. Note mild hypertrophy of hepatocytes that extends from central vein (C) nearly to periportal (P) area of lobule. Focal areas of cystic degeneration are also present (arrows). H&E, 100X

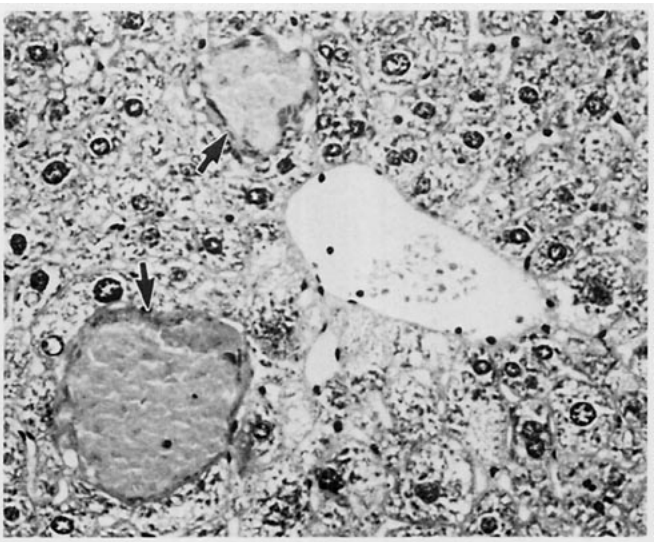


PLATE 10
Higher magnification of liver from male B6C3F$_1$ mouse exposed to 0:300 ppm diphenylhydantoin in the 2-year feed study shows two foci of cystic degeneration (arrows) adjacent to a central vein. H&E, 200X

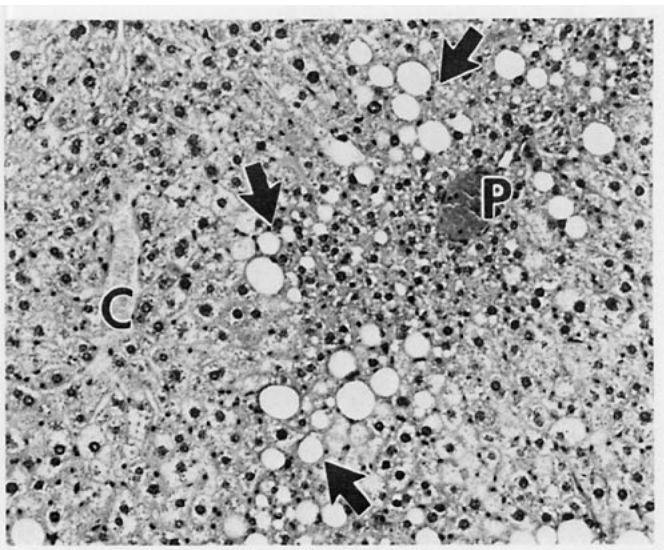


PLATE 11
Liver from female B6C3F$_1$ mouse exposed to 0:200 ppm diphenylhydantoin in the 2-year feed study shows mild fatty change characterized by clear vacuoles (arrows) in hepatocytes midway between periportal (P) and centrilobular (C) areas of lobule. H&E, 100X

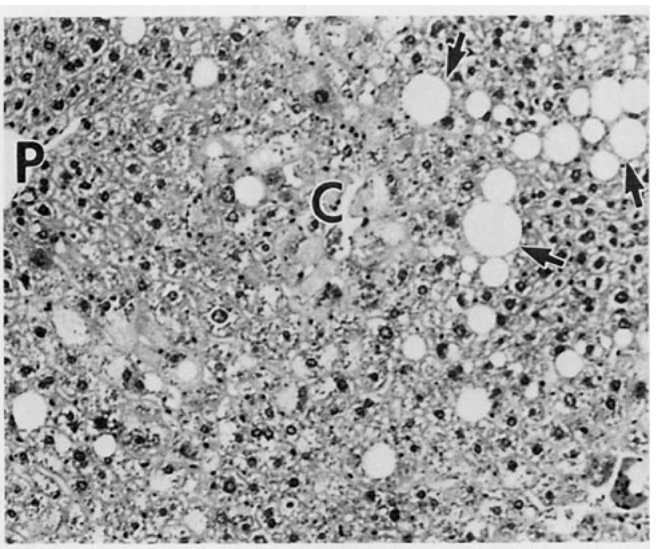


PLATE 12
Liver from male B6C3F$_1$ mouse exposed to 0:100 ppm diphenylhydantoin in the 2-year feed study shows mild hypertrophy of hepatocytes around central vein (C) and fatty change characterized by the accumulation of large vacuoles (arrows); periportal (P) area. H&E, 100X

DISCUSSION AND CONCLUSIONS

TOXICITY OF 5,5-DIPHENYLHYDANTOIN

The present study was designed to compare the carcinogenicity of 5,5-diphenylhydantoin in rats and mice by a combined perinatal and adult exposure regimen versus a conventional bioassay study in which animals were exposed to the chemical for 2 years, beginning at 8 weeks of age. The combined perinatal and adult protocol included exposure of females prior to breeding, through gestation, lactation, and weaning of the offspring followed by the continued dietary exposure of offspring beginning at the age of 8 weeks and continuing for 2 years.

The 13-week and gestational studies were conducted to determine the exposure levels for the adult and perinatal phases of chronic studies, respectively. Chemical-related microscopic lesions in the 13-week studies were confined to the liver in rats and mice and consisted of centrilobular hypertrophy of hepatocytes. No histopathologic differences in the liver or in other organs were seen in the gestational studies, possibly due to the shorter exposure period and to the use of exposure levels that were below hepatotoxic levels as determined in the 13-week studies. Based on the results of the gestational studies, the maximum perinatal exposure levels estimated for rats and mice were not predicted to have any adverse effects on embryonic, fetal, or neonatal development during the perinatal exposure portion of the 2-year carcinogenicity studies.

The only significant finding at the interim evaluation of animals at 9 months during the 2-year studies was centrilobular hypertrophy of hepatocytes in rats and mice. Hematology studies in rats indicated mild, but consistent, increased erythrocyte and platelet counts related to 5,5-diphenylhydantoin treatment in males and females. In the 2-year studies, the survival of animals in the groups exposed to 5,5-diphenylhydantoin was similar to that of the controls. However, the mean body weights of the exposed groups were lower than those of the controls, and these decreases were most severe in the groups receiving the highest exposure levels. Such weight differences could not have been predicted from the results of the 13-week studies. Negative trends in the incidences of a number of neoplasms, including neoplasms of the mammary gland, pituitary gland (females), and thyroid gland (females) occurred in exposed male and female rats; these rats also had mean body weight gains significantly less than those of controls. An association between lower body weight gain and decreased incidences of spontaneous neoplasms has been reported for rats (Rao *et al.*, 1987).

The only significant nonneoplastic effect in the liver of male and female rats was a chemical- and exposure-related increase in the incidence of centrilobular hypertrophy. At the end of the 2-year study, there was no difference between males and females in the mean severity of hypertrophy although the group incidences were slightly higher in females.

Associated with the increased incidence of hypertrophy was a decrease in the incidence of spontaneously occurring basophilic foci of cellular alteration. The treatment effect which resulted in the hypertrophy may have caused the decreased formation of spontaneous basophilic foci. Chemical administration has been reported to decrease the incidence of basophilic foci in some studies (Harada *et al.*, 1989). Other effects on liver morphology such as those seen with mononuclear cell leukemia may also decrease the number of basophilic foci (Harada *et al.*, 1990). In this study, increases and decreases in the incidence of several other nonneoplastic liver lesions in rats were small and not dose related, and their relationship to treatment was uncertain.

In mice, chemical-related nonneoplastic effects in the liver were more prominent than in rats. Additionally, unlike rats, there was a clear sex-related difference in hepatotoxicity in mice with more severe lesions present in males. The most common treatment effect was a centrilobular hypertrophy which was dose-related in incidence and severity. At higher doses a spectrum of degenerative vacuolar differences (cystic degeneration in males, fatty change in females) was also present. The difference in the terminology used for these degenerative differences in males and

females is a reflection of the greater severity of the degeneration and the larger cystic lesions seen in the liver of male mice compared to the smaller vacuoles of fatty degeneration seen more often in the liver of females. The only inconsistency in the pattern of nonneoplastic effects was observed at the highest exposure dose in females, where the severity of hypertrophy was less than in the mid-dose, and fatty degeneration was not present.

The toxicity data from the 13-week, gestational, and 2-year studies demonstrated that mice are more sensitive to 5,5-diphenylhydantoin than are rats. Species differences have been reported in past studies conducted for the determination of LD_{50} and developmental toxicity of 5,5-diphenylhydantoin in rats and mice (Woodbury and Swinyard, 1972; Schardein *et al.*, 1989). A higher LD_{50} in the rat could be due to the faster rate of 5,5-diphenylhydantoin metabolism in rats than in mice (Woodbury and Swinyard, 1972). In a placental transfer study, concentrations of 5,5-diphenylhydantoin in mouse fetuses were higher than those in rat fetuses (Stevens and Harbison, 1974). This may be at least part of the reason for the increased sensitivity of developing mice to 5,5-diphenylhydantoin toxicity.

CARCINOGENICITY OF 5,5-DIPHENYLHYDANTOIN

Epidemiology and laboratory animal studies have reported a possible association of lymphoid organ neoplasms with 5,5-diphenylhydantoin administration. In several epidemiology studies, an association has been observed between the incidence of lymphomas and long-term epilepsy treatment with 5,5-diphenylhydantoin (Anthony, 1970; Li *et al.*, 1975; IARC, 1977). Anguiar *et al.* (1987) suggested that the risk of developing lymphomas is four times higher for patients taking 5,5-diphenylhydantoin than for those who do not. However, in two follow-up studies of epilepsy patients, no significant increase in lymphoma incidences was reported (IARC, 1987). In laboratory animal studies, 5,5-diphenylhydantoin has been shown to induce thymic lymphoma in mice (IARC, 1977; Krueger *et al.*, 1978). Based on the results of epidemiology and animal studies, the International Agency for Research on Cancer (IARC) has classified 5,5-diphenylhydantoin as having limited evidence of carcinogenicity in humans and animals (IARC, 1977, 1987).

In contrast to the studies reported in the literature suggesting lymphoid organs as the sites of 5,5-diphenylhydantoin carcinogenicity in humans and mice, the liver was the major site of chemical-related toxicity and carcinogenicity in mice and rats in the current study. 5,5-Diphenylhydantoin exposure, with and without perinatal exposure, caused significant increases in the incidences of liver neoplasms in female mice. Marginal increases in the incidences of liver neoplasms were seen in the perinatal-only exposure group of female mice; there was no morphologic evidence of hypertrophy or other nonneoplastic chemical-related effects in this group. There were no increases in the incidences of liver neoplasms in male mice in the adult-only exposure groups or in the perinatal-only exposure group. Nonneoplastic chemical-related effects were evident in both the adult-only and perinatal-only exposure groups. The absence of increased neoplasm incidences in these groups of male mice could be due to the lower exposure levels used for the males (100 and 300 ppm for males versus 200 and 600 ppm for females). However, the perinatal and adult exposure combination caused increased incidences of liver neoplasms in males, showing a significant interaction for perinatal and adult exposures. Such enhancement of neoplasm incidence did not occur in the female mice.

Maeda *et al.* (1988) exposed groups of 50 B6C3F_1 mice of each sex to 0.006% (60 ppm) or 0.012% (120 ppm) 5,5-diphenylhydantoin in their diet for 78 weeks; they were then given a control diet for 8 weeks. Histopathologic examination of various tissues did not show any increase in the incidences of neoplasms related to 5,5-diphenylhydantoin administration in males or females. The lower exposure levels and reduced length of the study could explain the lack of a carcinogenic response.

The lower sensitivity of rats to 5,5-diphenylhydantoin toxicity as compared to mice was also reflected in the carcinogenicity results; only slight increases in liver neoplasm incidences were seen in male rats exposed to the highest adult-only or combined perinatal and adult doses. The historical incidence of hepatocellular adenomas or carcinomas in male rats in Battelle Columbus studies is 7/402 (1.7%; range 0%-10%) (Table A3). The control males in the current study did not have any liver neoplasms while there was a dose-related increase in neoplasms in adult-only males (0/50, 2/50, 4/50). Thus it was

considered that there was equivocal evidence of carcinogenic activity of 5,5-diphenylhydantoin in the male rats. This marginal effect was further supported by the increased incidence of neoplasms (5/49) in animals in the combined adult plus perinatal exposure group; four out of the five animals had multiple neoplasms. There were no increases in incidences of neoplasms in female rats exposed to 5,5-diphenylhydantoin; chemical-related nonneoplastic lesions were similar in incidence and severity for both sexes. Perinatal exposure alone, or its combination with adult exposure, did not result in increased incidences of liver neoplasms in male or female rats.

Jang *et al.* (1987) studied the carcinogenic potential of 5,5-diphenylhydantoin in male and female F344 rats at concentrations of 0.025% (250 ppm) and 0.05% (500 ppm) in the diet for 2 years. The incidences of neoplasms in the treated and control groups were similar, suggesting that 5,5-diphenylhydantoin was not carcinogenic in F344 rats. The marginally increased incidence of liver neoplasms in male rats could be due to the higher exposure levels used in the current study.

MECHANISM OF TOXICITY AND CARCINOGENICITY

5,5-Diphenylhydantoin is a suspected mutagen, but the existing evidence is contradictory or equivocal (Petter *et al.*, 1981; Schaumann *et al.*, 1985; McFee *et al.*, 1992). The weak mutagenic activity of 5,5-diphenylhydantoin is attributed to its arene oxide metabolite (Barcellona *et al.*, 1987). It has been proposed that chemical-induced immunosuppression or hypersensitivity may be responsible for the lymphomas seen in patients on long-term 5,5-diphenylhydantoin therapy (Anthony, 1970; Li *et al.*, 1975; Schwinghammer and Howrie, 1983; Aguiar *et al.*, 1987). Chemical-related increases in the incidences of thymic lymphomas in responsive strains of mice have also been related to immunotoxic effects of 5,5-diphenylhydantoin (Krüger *et al.*, 1972; Krueger and Bedoya, 1978). In the current studies, no increases in the incidences of lymphatic neoplasms were observed. This could be due to the different strain of mouse used in this study or to the use of exposure levels that were below the immunotoxic threshold.

5,5-Diphenylhydantoin hepatotoxicity has been reported in human patients of all ages. Although the incidence of chemical-related liver toxicity is less than 1%, liver toxicity can have serious consequences, including chronic hepatitis and death (Smyth and Umstead, 1989). Although most of the epidemiology studies have reported lymphadenopathy and malignant lymphoma related to 5,5-diphenylhydantoin use, an increase in the incidence of liver cancer has been reported in one study conducted in Denmark (Olsen *et al.*, 1989). The incidence of liver cancer among patients treated with anticonvulsive drugs, including 5,5-diphenylhydantoin, was elevated 10 years after hospitalization and was significantly increased among 30-year survivors (RR=2.9; n=6). The liver toxicity and carcinogenicity associated with 5,5-diphenylhydantoin in the current studies corroborate the potential for chemical-related hepatotoxicity in humans. Olsen *et al.* (1989) have given two possible mechanisms for 5,5-diphenylhydantoin hepatotoxicity in epileptic patients. The first explanation is that the hepatic toxicity in epileptic patients may be due to a hypersensitivity reaction. The other proposed mechanism for hepatic toxicity is related to an arene oxide, a toxic metabolite of 5,5-diphenylhydantoin. The toxicity and carcinogenicity observed in studies reported here are also most likely related to arene oxides formed during the metabolism of 5,5-diphenylhydantoin in mice (Figure 6). Excess arene oxides formed during the metabolism of some chemicals can bind covalently to essential cellular macromolecules and may lead to toxic or carcinogenic events. 5,5-Diphenylhydantoin is bioactivated to an arene oxide by hepatic microsomal cytochrome P_{450}-catalyzed mixed-function oxidases. Subsequent deactivation occurs via nonenzymatic rearrangement to the phenol metabolites (*p*-HPPH, *m*-HPPH, and their glucuronides and possibly sulfate conjugates) and oxidation to a dihydrodiol metabolite by hepatic microsomal epoxide hydrase enzymes, possibly involving reduced glutathione. Indirect evidence for an intermediate arene oxide metabolite includes the detection of the 5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl-5-phenylhydantoin) metabolite (dihydrodiol) in the urine of rats, mice, and humans (Wells and Harbison, 1980; Wong *et al.*, 1989). Further support for involvement of an intermediate arene oxide metabolite in 5,5-diphenylhydantoin toxicity comes from embryotoxicity and teratogenicity studies of 5,5-diphenylhydantoin which have related positive

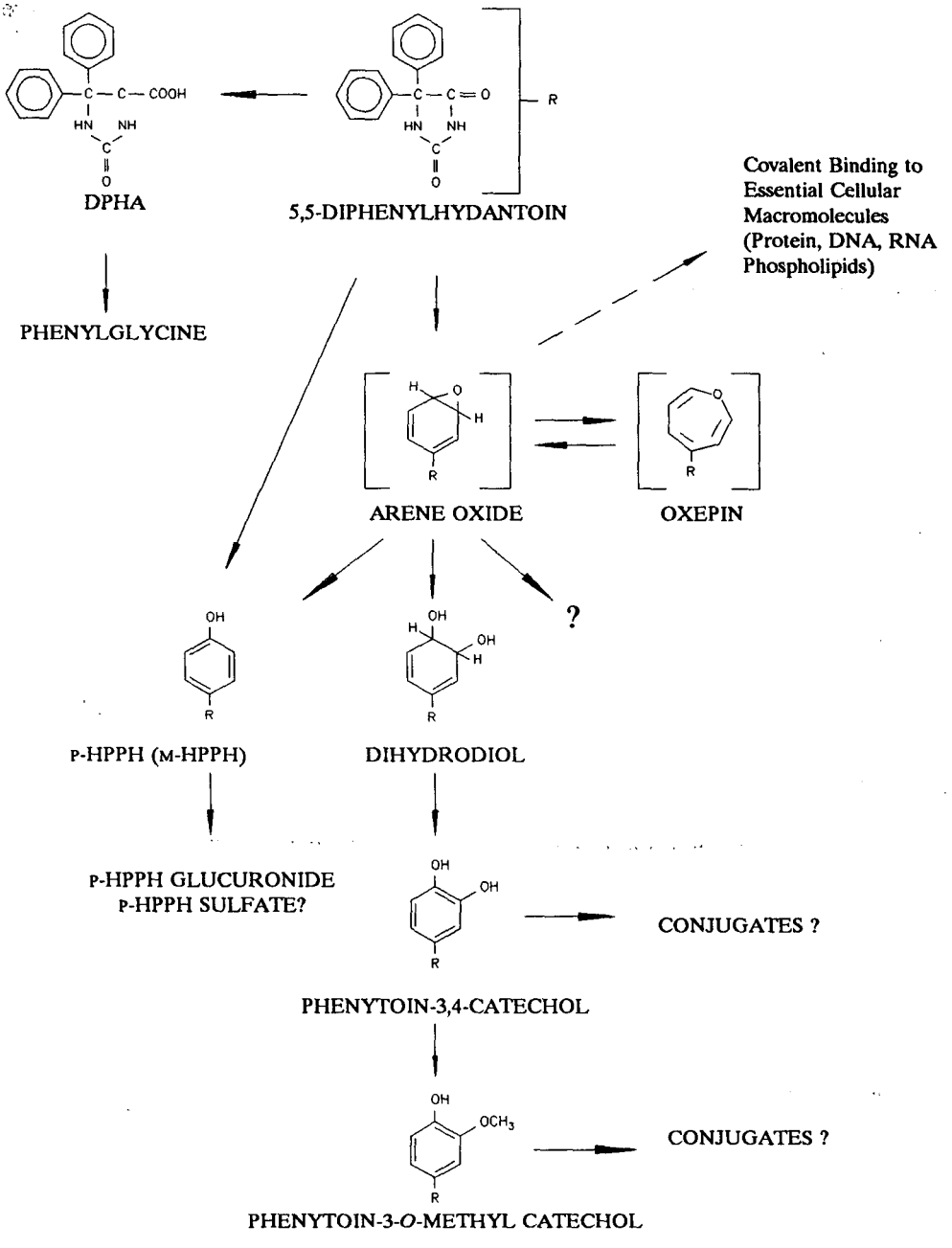


FIGURE 6
Proposed Biotransformation of 5,5-Diphenylhydantoin *In Vivo*
(Reproduced from Wells and Harbison, 1980)

findings to this metabolite (Barcellona *et al.*, 1987; Wong *et al.*, 1989). The relatively weak liver toxicity and carcinogenic activity of 5,5-diphenylhydantoin in rats are most likely related to high deactivation of 5,5-diphenylhydantoin arene oxide by rat liver microsomal enzymes (Wells and Harbison, 1980).

In general, the patterns of metabolism and toxicity of 5,5-diphenylhydantoin are similar in humans and laboratory animals. Adult dietary exposure levels of 800 or 2,400 ppm in the current carcinogenesis studies resulted in compound consumption levels of 35 to 125 mg/kg body weight in rats. Male mice exposed to 100 or 300 ppm as adults received 20 or 65 mg/kg body weight, and females exposed to 200 or 600 ppm received 50 or 165 mg/kg. The therapeutic dose levels of 5,5-diphenylhydantoin in adults and children vary from 3 to 8 mg/kg per day (*Goodman and Gilman's*, 1985). Though the therapeutic dose levels in humans are four to ten times lower than those that have been shown to cause liver carcinogenicity in mice, 5,5-diphenylhydantoin-mediated carcinogenic activity in humans appears possible, because the plasma half-life of 5,5-diphenylhydantoin is four to eight times higher in humans than in rats and mice (Khera, 1985).

CONCLUSIONS

Adult-Only Exposure

Under the conditions of these 2-year, adult-only, dietary exposure studies, there was *equivocal evidence of carcinogenic activity** of 5,5-diphenylhydantoin in male F344/N rats based on marginally increased incidences of hepatocellular neoplasms. There was *no evidence of carcinogenic activity* of 5,5-diphenylhydantoin in female F344/N rats given 240, 800, or 2,400 ppm. There was *no evidence of carcinogenic activity* of 5,5-diphenylhydantoin in male B6C3F$_1$ mice given 30, 100, or 300 ppm. There was *clear evidence of carcinogenic activity* of 5,5-diphenylhydantoin in female B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms.

Perinatal-Only Exposure

Perinatal exposure alone (through dietary administration of 210 ppm 5,5-diphenylhydantoin during the perinatal period) caused a marginal increase in the incidences of hepatocellular neoplasms in female B6C3F$_1$ mice evaluated 2 years after cessation of exposure. In male and female F344/N rats, exposure to 630 ppm during the perinatal period did not influence the incidences of hepatocellular or other neoplasms. Similarly, exposure of male B6C3F$_1$ mice to dietary levels of 210 ppm 5,5-diphenylhydantoin during the perinatal period did not affect neoplasm incidences. No teratologic effects were observed.

Combined Perinatal and Adult Exposure

Combined perinatal and adult dietary exposure to 5,5-diphenylhydantoin confirmed the findings of the increased incidences of hepatocellular neoplasms for adult-only exposures in male F344/N rats and female B6C3F$_1$ mice, although combined exposure did not enhance these neoplastic effects. However, in male B6C3F$_1$ mice, combined perinatal and adult exposure resulted in increased incidences of hepatocellular neoplasms (hepatocellular carcinomas and multiple adenomas) that were not seen when dietary exposure was limited to the adult exposure period only.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 11.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF 5,5-DIPHENYLHYDANTOIN

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	22	21	13	21	24	21
Natural deaths	2	5	4	3	4	6
Survivors						
Died last week of study	1	1			1	2
Terminal sacrifice	25	23	33	26	21	21
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, cecum	(49)	(22)	(13)	(22)	(26)	(21)
Intestine large, colon	(49)	(24)	(14)	(22)	(26)	(23)
Intestine large, rectum	(48)	(24)	(14)	(22)	(26)	(22)
Intestine small, duodenum	(48)	(25)	(15)	(23)	(26)	(25)
Intestine small, ileum	(46)	(22)	(13)	(23)	(26)	(23)
Intestine small, jejunum	(48)	(24)	(13)	(22)	(26)	(22)
Liver	(50)	(50)	(49)	(50)	(49)	(49)
Hepatocellular carcinoma			1 (2%)	1 (2%)		
Hepatocellular adenoma		1 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hepatocellular adenoma, multiple			1 (2%)		1 (2%)	
Histiocytic sarcoma	1 (2%)		1 (2%)		1 (2%)	
Mesentery	(7)	(3)	(3)	(3)	(7)	(2)
Pancreas	(50)	(27)	(16)	(23)	(28)	(25)
Salivary glands	(48)	(27)	(16)	(23)	(27)	(28)
Stomach, forestomach	(49)	(26)	(15)	(24)	(27)	(26)
Squamous cell carcinoma	1 (2%)					
Squamous cell papilloma		1 (4%)				
Stomach, glandular	(49)	(25)	(15)	(23)	(26)	(23)
Tongue				(1)	(2)	
Squamous cell papilloma				1 (100%)	1 (50%)	
Cardiovascular System						
Heart	(50)	(28)	(18)	(25)	(29)	(29)
Schwannoma benign	1 (2%)					
Endocrine System						
Adrenal gland	(50)	(50)	(50)	(50)	(48)	(49)
Adrenal gland, cortex	(49)	(50)	(48)	(49)	(47)	(49)
Adrenal gland, medulla	(49)	(50)	(50)	(49)	(48)	(49)
Pheochromocytoma malignant		1 (2%)	2 (4%)	1 (2%)	2 (4%)	4 (8%)
Pheochromocytoma benign	13 (27%)	11 (22%)	11 (22%)	19 (39%)	4 (8%)	16 (33%)
Bilateral, pheochromocytoma benign	5 (10%)	1 (2%)	5 (10%)	7 (14%)	7 (15%)	3 (6%)
Islets, pancreatic	(50)	(27)	(15)	(23)	(28)	(25)
Adenoma	3 (6%)	1 (4%)				1 (4%)
Carcinoma	1 (2%)				1 (4%)	1 (4%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	25	17
Natural deaths	3	2
Survivors		
Died last week of study	1	1
Terminal sacrifice	21	30
Animals examined microscopically	50	50
Alimentary System		
Esophagus	(49)	(50)
Intestine large, cecum	(47)	(48)
Intestine large, colon	(46)	(48)
Intestine large, rectum	(47)	(49)
Intestine small, duodenum	(48)	(48)
Intestine small, ileum	(47)	(48)
Intestine small, jejunum	(48)	(48)
Liver	(50)	(49)
Hepatocellular adenoma	3 (6%)	1 (2%)
Hepatocellular adenoma, multiple	1 (2%)	4 (8%)
Histiocytic sarcoma		1 (2%)
Mesentery	(6)	(4)
Pancreas	(48)	(50)
Salivary glands	(49)	(50)
Stomach, forestomach	(49)	(48)
Leiomyosarcoma		1 (2%)
Stomach, glandular	(49)	(48)
Tooth	(1)	
Molar, adamantinoma benign	1 (100%)	
Cardiovascular System		
Heart	(50)	(50)
Carcinoma, metastatic	1 (2%)	
Ventricle left, schwannoma benign	1 (2%)	
Endocrine System		
Adrenal gland	(49)	(50)
Adrenal gland, cortex	(49)	(50)
Adenoma	1 (2%)	2 (4%)
Histiocytic sarcoma		1 (2%)
Adrenal gland, medulla	(48)	(50)
Pheochromocytoma malignant		3 (6%)
Pheochromocytoma benign	16 (33%)	16 (32%)
Bilateral, pheochromocytoma benign	9 (19%)	11 (22%)
Parathyroid gland	(49)	(44)
Adenoma	1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Endocrine System (continued)						
Pituitary gland	(50)	(34)	(20)	(31)	(30)	(27)
Pars distalis, adenoma	14 (28%)	10 (29%)	6 (30%)	8 (26%)	10 (33%)	5 (19%)
Pars intermedia, adenoma				1 (3%)		
Pars nervosa, histiocytic sarcoma	1 (2%)					
Thyroid gland	(49)	(26)	(14)	(22)	(27)	(23)
Bilateral, C-cell, adenoma	3 (6%)		1 (7%)	1 (5%)		1 (4%)
C-cell, adenoma	8 (16%)	11 (42%)	6 (43%)	5 (23%)	5 (19%)	5 (22%)
C-cell, carcinoma	1 (2%)	1 (4%)			2 (7%)	1 (4%)
Follicular cell, adenocarcinoma	1 (2%)	2 (8%)				
General Body System						
Tissue NOS	(1)		(2)	(1)	(1)	
Chemodectoma benign			1 (50%)			
Genital System						
Epididymis	(48)	(27)	(17)	(24)	(27)	(30)
Preputial gland	(49)	(27)	(18)	(24)	(33)	(29)
Adenoma	2 (4%)	1 (4%)	1 (6%)	2 (8%)	1 (3%)	
Carcinoma	2 (4%)	1 (4%)			1 (3%)	1 (3%)
Prostate	(49)	(27)	(17)	(24)	(28)	(29)
Testes	(50)	(50)	(50)	(48)	(50)	(49)
Bilateral, interstitial cell, adenoma	42 (84%)	45 (90%)	42 (84%)	43 (90%)	42 (84%)	41 (84%)
Interstitial cell, adenoma	5 (10%)	5 (10%)	5 (10%)	5 (10%)	8 (16%)	5 (10%)
Interstitial cell, carcinoma			1 (2%)			
Hematopoietic System						
Bone marrow	(50)	(26)	(16)	(23)	(28)	(29)
Femoral, histiocytic sarcoma	1 (2%)		1 (6%)		1 (4%)	
Lymph node	(50)	(27)	(19)	(26)	(29)	(29)
Mandibular, histiocytic sarcoma	1 (2%)		1 (5%)		1 (3%)	
Mediastinal, histiocytic sarcoma			1 (5%)		1 (3%)	
Lymph node, mesenteric	(5)	(7)	(4)	(5)	(8)	(4)
Spleen	(49)	(33)	(29)	(31)	(35)	(29)
Hemangiosarcoma						1 (3%)
Histiocytic sarcoma			1 (3%)		1 (3%)	
Sarcoma						1 (3%)
Thymus	(40)	(23)	(15)	(23)	(28)	(25)
Thymoma benign					1 (4%)	
Integumentary System						
Mammary gland	(23)	(12)	(8)	(7)	(14)	(7)
Adenocarcinoma	1 (4%)					
Fibroadenoma	3 (13%)	1 (8%)	4 (50%)		1 (7%)	1 (14%)
Sarcoma						1 (14%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Endocrine System (continued)		
Pituitary gland	(48)	(49)
Pars distalis, adenoma	10 (21%)	9 (18%)
Thyroid gland	(48)	(49)
Bilateral, C-cell, adenoma		4 (8%)
C-cell, adenoma	10 (21%)	10 (20%)
C-cell, carcinoma	1 (2%)	4 (8%)
Follicular cell, adenocarcinoma	1 (2%)	
Follicular cell, adenoma	1 (2%)	
General Body System		
Tissue NOS	(1)	
Carcinoma	1 (100%)	
Genital System		
Epididymis	(49)	(48)
Preputial gland	(49)	(50)
Adenoma		2 (4%)
Prostate	(50)	(49)
Testes	(49)	(49)
Bilateral, interstitial cell, adenoma	40 (82%)	35 (71%)
Interstitial cell, adenoma	6 (12%)	10 (20%)
Hematopoietic System		
Bone marrow	(49)	(50)
Femoral, histiocytic sarcoma		1 (2%)
Sternal, histiocytic sarcoma		1 (2%)
Vertebral, histiocytic sarcoma		1 (2%)
Lymph node	(50)	(50)
Deep cervical, mediastinal, mandibular, carcinoma, metastatic, thyroid gland	1 (2%)	1 (2%)
Mandibular, carcinoma, metastatic, skin	1 (2%)	
Mediastinal, histiocytic sarcoma		1 (2%)
Lymph node, mesenteric	(8)	(8)
Spleen	(50)	(50)
Histiocytic sarcoma		1 (2%)
Sarcoma		1 (2%)
Thymus	(35)	(39)
Integumentary System		
Mammary gland	(19)	(18)
Abdominal, fibroadenoma		1 (6%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Integumentary System (continued)						
Skin	(49)	(29)	(20)	(31)	(29)	(30)
Basal cell adenoma				2 (6%)		
Keratoacanthoma	1 (2%)	1 (3%)		3 (10%)	1 (3%)	1 (3%)
Neoplasm NOS, metastatic	1 (2%)					
Squamous cell carcinoma	2 (4%)					1 (3%)
Squamous cell papilloma		1 (3%)		2 (6%)		
Subcutaneous tissue, fibroma	1 (2%)	1 (3%)	4 (20%)	2 (6%)	1 (3%)	
Subcutaneous tissue, fibrosarcoma	1 (2%)			2 (6%)		
Subcutaneous tissue, hemangiosarcoma				1 (3%)		1 (3%)
Subcutaneous tissue, osteosarcoma		1 (3%)				
Musculoskeletal System						
Bone	(49)	(27)	(18)	(25)	(29)	(27)
Osteosarcoma					1 (3%)	
Vertebra, osteosarcoma					1 (3%)	
Skeletal muscle	(1)		(1)		(1)	
Nervous System						
Brain	(50)	(27)	(17)	(24)	(29)	(29)
Astrocytoma benign	1 (2%)					
Astrocytoma malignant		1 (4%)				
Histiocytic sarcoma	1 (2%)					
Peripheral nerve		(1)			(1)	
Schwannoma malignant		1 (100%)			1 (100%)	
Respiratory System						
Lung	(50)	(27)	(18)	(24)	(30)	(29)
Alveolar/bronchiolar adenoma	1 (2%)	1 (4%)				1 (3%)
Alveolar/bronchiolar carcinoma			1 (6%)			
Carcinoma	1 (2%)					
Carcinoma, metastatic			1 (6%)			
Histiocytic sarcoma	1 (2%)		1 (6%)		1 (3%)	
Osteosarcoma, metastatic					1 (3%)	
Pheochromocytoma malignant, metastatic, adrenal gland						1 (3%)
Nose	(50)	(27)	(17)	(24)	(29)	(29)
Polyp	1 (2%)					
Nasolacrimal duct, histiocytic sarcoma	1 (2%)					
Trachea	(50)	(27)	(17)	(25)	(29)	(28)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Integumentary System (continued)		
Skin	(49)	(50)
Basal cell adenoma		1 (2%)
Fibroma	1 (2%)	
Keratoacanthoma	1 (2%)	1 (2%)
Schwannoma malignant		1 (2%)
Squamous cell carcinoma	2 (4%)	1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)	
Musculoskeletal System		
Bone	(50)	(50)
Left, femur, osteosarcoma	1 (2%)	
Skeletal muscle	(1)	
Nervous System		
Brain	(50)	(50)
Oligodendroglioma malignant		1 (2%)
Respiratory System		
Lung	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)	3 (6%)
Carcinoma, metastatic, thyroid gland	1 (2%)	2 (4%)
Pheochromocytoma malignant, metastatic		1 (2%)
Nose	(50)	(50)
Trachea	(50)	(50)
Lamina propria, carcinoma, metastatic, thyroid gland		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Special Senses System						
Ear	(1)	(1)			(2)	(3)
Pinna, basosquamous tumor benign						1 (33%)
Pinna, fibrosarcoma						1 (33%)
Eye	(9)	(3)	(6)	(4)	(3)	(6)
Squamous cell carcinoma						1 (17%)
Harderian gland	(2)	(1)	(1)		(1)	(1)
Zymbal's gland	(1)			(1)	(1)	(2)
Adenoma					1 (100%)	
Carcinoma				1 (100%)		2 (100%)
Urinary System						
Kidney	(50)	(28)	(21)	(28)	(31)	(30)
Sarcoma	1 (2%)					
Transitional epithelium, carcinoma					1 (3%)	1 (3%)
Urinary bladder	(48)	(26)	(15)	(23)	(27)	(24)
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		1 (2%)		1 (2%)	
Leukemia mononuclear	25 (50%)	25 (50%)	22 (44%)	24 (48%)	22 (44%)	9 (18%)
Mesothelioma malignant	2 (4%)	1 (2%)	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	50	50	49	50	50	49
Total primary neoplasms	144	126	118	135	119	111
Total animals with benign neoplasms	50	50	48	50	50	47
Total benign neoplasms	104	92	89	102	85	82
Total animals with malignant neoplasms	36	30	28	28	30	24
Total malignant neoplasms	40	34	29	33	34	29
Total animals with metastatic neoplasms	1		1		2	1
Total metastatic neoplasms	3		1		2	1

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Special Senses System		
Ear		(3)
Harderian gland	(4)	
Zymbal's gland	(1)	
Carcinoma	1 (100%)	
Urinary System		
Kidney	(50)	(50)
Renal tubule, adenoma	1 (2%)	
Urinary bladder	(49)	(49)
Systemic Lesions		
Multiple organs	(50)	(50)
Histiocytic sarcoma		1 (2%)
Leukemia mononuclear	19 (38%)	20 (40%)
Mesothelioma malignant	1 (2%)	2 (4%)
Neoplasm Summary		
Total animals with primary neoplasms	49	50
Total primary neoplasms	133	147
Total animals with benign neoplasms	48	49
Total benign neoplasms	104	109
Total animals with malignant neoplasms	26	30
Total malignant neoplasms	29	38
Total animals with metastatic neoplasms	2	3
Total metastatic neoplasms	5	4

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[a]	19/50 (38%)	26/49 (53%)	25/48 (52%)
Adjusted rate[b]	58.4%	73.1%	72.0%
Terminal rate[c]	13/26 (50%)	17/26 (65%)	12/21 (57%)
First incidence (days)	645	508	494
Life table test[d]	P=0.061	P=0.162	P=0.072
Logistic regression test[d]	P=0.066	P=0.140	P=0.060
Cochran-Armitage test[d]	P=0.141		
Fisher exact test[d]		P=0.096	P=0.115
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rate	19/50 (38%)	27/49 (55%)	25/48 (52%)
Adjusted rate	58.4%	73.7%	72.0%
Terminal rate	13/26 (50%)	17/26 (65%)	12/21 (57%)
First incidence (days)	645	508	494
Life table test	P=0.068	P=0.124	P=0.072
Logistic regression test	P=0.073	P=0.095	P=0.060
Cochran-Armitage test	P=0.151		
Fisher exact test		P=0.066	P=0.115
Liver: Hepatocellular Adenoma			
Overall rate	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rate	0.0%	3.8%	15.9%
Terminal rate	0/26 (0%)	1/26 (4%)	2/22 (9%)
First incidence (days)	–[e]	732 (T)	695
Life table test	P=0.017	P=0.500	P=0.053
Logistic regression test	P=0.018	P=0.500	P=0.054
Cochran-Armitage test	P=0.026		
Fisher exact test		P=0.500	P=0.059
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	7.7%	15.9%
Terminal rate	0/26 (0%)	2/26 (8%)	2/22 (9%)
First incidence (days)	–	732 (T)	695
Life table test	P=0.032	P=0.238	P=0.053
Logistic regression test	P=0.033	P=0.238	P=0.054
Cochran-Armitage test	P=0.047		
Fisher exact test		P=0.247	P=0.059
Mammary Gland: Fibroadenoma			
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.4%	0.0%	0.0%
Terminal rate	1/26 (4%)	0/26 (0%)	0/22 (0%)
First incidence (days)	617	–	–
Life table test	P=0.093N	P=0.125N	P=0.145N
Logistic regression test	P=0.072N	P=0.131N	P=0.114N
Cochran-Armitage test	P=0.080N		
Fisher exact test		P=0.121N	P=0.121N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
Mammary Gland: Fibroadenoma or Carcinoma			
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)
Adjusted rate	12.1%	0.0%	0.0%
Terminal rate	2/26 (8%)	0/26 (0%)	0/22 (0%)
First incidence (days)	617	–	–
Life table test	P=0.048N	P=0.066N	P=0.083N
Logistic regression test	P=0.038N	P=0.065N	P=0.065N
Cochran-Armitage test	P=0.039N		
Fisher exact test		P=0.059N	P=0.059N
Pancreatic Islets: Adenoma			
Overall rate	3/50 (6%)	0/23 (0%)[f]	0/47 (0%)
Adjusted rate	9.6%		0.0%
Terminal rate	2/26 (8%)		0/21 (0%)
First incidence (days)	543		–
Life table test			P=0.156N
Logistic regression test			P=0.125N
Cochran-Armitage test			
Fisher exact test			P=0.133N
Pancreatic Islets: Adenoma or Carcinoma			
Overall rate	4/50 (8%)	0/23 (0%)[f]	0/47 (0%)
Adjusted rate	13.4%		0.0%
Terminal rate	3/26 (12%)		0/21 (0%)
First incidence (days)	543		–
Life table test			P=0.090N
Logistic regression test			P=0.072N
Cochran-Armitage test			
Fisher exact test			P=0.066N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	14/50 (28%)	8/31 (26%)	10/48 (21%)
Adjusted rate	43.2%	62.5%	33.5%
Terminal rate	9/26 (35%)	4/7 (57%)	5/22 (23%)
First incidence (days)	611	676	629
Life table test	P=0.330N	P=0.481	P=0.359N
Logistic regression test	P=0.292N	P=0.569	P=0.332N
Cochran-Armitage test	P=0.248N		
Fisher exact test		P=0.520N	P=0.278N
Preputial Gland: Adenoma			
Overall rate	2/49 (4%)	2/24 (8%)[f]	0/49 (0%)
Adjusted rate	7.2%		0.0%
Terminal rate	1/25 (4%)		0/22 (0%)
First incidence (days)	690		–
Life table test			P=0.258N
Logistic regression test			P=0.247N
Cochran-Armitage test			
Fisher exact test			P=0.247N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
Preputial Gland: Adenoma or Carcinoma			
Overall rate	4/49 (8%)	2/24 (8%)[f]	0/49 (0%)
Adjusted rate	13.2%		0.0%
Terminal rate	2/25 (8%)		0/22 (0%)
First incidence (days)	634		–
Life table test			P=0.079N
Logistic regression test			P=0.068N
Cochran-Armitage test			
Fisher exact test			P=0.059N
Skin: Keratoacanthoma			
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	3.1%	10.5%	4.0%
Terminal rate	0/26 (0%)	2/26 (8%)	0/22 (0%)
First incidence (days)	683	709	722
Life table test	P=0.591N	P=0.338	P=0.756
Logistic regression test	P=0.581N	P=0.329	P=0.750
Cochran-Armitage test	P=0.548N		
Fisher exact test		P=0.309	P=0.753N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Squamous Cell Carcinoma			
Overall rate	3/50 (6%)	6/50 (12%)	3/50 (6%)
Adjusted rate	9.3%	21.7%	10.1%
Terminal rate	1/26 (4%)	5/26 (19%)	0/22 (0%)
First incidence (days)	658	709	666
Life table test	P=0.570N	P=0.266	P=0.638
Logistic regression test	P=0.548N	P=0.278	P=0.644
Cochran-Armitage test	P=0.500N		
Fisher exact test		P=0.243	P=0.661N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rate	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	7.3%	11.1%	0.0%
Terminal rate	1/26 (4%)	0/26 (0%)	0/22 (0%)
First incidence (days)	716	568	–
Life table test	P=0.192N	P=0.376	P=0.271N
Logistic regression test	P=0.164N	P=0.336	P=0.251N
Cochran-Armitage test	P=0.158N		
Fisher exact test		P=0.339	P=0.247N
Testes: Adenoma			
Overall rate	47/50 (94%)	48/48 (100%)	46/49 (94%)
Adjusted rate	97.9%	100.0%	100.0%
Terminal rate	25/26 (96%)	24/24 (100%)	22/22 (100%)
First incidence (days)	416	508	425
Life table test	P=0.286	P=0.498	P=0.329
Logistic regression test	P=0.587N	P=0.137	P=0.611
Cochran-Armitage test	P=0.495N		
Fisher exact test		P=0.129	P=0.651N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
Thyroid Gland (C-cell): Adenoma			
Overall rate	11/49 (22%)	6/22 (27%)[f]	10/48 (21%)
Adjusted rate	30.0%		33.9%
Terminal rate	4/26 (15%)		4/22 (18%)
First incidence (days)	532		631
Life table test			P=0.583
Logistic regression test			P=0.536N
Cochran-Armitage test			
Fisher exact test			P=0.521N
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rate	12/49 (24%)	6/22 (27%)[f]	11/48 (23%)
Adjusted rate	33.2%		36.4%
Terminal rate	5/26 (19%)		4/22 (18%)
First incidence (days)	532		631
Life table test			P=0.570
Logistic regression test			P=0.542N
Cochran-Armitage test			
Fisher exact test			P=0.523N
All Organs: Mononuclear Cell Leukemia			
Overall rate	25/50 (50%)	24/50 (48%)	19/50 (38%)
Adjusted rate	59.6%	57.9%	46.9%
Terminal rate	10/26 (38%)	10/26 (38%)	4/22 (18%)
First incidence (days)	416	571	425
Life table test	P=0.306N	P=0.427N	P=0.305N
Logistic regression test	P=0.087N	P=0.551N	P=0.118N
Cochran-Armitage test	P=0.127N		
Fisher exact test		P=0.500N	P=0.157N
All Organs: Malignant Mesothelioma			
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	6.7%	7.4%	4.2%
Terminal rate	1/26 (4%)	0/26 (0%)	0/22 (0%)
First incidence (days)	667	605	725
Life table test	P=0.406N	P=0.527	P=0.528N
Logistic regression test	P=0.359N	P=0.469	P=0.522N
Cochran-Armitage test	P=0.369N		
Fisher exact test		P=0.500	P=0.500N
All Organs: Benign Neoplasms			
Overall rate	50/50 (100%)	50/50 (100%)	48/50 (96%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	26/26 (100%)	26/26 (100%)	22/22 (100%)
First incidence (days)	416	508	425
Life table test	P=0.304	P=0.447N	P=0.375
Logistic regression test	P=0.424N	–[g]	P=0.598N
Cochran-Armitage test	P=0.091N		
Fisher exact test		P=1.000N	P=0.247N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
All Organs: Malignant Neoplasms			
Overall rate	36/50 (72%)	28/50 (56%)	26/50 (52%)
Adjusted rate	76.4%	62.3%	58.6%
Terminal rate	15/26 (58%)	10/26 (38%)	5/22 (23%)
First incidence (days)	416	568	209
Life table test	P=0.232N	P=0.117N	P=0.192N
Logistic regression test	P=0.019N	P=0.094N	P=0.018N
Cochran-Armitage test	P=0.041N		
Fisher exact test		P=0.072N	P=0.032N
All Organs: Benign or Malignant Neoplasms			
Overall rate	50/50 (100%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	26/26 (100%)	26/26 (100%)	22/22 (100%)
First incidence (days)	416	508	209
Life table test	P=0.253	P=0.447N	P=0.324
Logistic regression test	P=0.447N	–	P=0.639N
Cochran-Armitage test	P=0.296N		
Fisher exact test		P=1.000N	P=0.500N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group
[f] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not applicable.
[g] Value of statistic cannot be computed.

TABLE A2b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm
Adrenal Medulla: Benign Pheochromocytoma		
Overall rate[a]	19/50 (38%)	12/50 (24%)
Adjusted rate[b]	58.4%	36.6%
Terminal rate[c]	13/26 (50%)	5/24 (21%)
First incidence (days)	645	627
Life table test[d]		P=0.146N
Logistic regression test[d]		P=0.105N
Fisher exact test[d]		P=0.097N
Adrenal Medulla: Benign or Malignant Pheochromocytoma		
Overall rate	19/50 (38%)	13/50 (26%)
Adjusted rate	58.4%	38.1%
Terminal rate	13/26 (50%)	5/24 (21%)
First incidence (days)	645	627
Life table test		P=0.203N
Logistic regression test		P=0.154N
Fisher exact test		P=0.142N
Mammary Gland: Fibroadenoma		
Overall rate	3/50 (6%)	1/50 (2%)
Adjusted rate	8.4%	4.2%
Terminal rate	1/26 (4%)	1/24 (4%)
First incidence (days)	617	732 (T)
Life table test		P=0.335N
Logistic regression test		P=0.305N
Fisher exact test		P=0.309N
Mammary Gland: Fibroadenoma or Carcinoma		
Overall rate	4/50 (8%)	1/50 (2%)
Adjusted rate	12.1%	4.2%
Terminal rate	2/26 (8%)	1/24 (4%)
First incidence (days)	617	732 (T)
Life table test		P=0.206N
Logistic regression test		P=0.182N
Fisher exact test		P=0.181N
Pancreatic Islets: Adenoma		
Overall rate	3/50 (6%)	1/27 (4%)
Adjusted rate	9.6%	3.2%
Terminal rate	2/26 (8%)	0/1 (0%)
First incidence (days)	543	687
Life table test		P=0.716N
Logistic regression test		P=0.559N
Fisher exact test		P=0.561N

TABLE A2b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm
Pancreatic Islets: Adenoma or Carcinoma		
Overall rate	4/50 (8%)	1/27 (4%)
Adjusted rate	13.4%	3.2%
Terminal rate	3/26 (12%)	0/1 (0%)
First incidence (days)	543	687
Life table test		P=0.695N
Logistic regression test		P=0.472N
Fisher exact test		P=0.422N
Pituitary Gland (Pars Distalis): Adenoma		
Overall rate	14/50 (28%)	10/34 (29%)
Adjusted rate	43.2%	67.7%
Terminal rate	9/26 (35%)	5/8 (63%)
First incidence (days)	611	519
Life table test		P=0.263
Logistic regression test		P=0.363
Fisher exact test		P=0.539
Preputial Gland: Adenoma or Carcinoma		
Overall rate	4/49 (8%)	2/27 (7%)
Adjusted rate	13.2%	4.9%
Terminal rate	2/25 (8%)	0/1 (0%)
First incidence (days)	634	613
Life table test		P=0.677N
Logistic regression test		P=0.666N
Fisher exact test		P=0.640N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma		
Overall rate	3/50 (6%)	2/50 (4%)
Adjusted rate	9.3%	7.0%
Terminal rate	1/26 (4%)	1/24 (4%)
First incidence (days)	658	666
Life table test		P=0.520N
Logistic regression test		P=0.505N
Fisher exact test		P=0.500N
Testes: Adenoma		
Overall rate	47/50 (94%)	50/50 (100%)
Adjusted rate	97.9%	100.0%
Terminal rate	25/26 (96%)	24/24 (100%)
First incidence (days)	416	473
Life table test		P=0.263
Logistic regression test		P=0.117
Fisher exact test		P=0.121

TABLE A2b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm
Thyroid Gland (C-cell): Adenoma		
Overall rate	11/49 (22%)	11/26 (42%)
Adjusted rate	30.0%	28.7%
Terminal rate	4/26 (15%)	0/3 (0%)
First incidence (days)	532	519
Life table test		P=0.299
Logistic regression test		P=0.086
Fisher exact test		P=0.064
Thyroid Gland (C-cell): Adenoma or Carcinoma		
Overall rate	12/49 (24%)	12/26 (46%)
Adjusted rate	33.2%	52.4%
Terminal rate	5/26 (19%)	1/3 (33%)
First incidence (days)	532	519
Life table test		P=0.190
Logistic regression test		P=0.056
Fisher exact test		P=0.050
Thyroid Gland (Follicular Cell): Carcinoma		
Overall rate	1/49 (2%)	2/26 (8%)
Adjusted rate	2.5%	35.4%
Terminal rate	0/26 (0%)	1/3 (33%)
First incidence (days)	645	679
Life table test		P=0.300
Logistic regression test		P=0.255
Fisher exact test		P=0.274
All Organs: Mononuclear Cell Leukemia		
Overall rate	25/50 (50%)	25/50 (50%)
Adjusted rate	59.6%	57.5%
Terminal rate	10/26 (38%)	7/24 (29%)
First incidence (days)	416	473
Life table test		P=0.508
Logistic regression test		P=0.547N
Fisher exact test		P=0.579N
All Organs: Benign Neoplasms		
Overall rate	50/50 (100%)	50/50 (100%)
Adjusted rate	100.0%	100.0%
Terminal rate	26/26 (100%)	24/24 (100%)
First incidence (days)	416	473
Life table test		P=0.415
Logistic regression test		_[e]
Fisher exact test		P=1.000N

TABLE A2b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 Groups (continued)

F_0 Concentration	0 ppm	630 ppm
F_1 Concentration	0 ppm	0 ppm
All Organs: Malignant Neoplasms		
Overall rate	36/50 (72%)	30/50 (60%)
Adjusted rate	76.4%	66.0%
Terminal rate	15/26 (58%)	9/24 (38%)
First incidence (days)	416	473
Life table test		P=0.308N
Logistic regression test		P=0.125N
Fisher exact test		P=0.146N
All Organs: Benign or Malignant Neoplasms		
Overall rate	50/50 (100%)	50/50 (100%)
Adjusted rate	100.0%	100.0%
Terminal rate	26/26 (100%)	24/24 (100%)
First incidence (days)	416	473
Life table test		P=0.415
Logistic regression test		–
Fisher exact test		P=1.000N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Adrenal Medulla: Benign Pheochromocytoma					
Overall rate[a]	19/50 (38%)	16/50 (32%)	11/48 (23%)	19/49 (39%)	27/50 (54%)
Adjusted rate[b]	58.4%	43.9%	37.8%	54.9%	67.3%
Terminal rate[c]	13/26 (50%)	13/33 (39%)	6/22 (27%)	9/23 (39%)	18/31 (58%)
First incidence (days)	645	549	606	501	620
Life table test[d]	P=0.027	P=0.121N	P=0.132N	P=0.437	P=0.257
Logistic regression test[d]	P=0.011	P=0.233N	P=0.070N	P=0.458	P=0.103
Cochran-Armitage test[d]	P=0.015				
Fisher exact test[d]		P=0.338N	P=0.080N	P=0.551	P=0.080
Adrenal Medulla: Malignant Pheochromocytoma					
Overall rate	0/50 (0%)	2/50 (4%)	2/48 (4%)	4/49 (8%)	3/50 (6%)
Adjusted rate	0.0%	4.3%	7.8%	12.3%	8.0%
Terminal rate	0/26 (0%)	0/33 (0%)	1/22 (5%)	1/23 (4%)	1/31 (3%)
First incidence (days)	–[e]	340	689	307	399
Life table test	P=0.083	P=0.240	P=0.218	P=0.057	P=0.149
Logistic regression test	P=0.087	P=0.357	P=0.229	P=0.090	P=0.124
Cochran-Armitage test	P=0.073				
Fisher exact test		P=0.247	P=0.237	P=0.056	P=0.121
Adrenal Medulla: Benign or Malignant Pheochromocytoma					
Overall rate	19/50 (38%)	18/50 (36%)	13/48 (27%)	22/49 (45%)	29/50 (58%)
Adjusted rate	58.4%	46.3%	43.7%	60.7%	70.4%
Terminal rate	13/26 (50%)	13/33 (39%)	7/22 (32%)	10/23 (43%)	19/31 (61%)
First incidence (days)	645	340	606	307	399
Life table test	P=0.015	P=0.233N	P=0.256N	P=0.236	P=0.161
Logistic regression test	P=0.005	P=0.469N	P=0.157N	P=0.244	P=0.041
Cochran-Armitage test	P=0.006				
Fisher exact test		P=0.500N	P=0.175N	P=0.311	P=0.036
Liver: Hepatocellular Adenoma					
Overall rate	0/50 (0%)	3/49 (6%)	2/49 (4%)	1/49 (2%)	5/49 (10%)
Adjusted rate	0.0%	8.6%	9.1%	4.3%	15.0%
Terminal rate	0/26 (0%)	2/33 (6%)	2/22 (9%)	1/23 (4%)	3/31 (10%)
First incidence (days)	–	683	732 (T)	732 (T)	709
Life table test	P=0.073	P=0.162	P=0.201	P=0.476	P=0.055
Logistic regression test	P=0.067	P=0.133	P=0.201	P=0.476	P=0.044
Cochran-Armitage test	P=0.060				
Fisher exact test		P=0.117	P=0.242	P=0.495	P=0.027
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	0/50 (0%)	4/49 (8%)	2/49 (4%)	1/49 (2%)	5/49 (10%)
Adjusted rate	0.0%	11.3%	9.1%	4.3%	15.0%
Terminal rate	0/26 (0%)	2/33 (6%)	2/22 (9%)	1/23 (4%)	3/31 (10%)
First incidence (days)	–	683	732 (T)	732 (T)	709
Life table test	P=0.128	P=0.098	P=0.201	P=0.476	P=0.055
Logistic regression test	P=0.118	P=0.073	P=0.201	P=0.476	P=0.044
Cochran-Armitage test	P=0.108				
Fisher exact test		P=0.056	P=0.242	P=0.495	P=0.027

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Lung: Alveolar/bronchiolar Carcinoma					
Overall rate	1/50 (2%)	1/18 (6%)[f]	0/30 (0%)[f]	0/29 (0%)[f]	3/50 (6%)
Adjusted rate	3.8%				9.7%
Terminal rate	1/26 (4%)				3/31 (10%)
First incidence (days)	732 (T)				732 (T)
Life table test					P=0.369
Logistic regression test					P=0.369
Cochran-Armitage test					
Fisher exact test					P=0.309
Lung: Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate	2/50 (4%)	1/18 (6%)[f]	0/30 (0%)[f]	1/29 (3%)[f]	4/50 (8%)
Adjusted rate	7.7%				12.9%
Terminal rate	2/26 (8%)				4/31 (13%)
First incidence (days)	732 (T)				732 (T)
Life table test					P=0.419
Logistic regression test					P=0.419
Cochran-Armitage test					
Fisher exact test					P=0.339
Mammary Gland: Fibroadenoma					
Overall rate	3/50 (6%)	4/50 (8%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Adjusted rate	8.4%	10.9%	4.5%	2.3%	3.2%
Terminal rate	1/26 (4%)	2/33 (6%)	1/22 (5%)	0/23 (0%)	1/31 (3%)
First incidence (days)	617	654	732 (T)	597	732 (T)
Life table test	P=0.085N	P=0.577	P=0.337N	P=0.346N	P=0.277N
Logistic regression test	P=0.078N	P=0.501	P=0.305N	P=0.273N	P=0.306N
Cochran-Armitage test	P=0.077N				
Fisher exact test		P=0.500	P=0.309N	P=0.309N	P=0.309N
Mammary Gland: Fibroadenoma or Carcinoma					
Overall rate	4/50 (8%)	4/50 (8%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Adjusted rate	12.1%	10.9%	4.5%	2.3%	3.2%
Terminal rate	2/26 (8%)	2/33 (6%)	1/22 (5%)	0/23 (0%)	1/31 (3%)
First incidence (days)	617	654	732 (T)	597	732 (T)
Life table test	P=0.050N	P=0.554N	P=0.213N	P=0.218N	P=0.152N
Logistic regression test	P=0.045N	P=0.640N	P=0.181N	P=0.169N	P=0.178N
Cochran-Armitage test	P=0.043N				
Fisher exact test		P=0.643N	P=0.181N	P=0.181N	P=0.181N
Pancreatic Islets: Adenoma					
Overall rate	3/50 (6%)	0/15 (0%)[f]	0/28 (0%)[f]	1/25 (4%)[f]	0/50 (0%)
Adjusted rate	9.6%				0.0%
Terminal rate	2/26 (8%)				0/31 (0%)
First incidence (days)	543				–
Life table test					P=0.103N
Logistic regression test					P=0.121N
Cochran-Armitage test					
Fisher exact test					P=0.121N

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Pancreatic Islets: Adenoma or Carcinoma					
Overall rate	4/50 (8%)	0/15 (0%)[f]	1/28 (4%)[f]	2/25 (8%)[f]	0/50 (0%)
Adjusted rate	13.4%				0.0%
Terminal rate	3/26 (12%)				0/31 (0%)
First incidence (days)	543				–
Life table test					P=0.049N
Logistic regression test					P=0.063N
Cochran-Armitage test					
Fisher exact test					P=0.059N
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	14/50 (28%)	6/20 (30%)[f]	10/30 (33%)[f]	5/27 (19%)[f]	9/49 (18%)
Adjusted rate	43.2%				24.6%
Terminal rate	9/26 (35)%				5/31 (16%)
First incidence (days)	611				568
Life table test					P=0.108N
Logistic regression test					P=0.168N
Cochran-Armitage test					
Fisher exact test					P=0.185N
Preputial Gland: Adenoma					
Overall rate	2/49 (4%)	1/18 (6%)[f]	1/33 (3%)[f]	0/29 (0%)[f]	2/50 (4%)
Adjusted rate	7.2%				6.5%
Terminal rate	1/25 (4%)				2/31 (6%)
First incidence (days)	690				732 (T)
Life table test					P=0.620N
Logistic regression test					P=0.649N
Cochran-Armitage test					
Fisher exact test					P=0.684N
Preputial Gland: Adenoma or Carcinoma					
Overall rate	4/49 (8%)	1/18 (6%)[f]	2/33 (6%)[f]	1/29 (3%)[f]	2/50 (4%)
Adjusted rate	13.2%				6.5%
Terminal rate	2/25 (8%)				2/31 (6%)
First incidence (days)	634				732 (T)
Life table test					P=0.265N
Logistic regression test					P=0.307N
Cochran-Armitage test					
Fisher exact test					P=0.329N
Skin: Keratoacanthoma, Basal Cell Adenoma, or Squamous Cell Carcinoma					
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	9.3%	0.0%	4.2%	6.5%	8.4%
Terminal rate	1/26 (4%)	0/33 (0%)	0/22 (0%)	1/23 (4%)	1/31 (3%)
First incidence (days)	658	–	709	518	639
Life table test	P=0.266	P=0.101N	P=0.338N	P=0.545N	P=0.607N
Logistic regression test	P=0.255	P=0.120N	P=0.307N	P=0.486N	P=0.661N
Cochran-Armitage test	P=0.254				
Fisher exact test		P=0.121N	P=0.309N	P=0.500N	P=0.661N

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Skin (Subcutaneous Tissue): Fibroma					
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	3.8%	11.3%	2.9%	0.0%	0.0%
Terminal rate	1/26 (4%)	3/33 (9%)	0/22 (0%)	0/23 (0%)	0/31 (0%)
First incidence (days)	732 (T)	654	664	–	–
Life table test	P=0.040N	P=0.245	P=0.745	P=0.524N	P=0.465N
Logistic regression test	P=0.037N	P=0.203	P=0.761	P=0.524N	P=0.465N
Cochran-Armitage test	P=0.035N				
Fisher exact test		P=0.181	P=0.753N	P=0.500N	P=0.500N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma					
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	7.3%	11.3%	2.9%	0.0%	0.0%
Terminal rate	1/26 (4%)	3/33 (9%)	0/22 (0%)	0/23 (0%)	0/31 (0%)
First incidence (days)	716	654	664	–	–
Life table test	P=0.021N	P=0.435	P=0.538N	P=0.266N	P=0.203N
Logistic regression test	P=0.018N	P=0.375	P=0.505N	P=0.251N	P=0.210N
Cochran-Armitage test	P=0.017N				
Fisher exact test		P=0.339	P=0.500N	P=0.247N	P=0.247N
Testes: Adenoma					
Overall rate	47/50 (94%)	47/50 (94%)	50/50 (100%)	46/49 (94%)	45/49 (92%)
Adjusted rate	97.9%	100.0%	100.0%	100.0%	100.0%
Terminal rate	25/26 (96%)	33/33 (100%)	22/22 (100%)	22/22 (100%)	31/31 (100%)
First incidence (days)	416	549	426	307	399
Life table test	P=0.358N	P=0.111N	P=0.194	P=0.308	P=0.133N
Logistic regression test	P=0.396N	P=0.570	P=0.119	P=0.385	P=0.507N
Cochran-Armitage test	P=0.290N				
Fisher exact test		P=0.661N	P=0.121	P=0.651N	P=0.489N
Thyroid Gland (C-cell): Adenoma					
Overall rate	11/49 (22%)	7/14 (50%)[f]	5/27 (19%)[f]	6/23 (26%)[f]	14/49 (29%)
Adjusted rate	30.0%				37.8%
Terminal rate	4/26 (15%)				9/31 (29%)
First incidence (days)	532				568
Life table test					P=0.456
Logistic regression test					P=0.316
Cochran-Armitage test					
Fisher exact test					P=0.322
Thyroid Gland (C-cell): Carcinoma					
Overall rate	1/49 (2%)	0/14 (0%)[f]	2/27 (7%)[f]	1/23 (4%)[f]	4/49 (8%)
Adjusted rate	3.8%				10.1%
Terminal rate	1/26 (4%)				1/31 (3%)
First incidence (days)	732 (T)				428
Life table test					P=0.213
Logistic regression test					P=0.153
Cochran-Armitage test					
Fisher exact test					P=0.181

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Thyroid Gland (C-cell): Adenoma or Carcinoma					
Overall rate	12/49 (24%)	7/14 (50%)[f]	7/27 (26%)[f]	7/23 (30%)[f]	17/49 (35%)
Adjusted rate	33.2%				43.4%
Terminal rate	5/26 (19%)				10/31 (32%)
First incidence (days)	532				428
Life table test					P=0.321
Logistic regression test					P=0.170
Cochran-Armitage test					
Fisher exact test					P=0.188
All Organs: Mononuclear Cell Leukemia					
Overall rate	25/50 (50%)	22/50 (44%)	22/50 (44%)	9/50 (18%)	20/50 (40%)
Adjusted rate	59.6%	51.7%	58.9%	27.5%	45.3%
Terminal rate	10/26 (38%)	13/33 (39%)	9/22 (41%)	2/23 (9%)	8/31 (26%)
First incidence (days)	416	549	426	605	561
Life table test	P=0.058N	P=0.170N	P=0.477N	P=0.008N	P=0.154N
Logistic regression test	P=0.034N	P=0.347N	P=0.335N	P<0.001N	P=0.317N
Cochran-Armitage test	P=0.028N				
Fisher exact test		P=0.344N	P=0.344N	P<0.001N	P=0.211N
All Organs: Malignant Mesothelioma					
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	6.7%	2.6%	3.6%	8.3%	6.5%
Terminal rate	1/26 (4%)	0/33 (0%)	0/22 (0%)	1/23 (4%)	2/31 (6%)
First incidence (days)	667	673	695	410	732 (T)
Life table test	P=0.334	P=0.438N	P=0.523N	P=0.465	P=0.641N
Logistic regression test	P=0.341	P=0.499N	P=0.503N	P=0.611	P=0.672N
Cochran-Armitage test	P=0.327				
Fisher exact test		P=0.500N	P=0.500N	P=0.500	P=0.691N
All Organs: Benign Neoplasms					
Overall rate	50/50 (100%)	48/50 (96%)	50/50 (100%)	47/50 (94%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%	100.0%
Terminal rate	26/26 (100%)	33/33 (100%)	22/22 (100%)	23/23 (100%)	31/31 (100%)
First incidence (days)	416	549	426	307	399
Life table test	P=0.436N	P=0.063N	P=0.328	P=0.465	P=0.175N
Logistic regression test	P=0.376N	–	–	P=0.412N	P=0.651N
Cochran-Armitage test	P=0.283N				
Fisher exact test		P=0.247N	P=1.000N	P=0.121N	P=0.500N
All Organs: Malignant Neoplasms					
Overall rate	36/50 (72%)	29/50 (58%)	31/50 (62%)	24/50 (48%)	30/50 (60%)
Adjusted rate	76.4%	61.3%	75.0%	54.9%	61.0%
Terminal rate	15/26 (58%)	15/33 (45%)	13/22 (59%)	5/23 (22%)	12/31 (39%)
First incidence (days)	416	340	426	307	399
Life table test	P=0.196N	P=0.059N	P=0.410N	P=0.119N	P=0.124N
Logistic regression test	P=0.106N	P=0.232N	P=0.184N	P=0.009N	P=0.199
Cochran-Armitage test	P=0.104N				
Fisher exact test		P=0.104N	P=0.198N	P=0.012N	P=0.146N

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration	0 ppm	63 ppm	210 ppm	630 ppm	630 ppm
F_1 Concentration	0 ppm	240 ppm	800 ppm	800 ppm	2,400 ppm
All Organs: Benign or Malignant Neoplasms					
Overall rate	50/50 (100%)	49/50 (98%)	50/50 (100%)	49/50 (98%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%	100.0%
Terminal rate	26/26 (100%)	33/33 (100%)	22/22 (100%)	23/23 (100%)	31/31 (100%)
First incidence (days)	416	340	426	307	399
Life table test	P=0.513N	P=0.088N	P=0.328	P=0.353	P=0.220N
Logistic regression test	P=0.309	–	–	–	–
Cochran-Armitage test	P=0.571				
Fisher exact test		P=0.500N	P=1.000N	P=0.500N	P=1.000N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group
[f] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not applicable.

TABLE A2d
Statistical Analysis of Liver Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:2,400 and 630:2,400 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Liver: Hepatocellular Adenoma		
Overall rate[a]	4/50 (8%)	5/49 (10%)
Adjusted rate[b]	15.9%	15.0%
Terminal rate[c]	2/22 (9%)	3/31 (10%)
First incidence (days)	695	709
Life table test[d]		P=0.585N
Logistic regression test[d]		P=0.614
Fisher exact test[d]		P=0.487

[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

TABLE A2e
Statistical Analysis of Selected Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:800, 210:800, and 630:800 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Liver: Hepatocellular Adenoma			
Overall rate[a]	1/50 (2%)	2/49 (4%)	1/49 (2%)
Adjusted rate[b]	3.8%	9.1%	4.3%
Terminal rate[c]	1/26 (4%)	2/22 (9%)	1/23 (4%)
First incidence (days)	732 (T)	732 (T)	732 (T)
Life table test[d]	P=0.634N	P=0.441	P=0.735
Logistic regression test[d]	P=0.634N	P=0.441	P=0.735
Cochran-Armitage test[d]	P=0.609N		
Fisher exact test[d]		P=0.492	P=0.747
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	2/50 (4%)	2/49 (4%)	1/49 (2%)
Adjusted rate	7.7%	9.1%	4.3%
Terminal rate	2/26 (8%)	2/22 (9%)	1/23 (4%)
First incidence (days)	732 (T)	732 (T)	732 (T)
Life table test	P=0.442N	P=0.635	P=0.543N
Logistic regression test	P=0.442N	P=0.637	P=0.543N
Cochran-Armitage test	P=0.413N		
Fisher exact test		P=0.684	P=0.508N
All Organs: Mononuclear Cell Leukemia			
Overall rate	24/50 (48%)	22/50 (44%)	9/50 (18%)
Adjusted rate	57.9%	58.9%	27.5%
Terminal rate	10/26 (38%)	9/22 (41%)	2/23 (9%)
First incidence (days)	571	426	605
Life table test	P=0.011N	P=0.504	P=0.016N
Logistic regression test	P<0.001N	P=0.372	P=0.002N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.421N	P=0.001N

(T)Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

TABLE A2f
Statistical Analysis of Liver Neoplasms in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 630:0, 630:800, and 630:2,400 ppm Groups

F_0 Concentration F_1 Concentration	630 ppm 0 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Liver: Hepatocellular Adenoma			
Overall rate[a]	1/50 (2%)	1/49 (2%)	5/49 (10%)
Adjusted rate[b]	2.8%	4.3%	15.0%
Terminal rate[c]	0/24 (0%)	1/23 (4%)	3/31 (10%)
First incidence (days)	662	732 (T)	709
Life table test[d]	P=0.079	P=0.746	P=0.163
Logistic regression test[d]	P=0.055	P=0.751	P=0.122
Cochran-Armitage test[d]	P=0.038		
Fisher exact test[d]		P=0.747	P=0.098

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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.

TABLE A3
Historical Incidence of Hepatocellular Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
2,4-Dichlorophenol	4/50	3/50	5/50
5,5-Diphenylhydantoin	0/50	0/50	0/50
Ethylene thiourea	0/50	0/50	0/50
Polybrominated biphenyls (Firemaster FF-1®)	1/50	0/50	1/50
Manganese sulfate monohydrate	0/52	0/52	0/52
Triamterene	0/50	0/50	0/50
Overall Historical Incidence			
Total	26/1,001 (2.6%)	9/1,001 (0.9%)	32/1,001 (3.2%)
Standard deviation	3.2%	1.7%	3.6%
Range	0%–10%	0%–6%	0%–10%

[a] Data as of 17 December 1991

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	22	21	13	21	24	21
Natural deaths	2	5	4	3	4	6
Survivors						
Died last week of study	1	1			1	2
Terminal sacrifice	25	23	33	26	21	21
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, cecum	(49)	(22)	(13)	(22)	(26)	(21)
Inflammation, chronic, focal				1 (5%)		
Arteriole, inflammation, chronic	1 (2%)					
Arteriole, thrombosis	1 (2%)					
Intestine large, colon	(49)	(24)	(14)	(22)	(26)	(23)
Diverticulum			1 (7%)			
Parasite metazoan	3 (6%)	2 (8%)			3 (12%)	2 (9%)
Intestine large, rectum	(48)	(24)	(14)	(22)	(26)	(22)
Parasite metazoan	3 (6%)			1 (5%)		
Ulcer						1 (5%)
Intestine small, ileum	(46)	(22)	(13)	(23)	(26)	(23)
Inflammation, necrotizing				1 (4%)		1 (4%)
Ulcer	1 (2%)					1 (4%)
Intestine small, jejunum	(48)	(24)	(13)	(22)	(26)	(22)
Inflammation, chronic, focal	1 (2%)					
Peyer's patch, inflammation, suppurative					1 (4%)	
Liver	(50)	(50)	(49)	(50)	(49)	(49)
Basophilic focus	17 (34%)	17 (34%)	24 (49%)	15 (30%)	12 (24%)	26 (53%)
Clear cell focus	2 (4%)	5 (10%)	10 (20%)	8 (16%)	6 (12%)	3 (6%)
Congestion						1 (2%)
Degeneration, cystic	6 (12%)	5 (10%)	9 (18%)	3 (6%)	5 (10%)	2 (4%)
Eosinophilic focus	5 (10%)	2 (4%)	5 (10%)	6 (12%)	8 (16%)	11 (22%)
Fatty change	3 (6%)	1 (2%)	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Hepatodiaphragmatic nodule		1 (2%)			1 (2%)	1 (2%)
Mixed cell focus	2 (4%)				1 (2%)	5 (10%)
Necrosis, coagulative, focal		1 (2%)	2 (4%)			1 (2%)
Necrosis, coagulative, multifocal	4 (8%)	5 (10%)	1 (2%)	1 (2%)		1 (2%)
Necrosis, caseous, focal	1 (2%)					
Regeneration, focal			1 (2%)		2 (4%)	2 (4%)
Regeneration, multifocal					1 (2%)	
Centrilobular, hepatocyte hypertrophy[b]			24 (49%)	13 (26%)	14 (29%)	29 (59%)
Centrilobular, hemorrhage				1 (2%)		
Centrilobular, necrosis, coagulative		1 (2%)	2 (4%)	2 (4%)	1 (2%)	
Centrilobular, necrosis, caseous				1 (2%)		
Periportal, vacuolization cytoplasmic		1 (2%)				
Sinusoid, angiectasis	1 (2%)	4 (8%)	3 (6%)	1 (2%)	3 (6%)	5 (10%)
Sinusoid, angiectasis, focal		1 (2%)	1 (2%)			
Sinusoid, angiectasis, multifocal	1 (2%)					

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	25	17
Natural deaths	3	2
Survivors		
Died last week of study	1	1
Terminal sacrifice	21	30
Animals examined microscopically	50	50
Alimentary System		
Intestine large, cecum	(47)	(48)
Ulcer		1 (2%)
Submucosa, edema	1 (2%)	1 (2%)
Intestine large, colon	(46)	(48)
Inflammation, chronic active, focal	1 (2%)	
Parasite metazoan	6 (13%)	8 (17%)
Ulcer	1 (2%)	
Intestine large, rectum	(47)	(49)
Parasite metazoan	2 (4%)	3 (6%)
Intestine small, ileum	(47)	(48)
Inflammation, chronic, diffuse		1 (2%)
Inflammation, chronic, focal	2 (4%)	
Ulcer	1 (2%)	
Intestine small, jejunum	(48)	(48)
Inflammation, chronic, focal		2 (4%)
Peyer's patch, hyperplasia		2 (4%)
Liver	(50)	(49)
Basophilic focus	4 (8%)	7 (14%)
Clear cell focus	1 (2%)	7 (14%)
Degeneration, cystic	4 (8%)	7 (14%)
Eosinophilic focus	8 (16%)	7 (14%)
Fatty change	2 (4%)	3 (6%)
Hepatodiaphragmatic nodule	1 (2%)	
Mixed cell focus	3 (6%)	6 (12%)
Necrosis, coagulative, focal	2 (4%)	1 (2%)
Necrosis, coagulative, multifocal	2 (4%)	2 (4%)
Regeneration, focal		1 (2%)
Regeneration, multifocal	2 (4%)	
Centrilobular, congestion		1 (2%)
Centrilobular, hepatocyte hypertrophy	27 (54%)	31 (63%)
Serosa, inflammation, chronic, multifocal	1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Alimentary System (continued)						
Mesentery	(7)	(3)	(3)	(3)	(7)	(2)
Inflammation, chronic	2 (29%)					
Fat, necrosis	2 (29%)	2 (67%)			2 (29%)	1 (50%)
Pancreas	(50)	(27)	(16)	(23)	(28)	(25)
Autolysis		1 (4%)				
Acinus, atrophy	27 (54%)	14 (52%)	11 (69%)	13 (57%)	17 (61%)	14 (56%)
Acinus, hyperplasia				1 (4%)		
Acinus, hypertrophy, focal			1 (6%)			
Artery, inflammation, acute						1 (4%)
Artery, inflammation, chronic	1 (2%)			1 (4%)	2 (7%)	2 (8%)
Duct, concretion	1 (2%)					
Duct, ectasia	1 (2%)	2 (7%)			1 (4%)	
Salivary glands	(48)	(27)	(16)	(23)	(27)	(28)
Autolysis			1 (6%)			
Acinus, submandibular gland, atrophy	1 (2%)	1 (4%)				
Parotid gland, inflammation, chronic	1 (2%)					
Stomach, forestomach	(49)	(26)	(15)	(24)	(27)	(26)
Edema		1 (4%)				
Ulcer	2 (4%)	1 (4%)		2 (8%)	3 (11%)	
Epithelium, acanthosis, diffuse						1 (4%)
Stomach, glandular	(49)	(25)	(15)	(23)	(26)	(23)
Edema		1 (4%)				
Ulcer	3 (6%)	1 (4%)			1 (4%)	
Arteriole, submucosa, thrombosis	1 (2%)					
Mucosa, mineralization, multifocal					1 (4%)	
Tongue				(1)	(2)	
Cyst					1 (50%)	
Tooth		(1)				
Peridontal tissue, inflammation, chronic active		1 (100%)				
Cardiovascular System						
Heart	(50)	(28)	(18)	(25)	(29)	(29)
Dilatation			1 (6%)			
Infarct					1 (3%)	
Atrium, dilatation			2 (11%)		1 (3%)	
Atrium left, thrombosis	1 (2%)	1 (4%)	1 (6%)	3 (12%)	2 (7%)	2 (7%)
Endocardium, proliferation					1 (3%)	
Mitral valve, thrombosis				1 (4%)		
Myocardium, degeneration, multifocal	39 (78%)	22 (79%)	14 (78%)	16 (64%)	25 (86%)	27 (93%)
Myocardium, fibrosis, focal	1 (2%)					
Myocardium, inflammation, chronic, focal	1 (2%)		2 (11%)			
Myocardium, inflammation, chronic, multifocal	1 (2%)		2 (11%)			
Myocardium, inflammation, chronic active, multifocal				1 (4%)		
Myocardium, inflammation, multifocal, necrotizing	1 (2%)					
Myocardium, mineralization, focal		1 (4%)				
Valve, bacterium	2 (4%)				1 (3%)	
Valve, inflammation, chronic active	2 (4%)					
Valve, thrombosis	1 (2%)				1 (3%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Alimentary System (continued)		
Mesentery	(6)	(4)
Inflammation, chronic		1 (25%)
Inflammation, chronic active	1 (17%)	
Necrosis	1 (17%)	1 (25%)
Fat, necrosis	1 (17%)	1 (25%)
Pancreas	(48)	(50)
Acinus, atrophy	18 (38%)	24 (48%)
Artery, inflammation, chronic	2 (4%)	2 (4%)
Salivary glands	(49)	(50)
Acinus, sublingual gland, atrophy		1 (2%)
Arteriole, thrombosis	1 (2%)	
Parotid gland, hyperplasia, lobular	1 (2%)	
Parotid gland, inflammation, chronic	1 (2%)	
Submandibular gland, necrosis, caseous		1 (2%)
Stomach, forestomach	(49)	(48)
Hyperkeratosis	1 (2%)	3 (6%)
Ulcer	2 (4%)	1 (2%)
Submucosa, edema	1 (2%)	1 (2%)
Stomach, glandular	(49)	(48)
Inflammation, acute		1 (2%)
Inflammation, chronic	1 (2%)	
Ulcer	2 (4%)	
Submucosa, edema	1 (2%)	
Tooth	(1)	
Gingiva, inflammation, necrotizing	1 (100%)	
Cardiovascular System		
Heart	(50)	(50)
Arteriole, coronary artery, intima, necrosis, fibrinoid		1 (2%)
Atrium, dilatation	2 (4%)	1 (2%)
Atrium left, thrombosis	1 (2%)	1 (2%)
Coronary artery, inflammation, chronic		1 (2%)
Endocardium, proliferation		1 (2%)
Myocardium, degeneration, multifocal	34 (68%)	40 (80%)
Myocardium, fibrosis, focal		1 (2%)
Myocardium, infarct		1 (2%)
Myocardium, inflammation, chronic, multifocal		1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Endocrine System						
Adrenal gland, cortex	(49)	(50)	(48)	(49)	(47)	(49)
Hyperplasia, nodular	19 (39%)	10 (20%)	13 (27%)	8 (16%)	10 (21%)	19 (39%)
Hypertrophy, focal	1 (2%)	1 (2%)	2 (4%)		3 (6%)	3 (6%)
Inflammation, necrotizing				1 (2%)		
Mineralization, multifocal			1 (2%)			
Necrosis, coagulative, diffuse					1 (2%)	
Vacuolization cytoplasmic, focal	1 (2%)		1 (2%)			
Adrenal gland, medulla	(49)	(50)	(50)	(49)	(48)	(49)
Hyperplasia		1 (2%)		1 (2%)		
Hyperplasia, nodular	14 (29%)	25 (50%)	26 (52%)	24 (49%)	30 (63%)	21 (43%)
Inflammation, necrotizing				1 (2%)		
Necrosis, coagulative, diffuse					1 (2%)	
Parathyroid gland	(47)	(26)	(17)	(24)	(26)	(29)
Hyperplasia, diffuse	2 (4%)					
Pituitary gland	(50)	(34)	(20)	(31)	(30)	(27)
Pars distalis, angiectasis, focal	1 (2%)	1 (3%)			1 (3%)	
Pars distalis, cyst	1 (2%)			2 (6%)	1 (3%)	
Pars distalis, hyperplasia	1 (2%)					
Pars distalis, hyperplasia, nodular	6 (12%)	2 (6%)	1 (5%)	4 (13%)	3 (10%)	2 (7%)
Pars nervosa, inflammation, multifocal, subacute	1 (2%)					
Thyroid gland	(49)	(26)	(14)	(22)	(27)	(23)
Necrosis, caseous, focal	1 (2%)					
C-cell, hyperplasia	37 (76%)	13 (50%)	7 (50%)	17 (77%)	19 (70%)	16 (70%)
Follicle, dilatation, focal		1 (4%)				
Follicle, dilatation, multifocal				1 (5%)	1 (4%)	
General Body System						
None						
Genital System						
Epididymis	(48)	(27)	(17)	(24)	(27)	(30)
Granuloma sperm	1 (2%)				1 (4%)	
Inflammation, chronic			1 (6%)			
Preputial gland	(49)	(27)	(18)	(24)	(33)	(29)
Autolysis, diffuse					1 (3%)	
Hyperplasia	1 (2%)	1 (4%)	1 (6%)			1 (3%)
Inflammation, chronic	28 (57%)	18 (67%)	15 (83%)	21 (88%)	25 (76%)	21 (72%)
Inflammation, chronic active	4 (8%)	5 (19%)	2 (11%)	2 (8%)	4 (12%)	7 (24%)
Duct, ectasia				1 (4%)	2 (6%)	1 (3%)
Prostate	(49)	(27)	(17)	(24)	(28)	(29)
Granuloma	1 (2%)					1 (3%)
Hemorrhage	1 (2%)					
Seminal vesicle	(3)	(1)	(1)			
Hemorrhage	1 (33%)					

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Endocrine System		
Adrenal gland, cortex	(49)	(50)
Hyperplasia, nodular	10 (20%)	11 (22%)
Adrenal gland, medulla	(48)	(50)
Hyperplasia		1 (2%)
Hyperplasia, nodular	17 (35%)	15 (30%)
Parathyroid gland	(49)	(44)
Hyperplasia, diffuse	2 (4%)	2 (5%)
Pituitary gland	(48)	(49)
Pars distalis, hyperplasia, nodular	4 (8%)	4 (8%)
Pars nervosa, inflammation, chronic	4 (8%)	1 (2%)
Thyroid gland	(48)	(49)
C-cell, hyperplasia	36 (75%)	30 (61%)
C-cell, mineralization, multifocal		1 (2%)
Follicle, dilatation, multifocal	1 (2%)	1 (2%)
Follicular cell, hyperplasia, nodular	1 (2%)	
Follicular cell, vacuolization cytoplasmic, focal		1 (2%)
General Body System		
None		
Genital System		
Epididymis	(49)	(48)
Granuloma sperm	1 (2%)	
Inflammation, chronic		1 (2%)
Preputial gland	(49)	(50)
Hyperplasia	3 (6%)	2 (4%)
Inflammation, chronic	44 (90%)	29 (58%)
Inflammation, chronic active	1 (2%)	5 (10%)
Duct, ectasia	3 (6%)	2 (4%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Genital System (continued)						
Testes	(50)	(50)	(50)	(48)	(50)	(49)
Autolysis, diffuse					1 (2%)	
Granuloma sperm	1 (2%)					
Necrosis	2 (4%)					
Artery, inflammation, chronic	1 (2%)					
Interstitial cell, hyperplasia	1 (2%)	1 (2%)	2 (4%)			1 (2%)
Seminiferous tubule, atrophy	4 (8%)	9 (18%)	15 (30%)	12 (25%)	13 (26%)	9 (18%)
Hematopoietic System						
Bone marrow	(50)	(26)	(16)	(23)	(28)	(29)
Femoral, hyperplasia	21 (42%)	20 (77%)	11 (69%)	15 (65%)	16 (57%)	16 (55%)
Femoral, myelofibrosis	1 (2%)				1 (4%)	
Lymph node	(50)	(27)	(19)	(26)	(29)	(29)
Lumbar, sinus, ectasia		1 (4%)				
Mandibular, hyperplasia, lymphoid			1 (5%)			
Mandibular, hyperplasia, plasma cell						1 (3%)
Mandibular, inflammation, necrotizing				1 (4%)		
Mediastinal, hematopoietic cell proliferation	1 (2%)					
Mediastinal, hyperplasia, lymphoid			1 (5%)			1 (3%)
Mediastinal, infiltration cellular, histiocyte						1 (3%)
Mediastinal, medulla, fibrosis	1 (2%)					
Mediastinal, sinus, ectasia	1 (2%)		1 (5%)	1 (4%)		1 (3%)
Sinus, mandibular, ectasia	1 (2%)		1 (5%)			1 (3%)
Lymph node, mesenteric	(5)	(7)	(4)	(5)	(8)	(4)
Infiltration cellular, histiocyte		1 (14%)				
Sinus, ectasia	2 (40%)		2 (50%)	2 (40%)	1 (13%)	1 (25%)
Spleen	(49)	(33)	(29)	(31)	(35)	(29)
Fibrosis, diffuse				1 (3%)		
Fibrosis, focal	2 (4%)	2 (6%)	3 (10%)	1 (3%)	3 (9%)	
Hematopoietic cell proliferation	3 (6%)	1 (3%)	2 (7%)		1 (3%)	1 (3%)
Necrosis, focal						1 (3%)
Capsule, fibrosis, focal		1 (3%)				
Capsule, pigmentation, focal, hemosiderin	1 (2%)					
Red pulp, pigmentation, diffuse, hemosiderin		1 (3%)				
Thymus	(40)	(23)	(15)	(23)	(28)	(25)
Ectopic parathyroid gland	1 (3%)					
Mediastinum, inflammation, chronic, multifocal		1 (4%)				
Vein, congestion		1 (4%)				
Integumentary System						
Mammary gland	(23)	(12)	(8)	(7)	(14)	(7)
Granuloma	1 (4%)					
Hyperplasia, cystic	20 (87%)	11 (92%)	5 (63%)	5 (71%)	12 (86%)	6 (86%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Genital System (continued)		
Testes	(49)	(49)
Artery, inflammation, chronic		1 (2%)
Interstitial cell, hyperplasia	4 (8%)	5 (10%)
Seminiferous tubule, atrophy	1 (2%)	3 (6%)
Hematopoietic System		
Bone marrow	(49)	(50)
Femoral, hyperplasia	22 (45%)	26 (52%)
Lymph node	(50)	(50)
Inguinal, sinus, ectasia		1 (2%)
Lumbar, necrosis, caseous		1 (2%)
Mandibular, hyperplasia, plasma cell	1 (2%)	
Mandibular, necrosis, caseous		1 (2%)
Mediastinal, sinus, ectasia	1 (2%)	
Renal, sinus, ectasia	3 (6%)	
Lymph node, mesenteric	(8)	(8)
Fibrosis	1 (13%)	
Giant cell	1 (13%)	
Metaplasia, focal, osseous	1 (13%)	
Thrombosis	1 (13%)	
Sinus, ectasia	2 (25%)	3 (38%)
Spleen	(50)	(50)
Fibrosis, focal	3 (6%)	2 (4%)
Hematopoietic cell proliferation		1 (2%)
Thymus	(35)	(39)
Cyst		1 (3%)
Ectopic parathyroid gland		1 (3%)
Arteriole, inflammation, chronic	1 (3%)	1 (3%)
Arteriole, thrombosis	1 (3%)	
Integumentary System		
Mammary gland	(19)	(18)
Hyperplasia, cystic	17 (89%)	16 (89%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Integumentary System (continued)						
Skin	(49)	(29)	(20)	(31)	(29)	(30)
Acanthosis				1 (3%)		
Cyst epithelial inclusion			1 (5%)			
Fungus				1 (3%)		
Hyperkeratosis	1 (2%)	1 (3%)		1 (3%)		
Inflammation, chronic	2 (4%)		1 (5%)	1 (3%)		
Inflammation, granulomatous			1 (5%)	1 (3%)		
Inflammation, necrotizing		1 (3%)				
Thrombosis			1 (5%)			
Subcutaneous tissue, edema					1 (3%)	
Tail, necrosis, coagulative, diffuse					1 (3%)	
Tail, dermis, epidermis, bacterium					1 (3%)	
Musculoskeletal System						
Bone	(49)	(27)	(18)	(25)	(29)	(27)
Cranium, inflammation, chronic, focal		1 (4%)				
Femur, fibrous osteodystrophy	1 (2%)					
Femur, osteopetrosis					1 (3%)	
Tibia, fracture healed			1 (6%)			
Skeletal muscle	(1)		(1)		(1)	
Hindlimb, inflammation, subacute	1 (100%)					
Nervous System						
Brain	(50)	(27)	(17)	(24)	(29)	(29)
Abscess					1 (3%)	
Hemorrhage		1 (4%)				
Infarct	2 (4%)	4 (15%)	2 (12%)			
Mineralization, focal	1 (2%)					
Artery, bacterium					1 (3%)	
Cerebellum, neuron, necrosis, focal					1 (3%)	
Hypothalamus, compression	8 (16%)	4 (15%)	1 (6%)	3 (13%)	5 (17%)	2 (7%)
Ventricle, hydrocephalus					1 (3%)	
Respiratory System						
Lung	(50)	(27)	(18)	(24)	(30)	(29)
Bacterium			1 (6%)			
Congestion		2 (7%)	2 (11%)			2 (7%)
Alveolar epithelium, hyperplasia				1 (4%)		
Alveolus, hemorrhage		1 (4%)	4 (22%)	3 (13%)	1 (3%)	
Alveolus, hemorrhage, multifocal					1 (3%)	
Artery, mineralization	2 (4%)			2 (8%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Integumentary System (continued)		
Skin	(49)	(50)
Acanthosis	1 (2%)	
Inflammation, chronic	1 (2%)	
Inflammation, necrotizing	2 (4%)	
Sebaceous gland, hyperplasia	1 (2%)	
Musculoskeletal System		
Bone	(50)	(50)
Femur, fibrous osteodystrophy		1 (2%)
Nervous System		
Brain	(50)	(50)
Infarct	2 (4%)	2 (4%)
Mineralization, focal		1 (2%)
Fourth ventricle, hydrocephalus		1 (2%)
Hypothalamus, compression	3 (6%)	2 (4%)
Third ventricle, hydrocephalus	1 (2%)	
Respiratory System		
Lung	(50)	(50)
Inflammation, focal, subacute		1 (2%)
Artery, mineralization		3 (6%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Respiratory System (continued)						
Lung (continued)	(50)	(27)	(18)	(24)	(30)	(29)
Interstitium, hemorrhage, chronic, multifocal						1 (3%)
Interstitium, inflammation, chronic						1 (3%)
Interstitium, inflammation, chronic, focal	2 (4%)	1 (4%)	2 (11%)		1 (3%)	
Interstitium, inflammation, chronic, multifocal	2 (4%)	3 (11%)	1 (6%)	2 (8%)	1 (3%)	2 (7%)
Nose	(50)	(27)	(17)	(24)	(29)	(29)
Sinus, inflammation, suppurative						1 (3%)
Special Senses System						
Ear	(1)	(1)			(2)	(3)
External ear, inflammation, chronic active	1 (100%)					
Middle ear, inflammation, chronic					1 (50%)	
Pinna, acanthosis						1 (33%)
Pinna, inflammation, necrotizing		1 (100%)			1 (50%)	
Eye	(9)	(3)	(6)	(4)	(3)	(6)
Anterior, synechia			1 (17%)			
Anterior chamber, hemorrhage	1 (11%)					
Lens capsule, cataract					1 (33%)	
Lens crystalline, cataract	9 (100%)	2 (67%)	6 (100%)	4 (100%)	2 (67%)	5 (83%)
Posterior chamber, inflammation, chronic	1 (11%)	2 (67%)		2 (50%)	2 (67%)	2 (33%)
Retina, atrophy	7 (78%)	1 (33%)	6 (100%)	2 (50%)		3 (50%)
Sclera, mineralization, multifocal			1 (17%)		2 (67%)	
Harderian gland	(2)	(1)	(1)		(1)	(1)
Inflammation, chronic, multifocal					1 (100%)	
Urinary System						
Kidney	(50)	(28)	(21)	(28)	(31)	(30)
Hemorrhage, focal					1 (3%)	
Infarct		1 (4%)				
Inflammation, multifocal, necrotizing					1 (3%)	
Nephropathy, multifocal	50 (100%)	26 (93%)	20 (95%)	26 (93%)	28 (90%)	27 (90%)
Pelvis, inflammation, suppurative					1 (3%)	
Renal tubule, bacterium					1 (3%)	
Renal tubule, cyst						1 (3%)
Renal tubule, cytoplasmic alteration	1 (2%)					
Urinary bladder	(48)	(26)	(15)	(23)	(27)	(24)
Dilatation		1 (4%)				
Inflammation, acute					1 (4%)	
Lamina propria, edema					1 (4%)	
Mucosa, inflammation, proliferative				1 (4%)		
Transitional epithelium, metaplasia, focal, squamous						1 (4%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Respiratory System (continued)		
Lung (continued)	(50)	(50)
Interstitium, inflammation, chronic, diffuse	1 (2%)	1 (2%)
Interstitium, inflammation, chronic, focal	2 (4%)	
Interstitium, inflammation, chronic, multifocal	1 (2%)	1 (2%)
Trachea	(50)	(50)
Lamina propria, inflammation, chronic	1 (2%)	2 (4%)
Special Senses System		
Ear		(3)
Middle ear, inflammation, chronic		1 (33%)
Eye	(6)	(8)
Lens crystalline, cataract	4 (67%)	7 (88%)
Posterior chamber, inflammation, chronic	1 (17%)	3 (38%)
Retina, atrophy	2 (33%)	5 (63%)
Retina, mineralization, focal		1 (13%)
Harderian gland	(4)	
Inflammation, chronic, multifocal	2 (50%)	
Zymbal's gland	(1)	
Inflammation, suppurative	1 (100%)	
Urinary System		
Kidney	(50)	(50)
Cyst		2 (4%)
Hemorrhage		1 (2%)
Nephropathy, multifocal	48 (96%)	49 (98%)
Fat, hematopoietic cell proliferation		1 (2%)
Urinary bladder	(49)	(49)
Dilatation	2 (4%)	
Inflammation, chronic	1 (2%)	

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report because it is more widely used and understood.

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF 5,5-DIPHENYLHYDANTOIN

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	17	19	19	9	19	15
Natural deaths	2	2	4	4	2	2
Survivors						
Died last week of study					1	
Terminal sacrifice	31	29	27	37	28	33
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Esophagus	(50)	(21)	(22)	(13)	(22)	(17)
Intestine large, cecum	(48)	(20)	(20)	(9)	(19)	(16)
Intestine large, colon	(49)	(20)	(20)	(10)	(20)	(16)
Intestine large, rectum	(49)	(20)	(20)	(9)	(20)	(16)
Intestine small, duodenum	(49)	(20)	(20)	(10)	(20)	(16)
Intestine small, ileum	(49)	(20)	(20)	(9)	(20)	(15)
Intestine small, jejunum	(49)	(20)	(20)	(9)	(20)	(17)
Adenocarcinoma						1 (6%)
Liver	(50)	(49)	(50)	(50)	(50)	(50)
Hepatocellular adenoma				1 (2%)		
Hepatocellular adenoma, multiple					1 (2%)	
Mesentery	(3)	(6)	(3)	(1)	(1)	(2)
Pancreas	(50)	(20)	(23)	(10)	(21)	(16)
Salivary glands	(50)	(21)	(22)	(12)	(22)	(17)
Carcinoma, metastatic		1 (5%)				
Stomach, forestomach	(49)	(20)	(23)	(11)	(22)	(16)
Stomach, glandular	(49)	(20)	(20)	(11)	(20)	(16)
Tongue						(1)
Squamous cell carcinoma						1 (100%)
Cardiovascular System						
Heart	(50)	(21)	(24)	(13)	(22)	(17)
Endocardium, schwannoma benign					1 (5%)	
Pericardium, alveolar/bronchiolar carcinoma, metastatic, lung						1 (6%)
Endocrine System						
Adrenal gland, cortex	(50)	(21)	(23)	(13)	(20)	(17)
Adenoma	2 (4%)					
Carcinoma						1 (6%)
Adrenal gland, medulla	(49)	(21)	(23)	(13)	(22)	(17)
Pheochromocytoma malignant		1 (5%)				
Pheochromocytoma benign	4 (8%)	1 (5%)			1 (5%)	1 (6%)
Islets, pancreatic	(50)	(20)	(23)	(11)	(21)	(16)
Adenoma	1 (2%)	1 (5%)	1 (4%)	1 (9%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Animals initially in study	50	50
Early deaths		
Accidental deaths		5
Moribund	11	13
Natural deaths	1	1
Survivors		
Died last week of study	1	
Terminal sacrifice	37	31
Animals examined microscopically	50	50
Alimentary System		
Esophagus	(50)	(50)
Intestine large, colon	(50)	(50)
Intestine large, rectum	(50)	(50)
Intestine small, ileum	(48)	(50)
Liver	(50)	(50)
Hepatocellular adenoma	1 (2%)	
Mesentery	(1)	(1)
Pancreas	(50)	(50)
Salivary glands	(49)	(50)
Stomach, forestomach	(50)	(50)
Stomach, glandular	(50)	(50)
Cardiovascular System		
Heart	(50)	(50)
Endocardium, schwannoma benign	1 (2%)	
Endocrine System		
Adrenal gland, cortex	(50)	(50)
Adrenal gland, medulla	(50)	(50)
Pheochromocytoma benign	2 (4%)	3 (6%)
Parathyroid gland	(48)	(47)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Endocrine System (continued)						
Pituitary gland	(50)	(41)	(43)	(32)	(35)	(33)
Pars distalis, adenoma	25 (50%)	26 (63%)	15 (35%)	19 (59%)	15 (43%)	13 (39%)
Pars distalis, carcinoma	1 (2%)			1 (3%)		
Thyroid gland	(48)	(20)	(19)	(9)	(20)	(17)
Bilateral, C-cell, adenoma	1 (2%)		1 (5%)			
C-cell, adenoma	12 (25%)	7 (35%)	4 (21%)	1 (11%)	3 (15%)	2 (12%)
C-cell, carcinoma	2 (4%)					
Follicular cell, adenocarcinoma	1 (2%)				1 (5%)	
General Body System						
None						
Genital System						
Clitoral gland	(45)	(21)	(24)	(14)	(24)	(20)
Adenoma	4 (9%)		4 (17%)	2 (14%)	4 (17%)	
Carcinoma	2 (4%)					1 (5%)
Squamous cell papilloma				1 (7%)		
Ovary	(49)	(23)	(26)	(19)	(26)	(21)
Uterus	(50)	(26)	(26)	(25)	(35)	(31)
Adenocarcinoma					1 (3%)	1 (3%)
Leiomyoma					1 (3%)	
Leiomyosarcoma	1 (2%)		1 (4%)			
Polyp stromal	6 (12%)	4 (15%)	6 (23%)	11 (44%)	13 (37%)	11 (35%)
Polyp stromal, multiple		1 (4%)				
Sarcoma stromal		1 (4%)		1 (4%)	1 (3%)	1 (3%)
Cervix, carcinoma adenosquamous					1 (3%)	
Hematopoietic System						
Bone marrow	(50)	(21)	(22)	(12)	(22)	(17)
Lymph node	(49)	(21)	(26)	(12)	(23)	(22)
Deep cervical, carcinoma, metastatic, thyroid gland	1 (2%)					
Lymph node, mesenteric	(1)	(1)	(4)	(1)	(6)	(6)
Spleen	(50)	(26)	(29)	(17)	(22)	(21)
Hemangioma			1 (3%)			
Thymus	(46)	(18)	(21)	(12)	(19)	(17)
Integumentary System						
Mammary gland	(50)	(24)	(31)	(19)	(25)	(23)
Adenocarcinoma	3 (6%)	3 (13%)		2 (11%)		
Adenoma	1 (2%)	1 (4%)			1 (4%)	
Fibroadenoma	16 (32%)	9 (38%)	14 (45%)	6 (32%)	6 (24%)	6 (26%)
Fibroadenoma, multiple	1 (2%)					

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Endocrine System (continued)		
Pituitary gland	(50)	(50)
Pars distalis, adenoma	13 (26%)	13 (26%)
Thyroid gland	(50)	(49)
Bilateral, C-cell, adenoma	1 (2%)	1 (2%)
C-cell, adenoma	4 (8%)	4 (8%)
Follicular cell, adenocarcinoma		1 (2%)
General Body System		
None		
Genital System		
Clitoral gland	(50)	(49)
Adenoma	1 (2%)	
Carcinoma	1 (2%)	1 (2%)
Ovary	(50)	(50)
Granulosa cell tumor malignant	1 (2%)	
Uterus	(50)	(50)
Polyp stromal	12 (24%)	6 (12%)
Sarcoma stromal		1 (2%)
Endometrium, adenocarcinoma		1 (2%)
Endometrium, adenoma	1 (2%)	
Endometrium, sarcoma stromal		1 (2%)
Hematopoietic System		
Bone marrow	(50)	(50)
Lymph node	(49)	(50)
Lymph node, mesenteric	(4)	(3)
Spleen	(50)	(50)
Thymus	(45)	(48)
Integumentary System		
Mammary gland	(50)	(49)
Fibroadenoma	4 (8%)	2 (4%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Integumentary System (continued)						
Skin	(50)	(22)	(23)	(14)	(26)	(18)
Keratoacanthoma	1 (2%)				1 (4%)	
Squamous cell carcinoma	1 (2%)					
Squamous cell papilloma		1 (5%)		1 (7%)	2 (8%)	1 (6%)
Trichoepithelioma	1 (2%)					
Subcutaneous tissue, fibroma					1 (4%)	
Subcutaneous tissue, fibrosarcoma		1 (5%)			1 (4%)	
Subcutaneous tissue, fibrous histiocytoma		1 (5%)				
Musculoskeletal System						
None						
Nervous System						
Brain	(50)	(21)	(23)	(13)	(22)	(17)
Respiratory System						
Lung	(50)	(22)	(24)	(13)	(23)	(18)
Alveolar/bronchiolar adenoma	1 (2%)					
Alveolar/bronchiolar carcinoma	1 (2%)					1 (6%)
Carcinoma, metastatic, adrenal gland						1 (6%)
Sarcoma, metastatic						1 (6%)
Nose	(50)	(21)	(23)	(13)	(22)	(17)
Trachea	(50)	(21)	(22)	(12)	(22)	(17)
Special Senses System						
Ear	(3)			(1)	(1)	(1)
Pinna, sarcoma				1 (100%)	1 (100%)	
Eye	(9)	(6)	(6)	(7)	(7)	(9)
Lids, fibrosarcoma	1 (11%)					
Harderian gland	(3)	(1)	(3)	(1)	(1)	
Zymbal's gland		(1)	(1)	(1)		
Carcinoma		1 (100%)	1 (100%)	1 (100%)		
Urinary System						
Kidney	(50)	(21)	(23)	(13)	(22)	(17)
Urinary bladder	(50)	(21)	(20)	(11)	(20)	(16)
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)	(50)
Leukemia mononuclear	13 (26%)	16 (32%)	21 (42%)	10 (20%)	10 (20%)	14 (28%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Integumentary System (continued)		
Skin	(50)	(50)
Squamous cell papilloma	1 (2%)	
Musculoskeletal System		
Skeletal muscle	(1)	(1)
Nervous System		
Brain	(50)	(50)
Peripheral nerve		(1)
Spinal, schwannoma malignant		1 (100%)
Respiratory System		
Lung	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	
Carcinoma, metastatic, Zymbal's gland	1 (2%)	
Nose	(49)	(50)
Trachea	(50)	(50)
Special Senses System		
Harderian gland	(7)	(2)
Zymbal's gland	(1)	
Carcinoma	1 (100%)	
Urinary System		
Kidney	(50)	(50)
Urinary bladder	(50)	(50)
Systemic Lesions		
Multiple organs	(50)	(50)
Leukemia mononuclear	13 (26%)	8 (16%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Neoplasm Summary						
Total animals with primary neoplasms[c]	45	40	40	38	40	38
Total primary neoplasms	102	75	69	59	66	55
Total animals with benign neoplasms	41	33	27	34	33	27
Total benign neoplasms	76	51	46	43	50	34
Total animals with malignant neoplasms	21	22	23	16	14	20
Total malignant neoplasms	26	24	23	16	16	21
Total animals with metastatic neoplasms	1	1				3
Total metastatic neoplasms	1	1				3

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Neoplasm Summary		
Total animals with primary neoplasms	39	26
Total primary neoplasms	58	43
Total animals with benign neoplasms	31	21
Total benign neoplasms	42	29
Total animals with malignant neoplasms	16	14
Total malignant neoplasms	16	14
Total animals with metastatic neoplasms	1	
Total metastatic neoplasms	1	

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[a]	4/49 (8%)	0/13 (0%)[e]	2/50 (4%)
Adjusted rate[b]	12.7%		5.3%
Terminal rate[c]	3/30 (10%)		2/38 (5%)
First incidence (days)	700		729 (T)
Life table test[d]			P=0.239N
Logistic regression test[d]			P=0.242N
Cochran-Armitage test[d]			
Fisher exact test[d]			P=0.329N
Clitoral Gland: Adenoma			
Overall rate	4/45 (9%)	2/14 (14%)[e]	1/50 (2%)
Adjusted rate	13.8%		2.6%
Terminal rate	4/29 (14%)		1/38 (3%)
First incidence (days)	729 (T)		729 (T)
Life table test			P=0.107N
Logistic regression test			P=0.107N
Cochran-Armitage test			
Fisher exact test			P=0.150N
Clitoral Gland: Adenoma or Carcinoma			
Overall rate	6/45 (13%)	2/14 (14%)[e]	2/50 (4%)
Adjusted rate	19.7%		4.9%
Terminal rate	5/29 (17%)		1/38 (3%)
First incidence (days)	700		693
Life table test			P=0.071N
Logistic regression test			P=0.065N
Cochran-Armitage test			
Fisher exact test			P=0.103N
Mammary Gland: Fibroadenoma			
Overall rate	17/50 (34%)	6/50 (12%)	4/50 (8%)
Adjusted rate	46.3%	15.7%	9.8%
Terminal rate	12/31 (39%)	5/37 (14%)	2/38 (5%)
First incidence (days)	586	672	683
Life table test	P<0.001N	P=0.003N	P<0.001N
Logistic regression test	P<0.001N	P=0.005N	P<0.001N
Cochran-Armitage test	P=0.002N		
Fisher exact test		P=0.008N	P=0.001N
Mammary Gland: Fibroadenoma or Adenoma			
Overall rate	18/50 (36%)	6/50 (12%)	4/50 (8%)
Adjusted rate	49.2%	15.7%	9.8%
Terminal rate	13/31 (42%)	5/37 (14%)	2/38 (5%)
First incidence (days)	586	672	683
Life table test	P<0.001N	P=0.002N	P<0.001N
Logistic regression test	P<0.001N	P=0.003N	P<0.001N
Cochran-Armitage test	P=0.001N		
Fisher exact test		P=0.005N	P<0.001N

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
Mammary Gland: Carcinoma			
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	8.3%	5.3%	0.0%
Terminal rate	1/31 (3%)	1/37 (3%)	0/38 (0%)
First incidence (days)	655	711	–[f]
Life table test	P=0.071N	P=0.441N	P=0.096N
Logistic regression test	P=0.083N	P=0.486N	P=0.120N
Cochran-Armitage test	P=0.091N		
Fisher exact test		P=0.500N	P=0.121N
Mammary Gland: Adenoma or Carcinoma			
Overall rate	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted rate	11.3%	5.3%	0.0%
Terminal rate	2/31 (6%)	1/37 (3%)	0/38 (0%)
First incidence (days)	655	711	–
Life table test	P=0.035N	P=0.279N	P=0.046N
Logistic regression test	P=0.040N	P=0.317N	P=0.056N
Cochran-Armitage test	P=0.047N		
Fisher exact test		P=0.339N	P=0.059N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma			
Overall rate	21/50 (42%)	8/50 (16%)	4/50 (8%)
Adjusted rate	54.5%	20.5%	9.8%
Terminal rate	14/31 (45%)	6/37 (16%)	2/38 (5%)
First incidence (days)	586	672	683
Life table test	P<0.001N	P=0.002N	P<0.001N
Logistic regression test	P<0.001N	P=0.002N	P<0.001N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.004N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	25/50 (50%)	19/32 (59%)	13/50 (26%)
Adjusted rate	57.7%	72.6%	30.4%
Terminal rate	13/31 (42%)	13/19 (68%)	9/38 (24%)
First incidence (days)	470	470	575
Life table test	P=0.002N	P=0.541N	P=0.005N
Logistic regression test	P=0.003N	P=0.252	P=0.011N
Cochran-Armitage test	P=0.005N		
Fisher exact test		P=0.274	P=0.011N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rate	26/50 (52%)	20/32 (63%)	13/50 (26%)
Adjusted rate	60.0%	73.2%	30.4%
Terminal rate	14/31 (45%)	13/19 (68%)	9/38 (24%)
First incidence (days)	470	470	575
Life table test	P=0.001N	P=0.555N	P=0.003N
Logistic regression test	P=0.002N	P=0.220	P=0.006N
Cochran-Armitage test	P=0.002N		
Fisher exact test		P=0.240	P=0.007N

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Squamous Cell Carcinoma			
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	9.1%	2.7%	2.6%
Terminal rate	2/31 (6%)	1/37 (3%)	1/38 (3%)
First incidence (days)	677	729 (T)	729 (T)
Life table test	P=0.221N	P=0.254N	P=0.240N
Logistic regression test	P=0.229N	P=0.275N	P=0.255N
Cochran-Armitage test	P=0.272N		
Fisher exact test		P=0.309N	P=0.309N
Thyroid Gland (C-cell): Adenoma			
Overall rate	13/48 (27%)	1/9 (11%)[e]	5/50 (10%)
Adjusted rate	37.2%		11.6%
Terminal rate	10/31 (32%)		2/38 (5%)
First incidence (days)	484		575
Life table test			P=0.015N
Logistic regression test			P=0.030N
Cochran-Armitage test			
Fisher exact test			P=0.026N
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rate	15/48 (31%)	1/9 (11%)[e]	5/50 (10%)
Adjusted rate	40.7%		11.6%
Terminal rate	10/31 (32%)		2/38 (5%)
First incidence (days)	484		575
Life table test			P=0.005N
Logistic regression test			P=0.011N
Cochran-Armitage test			
Fisher exact test			P=0.009N
Uterus: Stromal Polyp			
Overall rate	6/50 (12%)	11/50 (22%)	12/50 (24%)
Adjusted rate	16.9%	28.1%	28.7%
Terminal rate	4/31 (13%)	9/37 (24%)	9/38 (24%)
First incidence (days)	388	672	622
Life table test	P=0.219	P=0.238	P=0.198
Logistic regression test	P=0.148	P=0.160	P=0.100
Cochran-Armitage test	P=0.112		
Fisher exact test		P=0.143	P=0.096
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rate	6/50 (12%)	12/50 (24%)	12/50 (24%)
Adjusted rate	16.9%	29.6%	28.7%
Terminal rate	4/31 (13%)	9/37 (24%)	9/38 (24%)
First incidence (days)	388	509	622
Life table test	P=0.242	P=0.175	P=0.198
Logistic regression test	P=0.147	P=0.103	P=0.100
Cochran-Armitage test	P=0.126		
Fisher exact test		P=0.096	P=0.096

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm
All Organs: Mononuclear Cell Leukemia			
Overall rate	13/50 (26%)	10/50 (20%)	13/50 (26%)
Adjusted rate	33.8%	23.9%	28.6%
Terminal rate	7/31 (23%)	6/37 (16%)	7/38 (18%)
First incidence (days)	484	602	499
Life table test	P=0.439N	P=0.223N	P=0.390N
Logistic regression test	P=0.490	P=0.303N	P=0.560
Cochran-Armitage test	P=0.500		
Fisher exact test		P=0.318N	P=0.590N
All Organs: Benign Neoplasms			
Overall rate	41/50 (82%)	34/50 (68%)	31/50 (62%)
Adjusted rate	87.1%	73.8%	67.2%
Terminal rate	25/31 (81%)	25/37 (68%)	23/38 (61%)
First incidence (days)	388	470	575
Life table test	P=0.008N	P=0.034N	P=0.006N
Logistic regression test	P=0.014N	P=0.063N	P=0.014N
Cochran-Armitage test	P=0.028N		
Fisher exact test		P=0.083N	P=0.022N
All Organs: Malignant Neoplasms			
Overall rate	21/50 (42%)	16/50 (32%)	16/50 (32%)
Adjusted rate	50.9%	35.2%	33.9%
Terminal rate	11/31 (35%)	8/37 (22%)	8/38 (21%)
First incidence (days)	484	509	499
Life table test	P=0.117N	P=0.128N	P=0.095N
Logistic regression test	P=0.241N	P=0.193N	P=0.217N
Cochran-Armitage test	P=0.219N		
Fisher exact test		P=0.204N	P=0.204N
All Organs: Benign or Malignant Neoplasms			
Overall rate	45/50 (90%)	38/50 (76%)	39/50 (78%)
Adjusted rate	93.7%	79.2%	78.0%
Terminal rate	28/31 (90%)	27/37 (73%)	27/38 (71%)
First incidence (days)	388	470	499
Life table test	P=0.037N	P=0.028N	P=0.022N
Logistic regression test	P=0.077N	P=0.037N	P=0.055N
Cochran-Armitage test	P=0.131N		
Fisher exact test		P=0.054N	P=0.086N

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:800, and 0:2,400 ppm Groups (continued)

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not applicable.
[f] Not applicable; no neoplasms in animal group

TABLE B2b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm
Adrenal Medulla: Benign Pheochromocytoma		
Overall rate[a]	4/49 (8%)	1/21 (5%)
Adjusted rate[b]	12.7%	2.2%
Terminal rate[c]	3/30 (10%)	0/1 (0%)
First incidence (days)	700	627
Life table test[d]		P=0.685N
Logistic regression test[d]		P=0.613N
Fisher exact test[d]		P=0.525N
Adrenal Medulla: Benign or Malignant Pheochromocytoma		
Overall rate	4/49 (8%)	2/21 (10%)
Adjusted rate	12.7%	100.0%
Terminal rate	3/30 (10%)	1/1 (100%)
First incidence (days)	700	627
Life table test		P=0.334
Logistic regression test		P=0.464
Fisher exact test		P=0.588
Clitoral Gland: Adenoma		
Overall rate	4/45 (9%)	0/21 (0%)
Adjusted rate	13.8%	0.0%
Terminal rate	4/29 (14%)	0/2 (0%)
First incidence (days)	729 (T)	–[e]
Life table test		P=0.698N
Logistic regression test		P=0.698N
Fisher exact test		P=0.207N
Clitoral Gland: Adenoma or Carcinoma		
Overall rate	6/45 (13%)	0/21 (0%)
Adjusted rate	19.7%	0.0%
Terminal rate	5/29 (17%)	0/2 (0%)
First incidence (days)	700	–
Life table test		P=0.326N
Logistic regression test		P=0.258N
Fisher exact test		P=0.090N
Mammary Gland: Fibroadenoma		
Overall rate	17/50 (34%)	9/50 (18%)
Adjusted rate	46.3%	22.9%
Terminal rate	12/31 (39%)	2/29 (7%)
First incidence (days)	586	627
Life table test		P=0.073N
Logistic regression test		P=0.043N
Fisher exact test		P=0.055N

TABLE B2b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm
Mammary Gland: Fibroadenoma or Adenoma		
Overall rate	18/50 (36%)	10/50 (20%)
Adjusted rate	49.2%	25.7%
Terminal rate	13/31 (42%)	3/29 (10%)
First incidence (days)	586	627
Life table test		P=0.079N
Logistic regression test		P=0.044N
Fisher exact test		P=0.059N
Mammary Gland: Carcinoma		
Overall rate	3/50 (6%)	3/50 (6%)
Adjusted rate	8.3%	8.2%
Terminal rate	1/31 (3%)	1/29 (3%)
First incidence (days)	655	388
Life table test		P=0.659
Logistic regression test		P=0.627
Fisher exact test		P=0.661N
Mammary Gland: Adenoma or Carcinoma		
Overall rate	4/50 (8%)	4/50 (8%)
Adjusted rate	11.3%	11.5%
Terminal rate	2/31 (6%)	2/29 (7%)
First incidence (days)	655	388
Life table test		P=0.631
Logistic regression test		P=0.627
Fisher exact test		P=0.643N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma		
Overall rate	21/50 (42%)	13/50 (26%)
Adjusted rate	54.5%	32.1%
Terminal rate	14/31 (45%)	4/29 (14%)
First incidence (days)	586	388
Life table test		P=0.099N
Logistic regression test		P=0.059N
Fisher exact test		P=0.069N
Pancreatic Islets: Adenoma		
Overall rate	1/50 (2%)	1/20 (5%)
Adjusted rate	3.2%	0.0%
Terminal rate	1/31 (3%)	0/0
First incidence (days)	729 (T)	652
Life table test		P=0.510
Logistic regression test		P=0.508
Fisher exact test		P=0.493

TABLE B2b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm
Pituitary Gland (Pars Distalis): Adenoma		
Overall rate	25/50 (50%)	26/41 (63%)
Adjusted rate	57.7%	78.4%
Terminal rate	13/31 (42%)	14/20 (70%)
First incidence (days)	470	627
Life table test		P=0.198
Logistic regression test		P=0.164
Fisher exact test		P=0.142
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma		
Overall rate	26/50 (52%)	26/41 (63%)
Adjusted rate	60.0%	78.4%
Terminal rate	14/31 (45%)	14/20 (70%)
First incidence (days)	470	627
Life table test		P=0.236
Logistic regression test		P=0.218
Fisher exact test		P=0.189
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Squamous Cell Carcinoma		
Overall rate	3/50 (6%)	1/50 (2%)
Adjusted rate	9.1%	3.4%
Terminal rate	2/31 (6%)	1/29 (3%)
First incidence (days)	677	729 (T)
Life table test		P=0.319N
Logistic regression test		P=0.285N
Fisher exact test		P=0.309N
Thyroid Gland (C-cell): Adenoma		
Overall rate	13/48 (27%)	7/20 (35%)
Adjusted rate	37.2%	0.0%
Terminal rate	10/31 (32%)	0/0
First incidence (days)	484	606
Life table test		P=0.201
Logistic regression test		P=0.274
Fisher exact test		P=0.354
Thyroid Gland (C-cell): Adenoma or Carcinoma		
Overall rate	15/48 (31%)	7/20 (35%)
Adjusted rate	40.7%	0.0%
Terminal rate	10/31 (32%)	0/0
First incidence (days)	484	606
Life table test		P=0.431
Logistic regression test		P=0.411
Fisher exact test		P=0.488

TABLE B2b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm
Uterus: Stromal Polyp		
Overall rate	6/50 (12%)	5/50 (10%)
Adjusted rate	16.9%	13.3%
Terminal rate	4/31 (13%)	2/29 (7%)
First incidence (days)	388	599
Life table test		P=0.504N
Logistic regression test		P=0.520N
Fisher exact test		P=0.500N
Uterus: Stromal Polyp or Stromal Sarcoma		
Overall rate	6/50 (12%)	6/50 (12%)
Adjusted rate	16.9%	15.8%
Terminal rate	4/31 (13%)	2/29 (7%)
First incidence (days)	388	599
Life table test		P=0.614
Logistic regression test		P=0.606
Fisher exact test		P=0.620N
All Organs: Mononuclear Cell Leukemia		
Overall rate	13/50 (26%)	16/50 (32%)
Adjusted rate	33.8%	39.7%
Terminal rate	7/31 (23%)	6/29 (21%)
First incidence (days)	484	599
Life table test		P=0.348
Logistic regression test		P=0.354
Fisher exact test		P=0.330
All Organs: Benign Neoplasms		
Overall rate	42/50 (84%)	33/50 (66%)
Adjusted rate	89.3%	71.5%
Terminal rate	26/31 (84%)	16/29 (55%)
First incidence (days)	388	599
Life table test		P=0.127N
Logistic regression test		P=0.019N
Fisher exact test		P=0.032N
All Organs: Malignant Neoplasms		
Overall rate	21/50 (42%)	22/50 (44%)
Adjusted rate	50.9%	50.5%
Terminal rate	11/31 (35%)	8/29 (28%)
First incidence (days)	484	388
Life table test		P=0.500
Logistic regression test		P=0.524
Fisher exact test		P=0.500

TABLE B2b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 630:0 ppm Groups (continued)

F_0 Concentration	0 ppm	630 ppm
F_1 Concentration	0 ppm	0 ppm
All Organs: Benign or Malignant Neoplasms		
Overall rate	45/50 (90%)	40/50 (80%)
Adjusted rate	93.7%	81.6%
Terminal rate	28/31 (90%)	20/29 (69%)
First incidence (days)	388	388
Life table test		P=0.297N
Logistic regression test		P=0.091N
Fisher exact test		P=0.131N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE B2c
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Adrenal Medulla: Benign Pheochromocytoma					
Overall rate[a]	4/49 (8%)	0/23 (0%)[e]	1/22 (5%)[e]	1/17 (6%)[e]	3/50 (6%)
Adjusted rate[b]	12.7%				8.9%
Terminal rate[c]	3/30 (10%)				1/31 (3%)
First incidence (days)	700				671
Life table test[d]					P=0.488N
Logistic regression test[d]					P=0.505N
Cochran-Armitage test[d]					
Fisher exact test[d]					P=0.489N
Clitoral Gland: Adenoma					
Overall rate	4/45 (9%)	4/24 (17%)[e]	4/24 (17%)[e]	0/20 (0%)[e]	0/49 (0%)
Adjusted rate	13.8%				0.0%
Terminal rate	4/29 (14%)				0/30 (0%)
First incidence (days)	729 (T)				–[f]
Life table test					P=0.058N
Logistic regression test					P=0.058N
Cochran-Armitage test					
Fisher exact test					P=0.049N
Clitoral Gland: Carcinoma					
Overall rate	2/45 (4%)	0/24 (0%)[e]	0/24 (0%)[e]	1/20 (5%)[e]	1/49 (2%)
Adjusted rate	6.4%				3.3%
Terminal rate	1/29 (3%)				1/30 (3%)
First incidence (days)	700				729 (T)
Life table test					P=0.492N
Logistic regression test					P=0.487N
Cochran-Armitage test					
Fisher exact test					P=0.468N
Clitoral Gland: Adenoma or Carcinoma					
Overall rate	6/45 (13%)	4/24 (17%)[e]	4/24 (17%)[e]	1/20 (5%)[e]	1/49 (2%)
Adjusted rate	19.7%				3.3%
Terminal rate	5/29 (17%)				1/30 (3%)
First incidence (days)	700				729 (T)
Life table test					P=0.054N
Logistic regression test					P=0.049N
Cochran-Armitage test					
Fisher exact test					P=0.043N
Lung: Alveolar/bronchiolar Carcinoma					
Overall rate	1/50 (2%)	0/24 (0%)[e]	0/23 (0%)[e]	1/18 (6%)[e]	0/50 (0%)
Adjusted rate	3.2%				0.0%
Terminal rate	1/31 (3%)				0/31 (0%)
First incidence (days)	729 (T)				–
Life table test					P=0.500N
Logistic regression test					P=0.500N
Cochran-Armitage test					
Fisher exact test					P=0.500N

TABLE B2c
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate	2/50 (4%)	0/24 (0%)[e]	0/23 (0%)[e]	1/18 (6%)[e]	0/50 (0%)
Adjusted rate	6.5%				0.0%
Terminal rate	2/31 (6%)				0/31 (0%)
First incidence (days)	729 (T)				–
Life table test					P=0.238N
Logistic regression test					P=0.238N
Cochran-Armitage test					
Fisher exact test					P=0.247N
Mammary Gland: Fibroadenoma					
Overall rate	17/50 (34%)	14/50 (28%)	6/50 (12%)	6/50 (12%)	2/50 (4%)
Adjusted rate	46.3%	41.2%	16.5%	16.2%	5.8%
Terminal rate	12/31 (39%)	8/27 (30%)	3/29 (10%)	4/33 (12%)	1/31 (3%)
First incidence (days)	586	630	600	595	630
Life table test	P<0.001N	P=0.457N	P=0.014N	P=0.007N	P<0.001N
Logistic regression test	P<0.001N	P=0.302N	P=0.006N	P=0.006N	P<0.001N
Cochran-Armitage test	P<0.001N				
Fisher exact test		P=0.333N	P=0.008N	P=0.008N	P<0.001N
Mammary Gland: Fibroadenoma or Adenoma					
Overall rate	18/50 (36%)	14/50 (28%)	7/50 (14%)	6/50 (12%)	2/50 (4%)
Adjusted rate	49.2%	41.2%	19.8%	16.2%	5.8%
Terminal rate	13/31 (42%)	8/27 (30%)	4/29 (14%)	4/33 (12%)	1/31 (3%)
First incidence (days)	586	630	600	595	630
Life table test	P<0.001N	P=0.383N	P=0.016N	P=0.004N	P<0.001N
Logistic regression test	P<0.001N	P=0.231N	P=0.007N	P=0.003N	P<0.001N
Cochran-Armitage test	P<0.001N				
Fisher exact test		P=0.260N	P=0.010N	P=0.005N	P<0.001N
Mammary Gland: Carcinoma					
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.3%	0.0%	0.0%	0.0%	0.0%
Terminal rate	1/31 (3%)	0/27 (0%)	0/29 (0%)	0/33 (0%)	0/31 (0%)
First incidence (days)	655	–	–	–	–
Life table test	P=0.041N	P=0.136N	P=0.119N	P=0.114N	P=0.130N
Logistic regression test	P=0.043N	P=0.119N	P=0.119N	P=0.119N	P=0.126N
Cochran-Armitage test	P=0.043N				
Fisher exact test		P=0.121N	P=0.121N	P=0.121N	P=0.121N
Mammary Gland: Adenoma or Carcinoma					
Overall rate	4/50 (8%)	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	11.3%	0.0%	3.4%	0.0%	0.0%
Terminal rate	2/31 (6%)	0/27 (0%)	1/29 (3%)	0/33 (0%)	0/31 (0%)
First incidence (days)	655	–	729 (T)	–	–
Life table test	P=0.023N	P=0.077N	P=0.186N	P=0.059N	P=0.069N
Logistic regression test	P=0.025N	P=0.060N	P=0.168N	P=0.058N	P=0.068N
Cochran-Armitage test	P=0.025N				
Fisher exact test		P=0.059N	P=0.181N	P=0.059N	P=0.059N

TABLE B2c
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma					
Overall rate	21/50 (42%)	14/50 (28%)	7/50 (14%)	6/50 (12%)	2/50 (4%)
Adjusted rate	54.5%	41.2%	19.8%	16.2%	5.8%
Terminal rate	14/31 (45%)	8/27 (30%)	4/29 (14%)	4/33 (12%)	1/31 (3%)
First incidence (days)	586	630	600	595	630
Life table test	P<0.001N	P=0.199N	P=0.004N	P<0.001N	P<0.001N
Logistic regression test	P<0.001N	P=0.084N	P=0.001N	P<0.001N	P<0.001N
Cochran-Armitage test	P<0.001N				
Fisher exact test		P=0.104N	P=0.002N	P<0.001N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	25/50 (50%)	15/43 (35%)	15/35 (43%)[e]	13/33 (39%)[e]	13/50 (26%)
Adjusted rate	57.7%	56.3%			37.3%
Terminal rate	13/31 (42%)	10/20 (50%)			10/31 (32%)
First incidence (days)	470	425			582
Life table test		P=0.228N			P=0.025N
Logistic regression test		P=0.096N			P=0.016N
Cochran-Armitage test					
Fisher exact test		P=0.104N			P=0.011N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma					
Overall rate	26/50 (52%)	15/43 (35%)	15/35 (43%)[e]	13/33 (39%)[e]	13/50 (26%)
Adjusted rate	60.0%	56.3%			37.3%
Terminal rate	14/31 (45%)	10/20 (50%)			10/31 (32%)
First incidence (days)	470	425			582
Life table test		P=0.187N			P=0.016N
Logistic regression test		P=0.067N			P=0.010N
Cochran-Armitage test					
Fisher exact test		P=0.073N			P=0.007N
Skin: Squamous Cell Papilloma					
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	6.9%	2.9%	0.0%
Terminal rate	0/31 (0%)	0/27 (0%)	2/29 (7%)	0/33 (0%)	0/31 (0%)
First incidence (days)	–	–	729 (T)	707	–
Life table test	P=0.583	–	P=0.223	P=0.518	–
Logistic regression test	P=0.565	–	P=0.223	P=0.513	–
Cochran-Armitage test	P=0.558				
Fisher exact test		–	P=0.247	P=0.500	–
Skin: Squamous Cell Papilloma or Squamous Cell Carcinoma					
Overall rate	3/50 (6%)	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	9.1%	0.0%	9.6%	2.9%	0.0%
Terminal rate	2/31 (6%)	0/27 (0%)	2/29 (7%)	0/33 (0%)	0/31 (0%)
First incidence (days)	677	–	699	707	–
Life table test	P=0.113N	P=0.139N	P=0.643	P=0.282N	P=0.128N
Logistic regression test	P=0.123N	P=0.119N	P=0.647N	P=0.281N	P=0.127N
Cochran-Armitage test	P=0.126N				
Fisher exact test		P=0.121N	P=0.661N	P=0.309N	P=0.121N

TABLE B2c
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Thyroid Gland (C-cell): Adenoma					
Overall rate	13/48 (27%)	5/19 (26%)[e]	3/20 (15%)[e]	2/17 (12%)[e]	5/49 (10%)
Adjusted rate	37.2%				14.9%
Terminal rate	10/31 (32%)				3/31 (10%)
First incidence (days)	484				643
Life table test					P=0.038N
Logistic regression test					P=0.040N
Cochran-Armitage test					
Fisher exact test					P=0.029N
Thyroid Gland (C-cell): Adenoma or Carcinoma					
Overall rate	15/48 (31%)	5/19 (26%)[e]	3/20 (15%)[e]	2/17 (12%)[e]	5/49 (10%)
Adjusted rate	40.7%				14.9%
Terminal rate	10/31 (32%)				3/31 (10%)
First incidence (days)	484				643
Life table test					P=0.016N
Logistic regression test					P=0.014N
Cochran-Armitage test					
Fisher exact test					P=0.010N
Uterus: Stromal Polyp					
Overall rate	6/50 (12%)	6/50 (12%)	13/50 (26%)	11/50 (22%)	6/50 (12%)
Adjusted rate	16.9%	17.6%	34.2%	30.7%	18.4%
Terminal rate	4/31 (13%)	2/27 (7%)	6/29 (21%)	9/33 (27%)	5/31 (16%)
First incidence (days)	388	593	512	616	643
Life table test	P=0.475	P=0.565	P=0.073	P=0.179	P=0.608
Logistic regression test	P=0.325	P=0.608N	P=0.086	P=0.140	P=0.574
Cochran-Armitage test	P=0.417				
Fisher exact test		P=0.620N	P=0.062	P=0.143	P=0.620N
Uterus: Stromal Polyp or Stromal Sarcoma					
Overall rate	6/50 (12%)	6/50 (12%)	14/50 (28%)	12/50 (24%)	8/50 (16%)
Adjusted rate	16.9%	17.6%	37.0%	32.6%	23.3%
Terminal rate	4/31 (13%)	2/27 (7%)	7/29 (24%)	9/33 (27%)	5/31 (16%)
First incidence (days)	388	593	512	616	643
Life table test	P=0.274	P=0.565	P=0.048	P=0.131	P=0.378
Logistic regression test	P=0.188	P=0.617	P=0.040	P=0.109	P=0.356
Cochran-Armitage test	P=0.216				
Fisher exact test		P=0.620N	P=0.039	P=0.096	P=0.387
All Organs: Mononuclear Cell Leukemia					
Overall rate	13/50 (26%)	21/50 (42%)	10/50 (20%)	14/50 (28%)	8/50 (16%)
Adjusted rate	33.8%	52.9%	24.6%	32.0%	23.0%
Terminal rate	7/31 (23%)	10/27 (37%)	2/29 (7%)	5/33 (15%)	5/31 (16%)
First incidence (days)	484	425	519	573	630
Life table test	P=0.037N	P=0.067	P=0.326N	P=0.575	P=0.188N
Logistic regression test	P=0.056N	P=0.070	P=0.237N	P=0.565N	P=0.189N
Cochran-Armitage test	P=0.032N				
Fisher exact test		P=0.069	P=0.318N	P=0.500	P=0.163N

TABLE B2c
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 63:240, 210:800, 630:800, and 630:2,400 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	63 ppm 240 ppm	210 ppm 800 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
All Organs: Benign Neoplasms					
Overall rate	41/50 (82%)	27/50 (54%)	33/50 (66%)	27/50 (54%)	22/50 (44%)
Adjusted rate	87.1%	64.5%	74.1%	63.6%	57.1%
Terminal rate	25/31 (81%)	13/27 (48%)	18/29 (62%)	18/33 (55%)	15/31 (48%)
First incidence (days)	388	425	439	558	540
Life table test	P=0.003N	P=0.054N	P=0.162N	P=0.007N	P=0.002N
Logistic regression test	P=0.001N	P=0.002N	P=0.048N	P=0.002N	P<0.001N
Cochran-Armitage test	P<0.001N				
Fisher exact test		P=0.002N	P=0.055N	P=0.002N	P<0.001N
All Organs: Malignant Neoplasms					
Overall rate	21/50 (42%)	24/50 (48%)	15/50 (30%)	20/50 (40%)	15/50 (30%)
Adjusted rate	50.9%	59.4%	34.9%	43.0%	38.9%
Terminal rate	11/31 (35%)	12/27 (44%)	3/29 (10%)	7/33 (21%)	8/31 (26%)
First incidence (days)	484	425	519	491	540
Life table test	P=0.090N	P=0.279	P=0.187N	P=0.401N	P=0.195N
Logistic regression test	P=0.149N	P=0.361	P=0.067N	P=0.340N	P=0.183N
Cochran-Armitage test	P=0.079N				
Fisher exact test		P=0.344	P=0.149N	P=0.500N	P=0.149N
All Organs: Benign or Malignant Neoplasms					
Overall rate	45/50 (90%)	40/50 (80%)	40/50 (80%)	38/50 (76%)	27/50 (54%)
Adjusted rate	93.7%	81.6%	81.5%	77.5%	67.2%
Terminal rate	28/31 (90%)	18/27 (67%)	20/29 (69%)	22/33 (67%)	18/31 (58%)
First incidence (days)	388	425	439	491	540
Life table test	P=0.002N	P=0.439N	P=0.309N	P=0.091N	P=0.004N
Logistic regression test	P<0.001N	P=0.037N	P=0.047N	P=0.018N	P<0.001N
Cochran-Armitage test	P<0.001N				
Fisher exact test		P=0.131N	P=0.131N	P=0.054N	P<0.001N

(T)Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not applicable.

[f] Not applicable; no neoplasms in animal group

TABLE B2d
Statistical Analysis of Liver Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:2,400 and 630:2,400 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Liver: Hepatocellular Adenoma		
Overall rate[a]	1/50 (2%)	0/50 (0%)
Adjusted rate[b]	2.6%	0.0%
Terminal rate[c]	1/38 (3%)	0/31 (0%)
First incidence (days)	729 (T)	-[e]
Life table test[d]		P=0.541N
Logistic regression test[d]		P=0.541N
Fisher exact test[d]		P=0.500N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE B2e
Statistical Analysis of Liver Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:800, and 210:800, and 630:800 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Liver: Hepatocellular Adenoma			
Overall rate[a]	1/50 (2%)	1/50 (2%)	0/50 (0%)
Adjusted rate[b]	2.7%	3.4%	0.0%
Terminal rate[c]	1/37 (3%)	1/29 (3%)	0/33 (0%)
First incidence (days)	729 (T)	729 (T)	-[e]
Life table test[d]	P=0.374N	P=0.707	P=0.523N
Logistic regression test[d]	P=0.374N	P=0.707	P=0.523N
Cochran-Armitage test[d]	P=0.356N		
Fisher exact test[d]		P=0.753N	P=0.500N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE B2f
Statistical Analysis of Liver Neoplasms in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 630:0, 630:800, and 630:2,400 ppm Groups

F_0 Concentration F_1 Concentration	630 ppm 0 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Liver: Hepatocellular Adenoma			
Overall rate[a]	0/49 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate[b]	0.0%	0.0%	0.0%
Terminal rate[c]	0/29 (0%)	0/33 (0%)	0/31 (0%)
First incidence (days)	–[e]	–	–
Life table test[d]	–	–	–
Logistic regression test[d]	–	–	–
Cochran-Armitage test[d]	–		
Fisher exact test[d]		–	–

[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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.
[e] Not applicable; no neoplasms in animal group

TABLE B3
Historical Incidence of Hepatocellular Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
2,4-Dichlorophenol	0/50	0/50	0/50
5,5-Diphenylhydantoin	0/50	0/50	0/50
Ethylene thiourea	0/50	0/50	0/50
Polybrominated biphenyls (Firemaster FF-1®)	0/50	0/50	0/50
Manganese sulfate monohydrate	0/50	0/50	0/50
Triamterene	0/50	0/50	0/50
Overall Historical Incidence			
Total	5/1,000 (0.5%)	1/1,000 (0.1%)	6/1,000 (0.6%)
Standard deviation	1.4%	0.5%	1.5%
Range	0%–6%	0%–2%	0%–6%

[a] Data as of 17 December 1991

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	17	19	19	9	19	15
Natural deaths	2	2	4	4	2	2
Survivors						
Died last week of study					1	
Terminal sacrifice	31	29	27	37	28	33
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, cecum	(48)	(20)	(20)	(9)	(19)	(16)
Edema		1 (5%)				
Inflammation, chronic active, focal	1 (2%)					
Ulcer	1 (2%)		1 (5%)			
Submucosa, edema	1 (2%)		1 (5%)			
Intestine large, colon	(49)	(20)	(20)	(10)	(20)	(16)
Parasite metazoan	1 (2%)		1 (5%)			1 (6%)
Intestine large, rectum	(49)	(20)	(20)	(9)	(20)	(16)
Parasite metazoan	2 (4%)					
Liver	(50)	(49)	(50)	(50)	(50)	(50)
Basophilic focus	40 (80%)	35 (71%)	30 (60%)	33 (66%)	35 (70%)	31 (62%)
Clear cell focus	1 (2%)	2 (4%)			1 (2%)	1 (2%)
Cyst		1 (2%)	1 (2%)	1 (2%)		
Eosinophilic focus				3 (6%)	1 (2%)	1 (2%)
Fatty change	5 (10%)	3 (6%)	3 (6%)		1 (2%)	2 (4%)
Fatty change, diffuse			2 (4%)			
Granuloma, multiple		1 (2%)				
Hepatodiaphragmatic nodule		1 (2%)	1 (2%)			
Mixed cell focus	1 (2%)		1 (2%)	1 (2%)		2 (4%)
Necrosis, coagulative, focal					1 (2%)	
Necrosis, coagulative, multifocal	1 (2%)	2 (4%)		1 (2%)	1 (2%)	1 (2%)
Necrosis, caseous		1 (2%)				
Necrosis, caseous, multifocal				1 (2%)	1 (2%)	
Regeneration, focal	2 (4%)	1 (2%)	2 (4%)			1 (2%)
Centrilobular, hepatocyte hypertrophy[b]			7 (14%)	22 (44%)	24 (48%)	29 (58%)
Sinusoid, angiectasis	3 (6%)	1 (2%)				
Sinusoid, angiectasis, multifocal		1 (2%)				
Mesentery	(3)	(6)	(3)	(1)	(1)	(2)
Inflammation, chronic	1 (33%)	1 (17%)				
Artery, inflammation, chronic	1 (33%)					
Fat, necrosis	1 (33%)	3 (50%)	3 (100%)	1 (100%)		1 (50%)
Pancreas	(50)	(20)	(23)	(10)	(21)	(16)
Fibrosis, focal			1 (4%)			
Acinus, atrophy	21 (42%)	7 (35%)	13 (57%)	5 (50%)	5 (24%)	5 (31%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Animals initially in study	50	50
Early deaths		
Accidental deaths		5
Moribund	11	13
Natural deaths	1	1
Survivors		
Died last week of study	1	
Terminal sacrifice	37	31
Animals examined microscopically	50	50
Alimentary System		
Intestine large, cecum	(50)	(49)
Ulcer		1 (2%)
Intestine large, colon	(50)	(50)
Dilatation		1 (2%)
Inflammation, chronic, focal		2 (4%)
Parasite metazoan	3 (6%)	3 (6%)
Intestine small, ileum	(48)	(50)
Inflammation, chronic active, focal	1 (2%)	
Liver	(50)	(50)
Basophilic focus	6 (12%)	3 (6%)
Clear cell focus	2 (4%)	3 (6%)
Eosinophilic focus	5 (10%)	2 (4%)
Hepatodiaphragmatic nodule		2 (4%)
Mixed cell focus	1 (2%)	4 (8%)
Necrosis, coagulative, multifocal	1 (2%)	
Regeneration, focal		1 (2%)
Centrilobular, hepatocyte hypertrophy	42 (84%)	38 (76%)
Sinusoid, angiectasis	1 (2%)	
Pancreas	(50)	(50)
Ectopic tissue		1 (2%)
Acinus, atrophy	22 (44%)	14 (28%)
Acinus, hypertrophy, focal		1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Alimentary System (continued)						
Salivary glands	(50)	(21)	(22)	(12)	(22)	(17)
Ectopic tissue					1 (5%)	
Acinus, parotid gland, atrophy	1 (2%)					
Parotid gland, inflammation, chronic						1 (6%)
Stomach, forestomach	(49)	(20)	(23)	(11)	(22)	(16)
Edema						1 (6%)
Inflammation, chronic					1 (5%)	
Epithelium, acanthosis, diffuse					1 (5%)	
Epithelium, hyperkeratosis, diffuse					1 (5%)	
Submucosa, edema	1 (2%)					
Stomach, glandular	(49)	(20)	(20)	(11)	(20)	(16)
Inflammation, chronic					1 (5%)	
Inflammation, subacute		1 (5%)	1 (5%)			
Ulcer				1 (9%)		
Mucosa, inflammation, multifocal, necrotizing					1 (5%)	
Mucosa, proliferation, focal		1 (5%)				
Tooth			(1)			
Gingiva, inflammation, chronic			1 (100%)			
Cardiovascular System						
Blood vessel	(1)					
Aorta, inflammation, chronic, focal	1 (100%)					
Heart	(50)	(21)	(24)	(13)	(22)	(17)
Autolysis			1 (4%)			
Coronary artery, inflammation, chronic	1 (2%)		1 (4%)			1 (6%)
Myocardium, degeneration, diffuse					1 (5%)	
Myocardium, degeneration, multifocal	32 (64%)	13 (62%)	13 (54%)	6 (46%)	8 (36%)	5 (29%)
Myocardium, fibrosis, focal	1 (2%)					
Myocardium, inflammation, chronic, multifocal	2 (4%)		1 (4%)			
Valve, inflammation, chronic			1 (4%)			
Endocrine System						
Adrenal gland, cortex	(50)	(21)	(23)	(13)	(20)	(17)
Atrophy						1 (6%)
Atypia cellular, focal	1 (2%)					
Congestion	1 (2%)					
Hyperplasia, nodular	19 (38%)	3 (14%)	7 (30%)	1 (8%)	9 (45%)	5 (29%)
Hypertrophy, focal	3 (6%)	1 (5%)				2 (12%)
Hypertrophy, multifocal					1 (5%)	
Necrosis, coagulative					1 (5%)	
Vacuolization cytoplasmic, focal		1 (5%)		2 (15%)		1 (6%)
Adrenal gland, medulla	(49)	(21)	(23)	(13)	(22)	(17)
Hyperplasia, nodular	4 (8%)	3 (14%)	6 (26%)		7 (32%)	4 (24%)
Necrosis, coagulative					1 (5%)	
Vacuolization cytoplasmic, focal					1 (5%)	

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Alimentary System (continued)		
Salivary glands	(49)	(50)
Acinus, parotid gland, atrophy	1 (2%)	
Stomach, forestomach	(50)	(50)
Hyperkeratosis	1 (2%)	
Inflammation, subacute		1 (2%)
Inflammation, suppurative	1 (2%)	
Stomach, glandular	(50)	(50)
Mucosa, proliferation, focal	1 (2%)	
Tooth	(3)	(1)
Dysplasia	1 (33%)	
Gingiva, inflammation, chronic active	1 (33%)	1 (100%)
Gingiva, inflammation, suppurative	1 (33%)	
Cardiovascular System		
Heart	(50)	(50)
Atrium left, thrombosis	1 (2%)	1 (2%)
Coronary artery, inflammation, chronic	1 (2%)	1 (2%)
Endocardium, proliferation	1 (2%)	
Epicardium, inflammation, chronic	2 (4%)	2 (4%)
Myocardium, degeneration, multifocal	27 (54%)	20 (40%)
Myocardium, fibrosis, focal	1 (2%)	
Myocardium, inflammation, chronic, multifocal		5 (10%)
Valve, inflammation, chronic		1 (2%)
Endocrine System		
Adrenal gland, cortex	(50)	(50)
Hyperplasia, nodular	22 (44%)	15 (30%)
Hypertrophy, focal	2 (4%)	
Mineralization, focal		1 (2%)
Vacuolization cytoplasmic, focal	1 (2%)	1 (2%)
Adrenal gland, medulla	(50)	(50)
Hyperplasia, nodular	18 (36%)	15 (30%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Endocrine System (continued)						
Parathyroid gland	(45)	(18)	(21)	(12)	(19)	(17)
Hyperplasia, diffuse	1 (2%)		1 (5%)		1 (5%)	
Hyperplasia, nodular	1 (2%)					
Pituitary gland	(50)	(41)	(43)	(32)	(35)	(33)
Angiectasis, focal				1 (3%)		
Autolysis				1 (3%)		
Pars distalis, angiectasis, focal		2 (5%)	5 (12%)	2 (6%)	4 (11%)	6 (18%)
Pars distalis, angiectasis, multifocal			1 (2%)			
Pars distalis, cyst	3 (6%)	4 (10%)	5 (12%)	6 (19%)	2 (6%)	5 (15%)
Pars distalis, hyperplasia, nodular	4 (8%)	3 (7%)	7 (16%)	3 (9%)	6 (17%)	2 (6%)
Pars intermedia, cyst						1 (3%)
Thyroid gland	(48)	(20)	(19)	(9)	(20)	(17)
Cyst					1 (5%)	
C-cell, hyperplasia	33 (69%)	14 (70%)	14 (74%)	8 (89%)	16 (80%)	16 (94%)
General Body System						
None						
Genital System						
Clitoral gland	(45)	(21)	(24)	(14)	(24)	(20)
Autolysis			1 (4%)			
Hyperplasia	2 (4%)	2 (10%)				
Inflammation, chronic	6 (13%)	4 (19%)	4 (17%)	4 (29%)	2 (8%)	4 (20%)
Inflammation, chronic active	3 (7%)	2 (10%)				1 (5%)
Duct, dilatation	1 (2%)	1 (5%)	2 (8%)	3 (21%)	1 (4%)	2 (10%)
Ovary	(49)	(23)	(26)	(19)	(26)	(21)
Inflammation, chronic	5 (10%)	1 (4%)		1 (5%)		
Bilateral, periovarian tissue, cyst					1 (4%)	
Corpus luteum, congestion	1 (2%)					
Follicle, cyst	1 (2%)	1 (4%)				
Periovarian tissue, cyst	5 (10%)	4 (17%)	5 (19%)	7 (37%)	4 (15%)	5 (24%)
Periovarian tissue, necrosis, caseous						1 (5%)
Vein, congestion						1 (5%)
Uterus	(50)	(26)	(26)	(25)	(35)	(31)
Dilatation		2 (8%)	1 (4%)	3 (12%)	2 (6%)	1 (3%)
Hemorrhage					1 (3%)	
Hyperplasia			1 (4%)			
Prolapse	3 (6%)		1 (4%)			
Cervix, inflammation, suppurative			1 (4%)		2 (6%)	5 (16%)
Endometrium, bacterium	1 (2%)					
Endometrium, hyperplasia, cystic	9 (18%)	2 (8%)		5 (20%)	6 (17%)	3 (9%)
Endometrium, hyperplasia, nodular					1 (3%)	
Endometrium, necrosis, coagulative	1 (2%)					1 (3%)
Lumen, hemorrhage					1 (3%)	
Wall, bacterium			1 (4%)			
Wall, cyst				1 (4%)		
Wall, inflammation, chronic active	2 (4%)					
Wall, inflammation, necrotizing	1 (2%)		1 (4%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Endocrine System (continued)		
Pituitary gland	(50)	(50)
Angiectasis, focal	1 (2%)	
Pars distalis, angiectasis, focal	3 (6%)	2 (4%)
Pars distalis, cyst		4 (8%)
Pars distalis, hyperplasia, nodular	10 (20%)	2 (4%)
Thyroid gland	(50)	(49)
C-cell, hyperplasia	43 (86%)	39 (80%)
Follicle, dilatation, focal	2 (4%)	
Follicle, dilatation, multifocal	2 (4%)	
Follicular cell, hyperplasia, focal	1 (2%)	1 (2%)
General Body System		
None		
Genital System		
Clitoral gland	(50)	(49)
Hyperplasia	3 (6%)	1 (2%)
Inflammation, chronic	7 (14%)	9 (18%)
Inflammation, chronic active	4 (8%)	1 (2%)
Duct, dilatation		3 (6%)
Ovary	(50)	(50)
Inflammation, chronic		2 (4%)
Follicle, cyst	1 (2%)	1 (2%)
Periovarian tissue, cyst		2 (4%)
Uterus	(50)	(50)
Prolapse	1 (2%)	1 (2%)
Endometrium, hyperplasia, cystic	6 (12%)	10 (20%)
Endometrium, necrosis, coagulative	2 (4%)	1 (2%)
Wall, bacterium		1 (2%)
Wall, inflammation, chronic active	2 (4%)	3 (6%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Genital System (continued)						
Vagina	(2)		(1)	(2)	(4)	(7)
Cyst			1 (100%)	1 (50%)	4 (100%)	7 (100%)
Lamina propria, inflammation, necrotizing	1 (50%)					
Lumen, exudate				1 (50%)		
Wall, edema	1 (50%)					
Hematopoietic System						
Blood	(1)					
Polychromasia	1 (100%)					
Bone marrow	(50)	(21)	(22)	(12)	(22)	(17)
Femoral, angiectasis	1 (2%)					
Femoral, hyperplasia	7 (14%)	8 (38%)	11 (50%)	4 (33%)	14 (64%)	8 (47%)
Femoral, hyperplasia, re cell	1 (2%)					
Femoral, hypoplasia	2 (4%)					
Femoral, myelofibrosis				1 (8%)		2 (12%)
Lymph node	(49)	(21)	(26)	(12)	(23)	(22)
Mandibular, hemorrhage, focal					1 (4%)	
Mandibular, infiltration cellular, polymorphonuclear						1 (5%)
Mandibular, infiltration cellular, histiocyte		1 (5%)				
Mandibular, inflammation, chronic			1 (4%)			
Mediastinal, pigmentation, hemosiderin			1 (4%)			
Renal, necrosis, caseous						2 (10%)
Sinus, ectasia			1 (4%)		2 (9%)	
Lymph node, mesenteric	(1)	(1)	(4)	(1)	(6)	(6)
Sinus, ectasia			1 (25%)		1 (17%)	3 (50%)
Spleen	(50)	(26)	(29)	(17)	(22)	(21)
Autolysis				1 (6%)		
Fibrosis, diffuse				1 (6%)		
Fibrosis, focal			1 (3%)			
Granuloma, single	1 (2%)					
Hematopoietic cell proliferation	3 (6%)	2 (8%)	1 (3%)	1 (6%)	1 (5%)	1 (5%)
Necrosis, coagulative	1 (2%)		1 (3%)			1 (5%)
Capsule, cyst				1 (6%)		
Capsule, hemorrhage		1 (4%)				
Capsule, inflammation, focal, subacute			1 (3%)			
Thymus	(46)	(18)	(21)	(12)	(19)	(17)
Hemorrhage, multifocal			1 (5%)			
Arteriole, inflammation, chronic	1 (2%)					
Integumentary System						
Mammary gland	(50)	(24)	(31)	(19)	(25)	(23)
Hyperplasia, cystic	32 (64%)	15 (63%)	12 (39%)	6 (32%)	15 (60%)	12 (52%)
Duct, inflammation, chronic active	1 (2%)					

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Genital System (continued)		
Vagina	(2)	(3)
Cyst	1 (50%)	3 (100%)
Prolapse	1 (50%)	
Hematopoietic System		
Bone marrow	(50)	(50)
Femoral, hyperplasia	8 (16%)	9 (18%)
Lymph node	(49)	(50)
Sinus, ectasia	3 (6%)	
Lymph node, mesenteric	(4)	(3)
Sinus, ectasia	1 (25%)	1 (33%)
Spleen	(50)	(50)
Fibrosis, focal	2 (4%)	
Integumentary System		
Mammary gland	(50)	(49)
Hyperplasia, cystic	16 (32%)	21 (43%)
Necrosis, coagulative		1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Integumentary System (continued)						
Skin	(50)	(22)	(23)	(14)	(26)	(18)
Acanthosis	1 (2%)	1 (5%)			1 (4%)	1 (6%)
Hyperkeratosis	1 (2%)	1 (5%)			1 (4%)	1 (6%)
Inflammation, chronic	1 (2%)	1 (5%)				
Inflammation, necrotizing		1 (5%)				
Tail, acanthosis, focal					1 (4%)	
Tail, hyperkeratosis, focal					1 (4%)	
Musculoskeletal System						
Bone	(50)	(21)	(24)	(13)	(22)	(17)
Hyperostosis	1 (2%)					
Femur, osteopetrosis	2 (4%)			1 (8%)		
Tibia, proliferation			1 (4%)			
Nervous System						
Brain	(50)	(21)	(23)	(13)	(22)	(17)
Infarct		1 (5%)	1 (4%)			
Infarct, multiple			1 (4%)			
Inflammation, multifocal, necrotizing		1 (5%)				
Hypothalamus, compression	15 (30%)	8 (38%)	5 (22%)	4 (31%)	4 (18%)	5 (29%)
Hypothalamus, hemorrhage, multifocal			1 (4%)			
Respiratory System						
Lung	(50)	(22)	(24)	(13)	(23)	(18)
Congestion	1 (2%)				1 (4%)	
Alveolar epithelium, hyperplasia	1 (2%)					
Alveolus, hemorrhage		3 (14%)				1 (6%)
Alveolus, hemorrhage, multifocal			2 (8%)			
Alveolus, inflammation, multifocal, suppurative				1 (8%)		
Bronchus, foreign body	1 (2%)			1 (8%)		
Bronchus, inflammation, multifocal, suppurative				1 (8%)		
Interstitium, inflammation, chronic, focal	3 (6%)	2 (9%)				
Interstitium, inflammation, chronic, multifocal			1 (4%)	2 (15%)	1 (4%)	
Nose	(50)	(21)	(23)	(13)	(22)	(17)
Nares, inflammation, chronic active					1 (5%)	
Sinus, inflammation, chronic active		1 (5%)				
Trachea	(50)	(21)	(22)	(12)	(22)	(17)
Inflammation, subacute						1 (6%)
Lamina propria, inflammation, chronic	1 (2%)					

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 2,400 ppm	630 ppm 2,400 ppm
Integumentary System (continued)		
Skin	(50)	(50)
Edema		1 (2%)
Infiltration cellular, polymorphonuclear		1 (2%)
Inflammation, chronic		1 (2%)
Musculoskeletal System		
None		
Nervous System		
Brain	(50)	(50)
Gliosis, focal	1 (2%)	
Infarct	1 (2%)	
Hypothalamus, compression	4 (8%)	2 (4%)
Respiratory System		
Lung	(50)	(50)
Congestion		3 (6%)
Alveolus, hemorrhage	2 (4%)	
Artery, mineralization	1 (2%)	1 (2%)
Interstitium, inflammation, chronic, multifocal	1 (2%)	1 (2%)
Trachea	(50)	(50)
Lamina propria, inflammation, chronic		2 (4%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	630 ppm 0 ppm	63 ppm 240 ppm	0 ppm 800 ppm	210 ppm 800 ppm	630 ppm 800 ppm
Special Senses System						
Ear	(3)			(1)	(1)	(1)
Middle ear, inflammation, chronic active						1 (100%)
Eye	(9)	(6)	(6)	(7)	(7)	(9)
Anterior, synechia					1 (14%)	
Bilateral, lens crystalline, cataract						1 (11%)
Cornea, inflammation, suppurative				1 (14%)		
Lens crystalline, cataract	7 (78%)	5 (83%)	6 (100%)	6 (86%)	4 (57%)	8 (89%)
Lids, inflammation, suppurative	1 (11%)					
Posterior chamber, inflammation, chronic		3 (50%)	4 (67%)	4 (57%)	5 (71%)	8 (89%)
Retina, atrophy	7 (78%)	2 (33%)	3 (50%)	1 (14%)	1 (14%)	
Sclera, mineralization, multifocal		1 (17%)			1 (14%)	
Harderian gland	(3)	(1)	(3)	(1)	(1)	
Inflammation, chronic, multifocal	2 (67%)		2 (67%)	1 (100%)	1 (100%)	
Urinary System						
Kidney	(50)	(21)	(23)	(13)	(22)	(17)
Hydronephrosis	1 (2%)					
Inflammation, multifocal, necrotizing	1 (2%)	1 (5%)				
Nephropathy, multifocal	32 (64%)	11 (52%)	10 (43%)	5 (38%)	9 (41%)	6 (35%)
Thrombosis				1 (8%)		
Glomerulus, inflammation, membranoproliferative					1 (5%)	
Papilla, bacterium		1 (5%)				
Pelvis, renal tubule, bacterium	1 (2%)					
Urinary bladder	(50)	(21)	(20)	(11)	(20)	(16)
Ectasia						1 (6%)
Inflammation, acute		1 (5%)				
Inflammation, chronic		1 (5%)				
Lumen, hemorrhage	1 (2%)					
Mucosa, hyperplasia, papillary		1 (5%)				
Transitional epithelium, hyperplasia, diffuse		1 (2%)				

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration	0 ppm	630 ppm
F_1 Concentration	2,400 ppm	2,400 ppm
Special Senses System		
Ear		(4)
Canal, external ear, exudate, subacute		1 (25%)
Middle ear, inflammation, chronic		1 (25%)
Middle ear, inflammation, chronic active		1 (25%)
Middle ear, inflammation, subacute		1 (25%)
Eye	(8)	(4)
Anterior chamber, inflammation, suppurative	1 (13%)	
Lens crystalline, cataract	6 (75%)	4 (100%)
Posterior chamber, inflammation, chronic	2 (25%)	
Retina, atrophy	6 (75%)	3 (75%)
Sclera, mineralization, multifocal	1 (13%)	
Harderian gland	(7)	(2)
Inflammation, chronic, multifocal	5 (71%)	2 (100%)
Urinary System		
Kidney	(50)	(50)
Infarct		1 (2%)
Inflammation, multifocal, necrotizing		1 (2%)
Nephropathy, multifocal	15 (30%)	15 (30%)
Pelvis, renal tubule, bacterium		1 (2%)
Urinary bladder	(50)	(50)
Inflammation, acute		1 (2%)
Inflammation, chronic	2 (4%)	
Transitional epithelium, hyperplasia, diffuse		1 (2%)
Transitional epithelium, hyperplasia, focal	1 (2%)	

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report because it is more widely used and understood.

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF 5,5-DIPHENYLHYDANTOIN

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 30 ppm	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	6	8	2	3	10	7
Natural deaths	5	5	9	6	7	6
Survivors						
Died last week of study	1	1	1	1		
Terminal sacrifice	38	36	38	39	33	36
Missing				1		1
Animals examined microscopically	50	50	50	49	50	49
Alimentary System						
Gallbladder	(46)	(11)	(8)	(5)	(10)	(11)
Intestine large, rectum	(47)	(13)	(11)	(8)	(15)	(12)
Intestine small, duodenum	(48)	(12)	(9)	(7)	(15)	(10)
Intestine small, ileum	(48)	(11)	(13)	(6)	(14)	(11)
Intestine small, jejunum	(50)	(12)	(17)	(8)	(16)	(10)
Liver	(50)	(50)	(50)	(49)	(50)	(49)
Cholangiocarcinoma			1 (2%)			
Hemangiosarcoma	4 (8%)	1 (2%)		1 (2%)		
Hemangiosarcoma, multiple	1 (2%)	2 (4%)		1 (2%)	1 (2%)	4 (8%)
Hepatoblastoma				1 (2%)	1 (2%)	
Hepatoblastoma, multiple			1 (2%)			
Hepatocellular carcinoma	11 (22%)	10 (20%)	11 (22%)	11 (22%)	14 (28%)	11 (22%)
Hepatocellular carcinoma, multiple	2 (4%)	4 (8%)	2 (4%)	3 (6%)	4 (8%)	7 (14%)
Hepatocellular adenoma	14 (28%)	11 (22%)	10 (20%)	11 (22%)	15 (30%)	10 (20%)
Hepatocellular adenoma, multiple	5 (10%)	12 (24%)	6 (12%)	8 (16%)	5 (10%)	13 (27%)
Hepatocholangiocarcinoma		1 (2%)				
Osteosarcoma, metastatic, uncertain primary site				1 (2%)		
Mesentery	(3)	(2)	(2)		(3)	
Hepatocholangiocarcinoma, metastatic		1 (50%)				
Sarcoma			1 (50%)			
Pancreas	(50)	(13)	(11)	(8)	(17)	(13)
Sarcoma, metastatic, mesentery			1 (9%)			
Salivary glands	(50)	(13)	(12)	(9)	(17)	(13)
Stomach, forestomach	(50)	(16)	(11)	(9)	(15)	(18)
Squamous cell papilloma		2 (13%)			2 (13%)	
Stomach, glandular	(50)	(15)	(9)	(9)	(13)	(16)
Adenoma				1 (11%)		
Tongue					(1)	
Squamous cell carcinoma					1 (100%)	
Cardiovascular System						
Heart	(50)	(14)	(12)	(10)	(17)	(13)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (8%)			
Cholangiocarcinoma, metastatic, liver			1 (8%)			
Hemangiosarcoma	1 (2%)					2 (15%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 300 ppm	210 ppm 300 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	9	4
Natural deaths	7	2
Survivors		
Terminal sacrifice	34	44
Animals examined microscopically	50	50
Alimentary System		
Gallbladder	(46)	(44)
Intestine large, cecum	(47)	(47)
Intestine large, colon	(50)	(48)
Intestine small, duodenum	(48)	(49)
Intestine small, ileum	(46)	(49)
Intestine small, jejunum	(48)	(48)
Liver	(49)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)
Hemangiosarcoma, multiple		2 (4%)
Hepatocellular carcinoma	7 (14%)	18 (36%)
Hepatocellular carcinoma, multiple		2 (4%)
Hepatocellular adenoma	11 (22%)	12 (24%)
Hepatocellular adenoma, multiple	11 (22%)	19 (38%)
Sarcoma, metastatic, uncertain primary site		1 (2%)
Mesentery	(4)	(3)
Sarcoma, metastatic, uncertain primary site		1 (33%)
Pancreas	(49)	(49)
Tooth	(39)	(44)
Cardiovascular System		
Heart	(49)	(50)
Hepatocellular carcinoma, metastatic, liver		1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 30 ppm	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Endocrine System						
Adrenal gland	(50)	(14)	(11)	(9)	(17)	(12)
Corticomedullary junction, hepatocellular carcinoma, metastatic, liver	1 (2%)					
Adrenal gland, cortex	(50)	(14)	(11)	(9)	(17)	(12)
Adenoma		1 (7%)				
Adrenal gland, medulla	(50)	(14)	(11)	(9)	(17)	(11)
Pheochromocytoma benign	1 (2%)					
Islets, pancreatic	(50)	(13)	(10)	(8)	(17)	(12)
Adenoma	1 (2%)					
Pituitary gland	(45)	(11)	(4)	(7)	(14)	(10)
Pars distalis, carcinoma			1 (25%)			
Pars intermedia, carcinoma						1 (10%)
Thyroid gland	(49)	(14)	(11)	(9)	(17)	(13)
Follicular cell, adenoma		1 (7%)	1 (9%)	1 (11%)		
General Body System						
None						
Genital System						
Preputial gland	(5)	(2)	(4)	(8)	(3)	(2)
Adenoma	1 (20%)					
Hemangiosarcoma				1 (13%)		
Prostate	(50)	(14)	(12)	(9)	(17)	(13)
Hematopoietic System						
Bone marrow	(50)	(14)	(11)	(9)	(18)	(13)
Femoral, hemangiosarcoma	2 (4%)					
Lymph node	(50)	(30)	(32)	(23)	(31)	(27)
Deep cervical, carcinoma, metastatic, ear		1 (3%)				
Mandibular, fibrosarcoma, metastatic, skin	1 (2%)					
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung						1 (4%)
Mediastinal, cholangiocarcinoma, metastatic, liver			1 (3%)			
Lymph node, mesenteric	(22)	(22)	(20)	(15)	(20)	(16)
Hemangiosarcoma	1 (5%)			1 (7%)		
Spleen	(49)	(21)	(18)	(17)	(25)	(20)
Hemangiosarcoma	4 (8%)	2 (10%)				3 (15%)
Thymus	(37)	(8)	(7)	(7)	(10)	(7)
Integumentary System						
Skin	(50)	(15)	(13)	(9)	(20)	(14)
Squamous cell carcinoma						1 (7%)
Subcutaneous tissue, fibroma		2 (13%)				
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (7%)		1 (11%)	1 (5%)	1 (7%)
Subcutaneous tissue, lipoma			1 (8%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 300 ppm	210 ppm 300 ppm
Endocrine System		
Adrenal gland	(48)	(49)
Capsule, adenoma	1 (2%)	
Adrenal gland, cortex	(49)	(49)
Adrenal gland, medulla	(45)	(49)
Pheochromocytoma benign	1 (2%)	
General Body System		
None		
Genital System		
Prostate	(50)	(50)
Testes	(50)	(50)
Interstitial cell, adenoma	2 (4%)	
Hematopoietic System		
Bone marrow	(50)	(50)
Femoral, mast cell tumor NOS		1 (2%)
Lymph node	(47)	(50)
Lymph node, mesenteric	(24)	(18)
Sarcoma, metastatic, uncertain primary site		1 (6%)
Spleen	(47)	(49)
Hemangiosarcoma		2 (4%)
Thymus	(41)	(43)
Integumentary System		
Skin	(50)	(50)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)
Subcutaneous tissue, hemangiosarcoma		1 (2%)
Subcutaneous tissue, sarcoma	1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 30 ppm	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Musculoskeletal System						
Bone	(50)	(30)	(40)	(35)	(37)	(30)
Osteosarcoma		1 (3%)				
Skeletal muscle	(1)	(1)	(1)			
Hemangiosarcoma		1 (100%)	1 (100%)			
Nervous System						
Brain	(50)	(14)	(12)	(10)	(18)	(13)
Carcinoma, metastatic, pituitary gland			1 (8%)			1 (8%)
Respiratory System						
Lung	(50)	(19)	(19)	(22)	(20)	(21)
Adenocarcinoma, metastatic, harderian gland					1 (5%)	
Alveolar/bronchiolar adenoma	6 (12%)	8 (42%)	5 (26%)	2 (9%)	3 (15%)	5 (24%)
Alveolar/bronchiolar carcinoma	4 (8%)	3 (16%)	2 (11%)	6 (27%)	1 (5%)	3 (14%)
Alveolar/bronchiolar carcinoma, multiple		2 (11%)	2 (11%)	3 (14%)	1 (5%)	1 (5%)
Carcinoma, metastatic, ear		1 (5%)				
Cholangiocarcinoma, metastatic, liver			1 (5%)			
Hepatocellular carcinoma, metastatic, liver	6 (12%)	2 (11%)	3 (16%)	2 (9%)	4 (20%)	5 (24%)
Hepatocholangiocarcinoma, metastatic		1 (5%)				
Osteosarcoma, metastatic, uncertain primary site				1 (5%)		
Special Senses System						
Ear		(1)				
Basal cell carcinoma		1 (100%)				
Harderian gland	(9)	(2)	(2)	(4)	(3)	(2)
Adenocarcinoma	1 (11%)		1 (50%)		1 (33%)	
Adenoma	5 (56%)	2 (100%)	1 (50%)	4 (100%)	2 (67%)	1 (50%)
Urinary System						
Kidney	(50)	(16)	(12)	(9)	(18)	(13)
Hepatocellular carcinoma, metastatic, liver	1 (2%)					1 (8%)
Osteosarcoma, metastatic, uncertain primary site				1 (11%)		
Sarcoma, metastatic, mesentery			1 (8%)			
Renal tubule, carcinoma					1 (6%)	
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(50)	(49)	(50)	(49)
Lymphoma malignant histiocytic	2 (4%)	2 (4%)	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Lymphoma malignant lymphocytic	5 (10%)				2 (4%)	2 (4%)
Lymphoma malignant mixed	5 (10%)	8 (16%)	5 (10%)	5 (10%)	5 (10%)	3 (6%)
Lymphoma malignant undifferentiated cell		1 (2%)				

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 300 ppm	210 ppm 300 ppm
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(49)	(50)
Adenocarcinoma, metastatic, harderian gland	1 (2%)	
Alveolar/bronchiolar adenoma	4 (8%)	7 (14%)
Alveolar/bronchiolar carcinoma	2 (4%)	4 (8%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	7 (14%)
Sarcoma, metastatic, uncertain primary site		1 (2%)
Mediastinum, hepatocellular carcinoma, metastatic, liver		1 (2%)
Special Senses System		
Harderian gland	(5)	(4)
Adenocarcinoma	1 (20%)	
Adenoma	3 (60%)	3 (75%)
Urinary System		
Kidney	(50)	(50)
Systemic Lesions		
Multiple organs	(50)	(50)
Lymphoma malignant histiocytic	3 (6%)	
Lymphoma malignant lymphocytic	3 (6%)	3 (6%)
Lymphoma malignant mixed	2 (4%)	3 (6%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 30 ppm	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Neoplasm Summary						
Total animals with primary neoplasms[c]	43	43	33	39	45	42
Total primary neoplasms	77	79	53	63	64	70
Total animals with benign neoplasms	25	32	20	22	24	26
Total benign neoplasms	33	39	24	27	27	29
Total animals with malignant neoplasms	29	31	24	31	34	29
Total malignant neoplasms	44	40	29	36	37	41
Total animals with metastatic neoplasms	7	4	7	3	5	6
Total metastatic neoplasms	9	6	10	5	5	8
Total animals with malignant neoplasms of uncertain primary site				1		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 300 ppm	210 ppm 300 ppm
Neoplasm Summary		
Total animals with primary neoplasms	36	45
Total primary neoplasms	53	79
Total animals with benign neoplasms	26	36
Total benign neoplasms	33	41
Total animals with malignant neoplasms	16	26
Total malignant neoplasms	20	37
Total animals with metastatic neoplasms	2	9
Total metastatic neoplasm	2	13
Total animals with malignant neoplasms uncertain primary site		1
Total animals with neoplasms uncertain benign or malignant		1
Total uncertain neoplasms		1

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:100, and 0:300 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 100 ppm	0 ppm 300 ppm
Harderian Gland: Adenoma			
Overall rate[a]	5/50 (10%)	4/49 (8%)	3/50 (6%)
Adjusted rate[b]	12.8%	10.0%	8.8%
Terminal rate[c]	5/39 (13%)	4/40 (10%)	3/34 (9%)
First incidence (days)	734 (T)	734 (T)	734 (T)
Life table test[d]	P=0.394N	P=0.484N	P=0.433N
Logistic regression test[d]	P=0.394N	P=0.484N	P=0.433N
Cochran-Armitage test[d]	P=0.313N		
Fisher exact test[d]		P=0.513N	P=0.357N
Harderian Gland: Adenoma or Carcinoma			
Overall rate	6/50 (12%)	4/49 (8%)	4/50 (8%)
Adjusted rate	15.4%	10.0%	11.4%
Terminal rate	6/39 (15%)	4/40 (10%)	3/34 (9%)
First incidence (days)	734 (T)	734 (T)	723
Life table test	P=0.444N	P=0.352N	P=0.456N
Logistic regression test	P=0.426N	P=0.352N	P=0.440N
Cochran-Armitage test	P=0.352N		
Fisher exact test		P=0.383N	P=0.370N
Liver: Hemangiosarcoma			
Overall rate	5/50 (10%)	2/49 (4%)	1/49 (2%)
Adjusted rate	12.2%	4.8%	2.9%
Terminal rate	4/39 (10%)	1/40 (3%)	1/34 (3%)
First incidence (days)	650	639	734 (T)
Life table test	P=0.118N	P=0.224N	P=0.141N
Logistic regression test	P=0.111N	P=0.226N	P=0.139N
Cochran-Armitage test	P=0.090N		
Fisher exact test		P=0.226N	P=0.107N
Liver: Hepatocellular Adenoma			
Overall rate	19/50 (38%)	19/49 (39%)	22/49 (45%)
Adjusted rate	43.7%	45.1%	59.3%
Terminal rate	15/39 (38%)	17/40 (43%)	19/34 (56%)
First incidence (days)	540	589	612
Life table test	P=0.123	P=0.560N	P=0.167
Logistic regression test	P=0.110	P=0.522	P=0.139
Cochran-Armitage test	P=0.271		
Fisher exact test		P=0.551	P=0.311
Liver: Hepatocellular Carcinoma			
Overall rate	13/50 (26%)	14/49 (29%)	7/49 (14%)
Adjusted rate	28.8%	33.2%	19.1%
Terminal rate	8/39 (21%)	12/40 (30%)	5/34 (15%)
First incidence (days)	463	590	612
Life table test	P=0.151N	P=0.507	P=0.198N
Logistic regression test	P=0.123N	P=0.479	P=0.154N
Cochran-Armitage test	P=0.079N		
Fisher exact test		P=0.475	P=0.115N

TABLE C2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:100, and 0:300 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 100 ppm	0 ppm 300 ppm
Liver: Hepatoblastoma or Hepatocellular Carcinoma			
Overall rate	13/50 (26%)	15/49 (31%)	7/49 (14%)
Adjusted rate	28.8%	35.6%	19.1%
Terminal rate	8/39 (21%)	13/40 (33%)	5/34 (15%)
First incidence (days)	463	590	612
Life table test	P=0.144N	P=0.424	P=0.198N
Logistic regression test	P=0.118N	P=0.388	P=0.154N
Cochran-Armitage test	P=0.074N		
Fisher exact test		P=0.387	P=0.115N
Liver: Hepatoblastoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma			
Overall rate	29/50 (58%)	29/49 (59%)	26/49 (53%)
Adjusted rate	60.1%	65.8%	68.3%
Terminal rate	20/39 (51%)	25/40 (63%)	22/34 (65%)
First incidence (days)	463	589	612
Life table test	P=0.478	P=0.554N	P=0.509
Logistic regression test	P=0.477	P=0.517	P=0.508
Cochran-Armitage test	P=0.333N		
Fisher exact test		P=0.534	P=0.385N
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	6/50 (12%)	2/22 (9%)[e]	4/49 (8%)
Adjusted rate	14.7%		11.8%
Terminal rate	5/39 (13%)		4/34 (12%)
First incidence (days)	639		734 (T)
Life table test			P=0.461N
Logistic regression test			P=0.464N
Cochran-Armitage test			
Fisher exact test			P=0.383N
Lung: Alveolar/bronchiolar Carcinoma			
Overall rate	4/50 (8%)	9/22 (41%)[e]	2/49 (4%)
Adjusted rate	10.3%		5.9%
Terminal rate	4/39 (10%)		2/34 (6%)
First incidence (days)	734 (T)		734 (T)
Life table test			P=0.401N
Logistic regression test			P=0.401N
Cochran-Armitage test			
Fisher exact test			P=0.349N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	10/50 (20%)	11/22 (50%)[e]	6/49 (12%)
Adjusted rate	24.7%		17.6%
Terminal rate	9/39 (23%)		6/34 (18%)
First incidence (days)	639		734 (T)
Life table test			P=0.301N
Logistic regression test			P=0.297N
Cochran-Armitage test			
Fisher exact test			P=0.220N

TABLE C2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:100, and 0:300 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 100 ppm	0 ppm 300 ppm
Spleen: Hemangiosarcoma			
Overall rate	4/49 (8%)	0/17 (0%)[e]	0/47 (0%)
Adjusted rate	9.7%		0.0%
Terminal rate	3/39 (8%)		0/33 (0%)
First incidence (days)	650		-[f]
Life table test			P=0.089N
Logistic regression test			P=0.082N
Cochran-Armitage test			
Fisher exact test			P=0.064N
All Organs: Hemangiosarcoma			
Overall rate	8/50 (16%)	3/49 (6%)	1/50 (2%)
Adjusted rate	19.7%	7.2%	2.9%
Terminal rate	7/39 (18%)	2/40 (5%)	1/34 (3%)
First incidence (days)	650	639	734 (T)
Life table test	P=0.024N	P=0.102N	P=0.031N
Logistic regression test	P=0.023N	P=0.112N	P=0.030N
Cochran-Armitage test	P=0.015N		
Fisher exact test		P=0.106N	P=0.015N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, or Mixed)			
Overall rate	12/50 (24%)	7/49 (14%)	8/50 (16%)
Adjusted rate	27.6%	16.3%	20.6%
Terminal rate	8/39 (21%)	5/40 (13%)	4/34 (12%)
First incidence (days)	609	412	408
Life table tests	P=0.372N	P=0.163N	P=0.348N
Logistic regression tests	P=0.288N	P=0.151N	P=0.302N
Cochran-Armitage test	P=0.245N		
Fisher exact test		P=0.166N	P=0.227N
All Organs: Benign Neoplasms			
Overall rate	25/50 (50%)	22/49 (45%)	26/50 (52%)
Adjusted rate	56.4%	51.0%	70.1%
Terminal rate	20/39 (51%)	19/40 (48%)	23/34 (68%)
First incidence (days)	540	589	612
Life table tests	P=0.170	P=0.333N	P=0.241
Logistic regression tests	P=0.148	P=0.413N	P=0.201
Cochran-Armitage test	P=0.422		
Fisher exact test		P=0.380N	P=0.500
All Organs: Malignant Neoplasms			
Overall rate	29/50 (58%)	32/49 (65%)	16/50 (32%)
Adjusted rate	61.5%	68.1%	39.8%
Terminal rate	21/39 (54%)	25/40 (63%)	10/34 (29%)
First incidence (days)	463	412	408
Life table tests	P=0.033N	P=0.382	P=0.058N
Logistic regression tests	P=0.009N	P=0.292	P=0.026N
Cochran-Armitage test	P=0.002N		
Fisher exact test		P=0.295	P=0.008N

TABLE C2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 0:100, and 0:300 ppm Groups (continued)

F_0 Concentration	0 ppm	0 ppm	0 ppm
F_1 Concentration	0 ppm	100 ppm	300 ppm
All Organs: Benign or Malignant Neoplasms			
Overall rate	43/50 (86%)	39/49 (80%)	36/50 (72%)
Adjusted rate	87.7%	83.0%	87.8%
Terminal rate	33/39 (85%)	32/40 (80%)	29/34 (85%)
First incidence (days)	463	412	408
Life table tests	P=0.487N	P=0.262N	P=0.463N
Logistic regression tests	P=0.479N	P=0.315N	P=0.495N
Cochran-Armitage test	P=0.061N		
Fisher exact test		P=0.282N	P=0.070N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, gallbladder, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not applicable.
[f] Not applicable; no neoplasms in animal group

TABLE C2b
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0 and 210:0 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm
Harderian Gland: Adenoma		
Overall rate[a]	5/50 (10%)	2/50 (4%)
Adjusted rate[b]	12.8%	5.4%
Terminal rate[c]	5/39 (13%)	2/37 (5%)
First incidence (days)	734 (T)	734 (T)
Life table test[d]		P=0.237N
Logistic regression test[d]		P=0.237N
Fisher exact test[d]		P=0.218N
Harderian Gland: Adenoma or Carcinoma		
Overall rate	6/50 (12%)	2/50 (4%)
Adjusted rate	15.4%	5.4%
Terminal rate	6/39 (15%)	2/37 (5%)
First incidence (days)	734 (T)	734 (T)
Life table test		P=0.150N
Logistic regression test		P=0.150N
Fisher exact test		P=0.134N
Liver: Hemangiosarcoma		
Overall rate	5/50 (10%)	3/50 (6%)
Adjusted rate	12.2%	7.6%
Terminal rate	4/39 (10%)	2/37 (5%)
First incidence (days)	650	642
Life table test		P=0.393N
Logistic regression test		P=0.358N
Fisher exact test		P=0.357N
Liver: Hepatocellular Adenoma		
Overall rate	19/50 (38%)	23/50 (46%)
Adjusted rate	43.7%	55.8%
Terminal rate	15/39 (38%)	19/37 (51%)
First incidence (days)	540	633
Life table test		P=0.216
Logistic regression test		P=0.253
Fisher exact test		P=0.272
Liver: Hepatocellular Carcinoma		
Overall rate	13/50 (26%)	14/50 (28%)
Adjusted rate	28.8%	32.6%
Terminal rate	8/39 (21%)	9/37 (24%)
First incidence (days)	463	575
Life table test		P=0.450
Logistic regression test		P=0.526
Fisher exact test		P=0.500

TABLE C2b
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0 and 210:0 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	29/50 (58%)	33/50 (66%)
Adjusted rate	60.1%	71.6%
Terminal rate	20/39 (51%)	24/37 (65%)
First incidence (days)	463	575
Life table test		P=0.228
Logistic regression test		P=0.281
Fisher exact test		P=0.268
Lung: Alveolar/bronchiolar Adenoma		
Overall rate	6/50 (12%)	8/19 (42%)
Adjusted rate	14.7%	55.6%
Terminal rate	5/39 (13%)	3/6 (50%)
First incidence (days)	639	491
Life table test		P=0.011
Logistic regression test		P=0.007
Fisher exact test		P=0.009
Lung: Alveolar/bronchiolar Carcinoma		
Overall rate	4/50 (8%)	5/19 (26%)
Adjusted rate	10.3%	52.2%
Terminal rate	4/39 (10%)	3/6 (50%)
First incidence (days)	734 (T)	633
Life table test		P=0.009
Logistic regression test		P=0.018
Fisher exact test		P=0.058
Lung: Alveolar/bronchiolar Adenoma or Carcinoma		
Overall rate	10/50 (20%)	12/19 (63%)
Adjusted rate	24.7%	85.8%
Terminal rate	9/39 (23%)	5/6 (83%)
First incidence (days)	639	491
Life table test		P<0.001
Logistic regression test		P<0.001
Fisher exact test		P=0.001
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma		
Overall rate	1/50 (2%)	3/50 (6%)
Adjusted rate	2.3%	7.8%
Terminal rate	0/39 (0%)	2/37 (5%)
First incidence (days)	673	696
Life table test		P=0.293
Logistic regression test		P=0.149
Fisher exact test		P=0.309

TABLE C2b
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0 and 210:0 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm
Spleen: Hemangiosarcoma		
Overall rate	4/49 (8%)	2/21 (10%)
Adjusted rate	9.7%	4.4%
Terminal rate	3/39 (8%)	0/8 (0%)
First incidence (days)	650	633
Life table test		P=0.666
Logistic regression test		P=0.670
Fisher exact test		P=0.588
Thyroid Gland (Follicular Cell): Adenoma		
Overall rate	0/49 (0%)	1/14 (7%)
Adjusted rate	0.0%	2.4%
Terminal rate	0/39 (0%)	0/1 (0%)
First incidence (days)	-[e]	646
Life table test		P=0.482
Logistic regression test		P=0.357
Fisher exact test		P=0.222
All Organs: Hemangiosarcoma		
Overall rate	8/50 (16%)	4/50 (8%)
Adjusted rate	19.7%	9.6%
Terminal rate	7/39 (18%)	2/37 (5%)
First incidence (days)	650	633
Life table test		P=0.211N
Logistic regression test		P=0.179N
Fisher exact test		P=0.178N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)		
Overall rate	12/50 (24%)	11/50 (22%)
Adjusted rate	27.6%	24.7%
Terminal rate	8/39 (21%)	5/37 (14%)
First incidence (days)	609	576
Life table test		P=0.548N
Logistic regression test		P=0.478N
Fisher exact test		P=0.500N
All Organs: Benign Neoplasms		
Overall rate	25/50 (50%)	32/50 (64%)
Adjusted rate	56.4%	68.0%
Terminal rate	20/39 (51%)	22/37 (59%)
First incidence (days)	540	491
Life table test		P=0.103
Logistic regression test		P=0.115
Fisher exact test		P=0.113

TABLE C2b
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0 and 210:0 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm
All Organs: Malignant Neoplasms		
Overall rate	29/50 (58%)	31/50 (62%)
Adjusted rate	61.5%	62.0%
Terminal rate	21/39 (54%)	18/37 (49%)
First incidence (days)	463	491
Life table test		P=0.350
Logistic regression test		P=0.466
Fisher exact test		P=0.419
All Organs: Benign or Malignant Neoplasms		
Overall rate	43/50 (86%)	43/50 (86%)
Adjusted rate	87.7%	86.0%
Terminal rate	33/39 (85%)	30/37 (81%)
First incidence (days)	463	491
Life table test		P=0.424
Logistic regression test		P=0.589N
Fisher exact test		P=0.613N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, gallbladder, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE C2c
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 21:30, 70:100, 210:100, and 210:300 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	21 ppm 30 ppm	70 ppm 100 ppm	210 ppm 100 ppm	210 ppm 300 ppm
Harderian Gland: Adenoma					
Overall rate[a]	5/50 (10%)	1/50 (2%)	2/50 (4%)	1/49 (2%)	3/50 (6%)
Adjusted rate[b]	12.8%	2.6%	6.1%	2.8%	6.8%
Terminal rate[c]	5/39 (13%)	1/39 (3%)	2/33 (6%)	1/36 (3%)	3/44 (7%)
First incidence (days)	734 (T)	734 (T)	734 (T)	734 (T)	734 (T)
Life table test[d]	P=0.318N	P=0.103N	P=0.287N	P=0.121N	P=0.292N
Logistic regression test[d]	P=0.318N	P=0.103N	P=0.287N	P=0.121N	P=0.292N
Cochran-Armitage test[d]	P=0.366N				
Fisher exact test[d]		P=0.102N	P=0.218N	P=0.107N	P=0.357N
Harderian Gland: Adenoma or Carcinoma					
Overall rate	6/50 (12%)	2/50 (4%)	3/50 (6%)	1/49 (2%)	3/50 (6%)
Adjusted rate	15.4%	5.1%	8.3%	2.8%	6.8%
Terminal rate	6/39 (15%)	2/39 (5%)	2/33 (6%)	1/36 (3%)	3/44 (7%)
First incidence (days)	734 (T)	734 (T)	645	734 (T)	734 (T)
Life table test	P=0.152N	P=0.133N	P=0.326N	P=0.071N	P=0.186N
Logistic regression test	P=0.152N	P=0.133N	P=0.283N	P=0.071N	P=0.186N
Cochran-Armitage test	P=0.184N				
Fisher exact test		P=0.134N	P=0.243N	P=0.059N	P=0.243N
Heart: Hemangiosarcoma					
Overall rate	1/50 (2%)	0/12 (0%)[e]	0/17 (0%)[e]	2/13 (15%)[e]	0/50 (0%)
Adjusted rate	2.6%				0.0%
Terminal rate	1/39 (3%)				0/44 (0%)
First incidence (days)	734 (T)				–[f]
Life table test					P=0.476N
Logistic regression test					P=0.476N
Cochran-Armitage test					
Fisher exact test					P=0.500N
Liver: Hemangiosarcoma					
Overall rate	5/50 (10%)	0/50 (0%)	1/50 (2%)	4/49 (8%)	3/50 (6%)
Adjusted rate	12.2%	0.0%	3.0%	9.6%	6.6%
Terminal rate	4/39 (10%)	0/39 (0%)	1/33 (3%)	1/36 (3%)	2/44 (5%)
First incidence (days)	650	–	734 (T)	633	710
Life table test	P=0.444	P=0.036N	P=0.146N	P=0.539N	P=0.298N
Logistic regression test	P=0.382	P=0.035N	P=0.118N	P=0.520N	P=0.347N
Cochran-Armitage test	P=0.383				
Fisher exact test		P=0.028N	P=0.102N	P=0.513N	P=0.357N
Liver: Hepatocellular Adenoma					
Overall rate	19/50 (38%)	16/50 (32%)	20/50 (40%)	23/49 (47%)	31/50 (62%)
Adjusted rate	43.7%	38.0%	50.1%	58.5%	65.9%
Terminal rate	15/39 (38%)	13/39 (33%)	14/33 (42%)	20/36 (56%)	28/44 (64%)
First incidence (days)	540	584	535	585	678
Life table test	P=0.011	P=0.357N	P=0.297	P=0.184	P=0.063
Logistic regression test	P=0.003	P=0.363N	P=0.471	P=0.241	P=0.020
Cochran-Armitage test	P=0.001				
Fisher exact test		P=0.338N	P=0.500	P=0.243	P=0.014

TABLE C2c
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 21:30, 70:100, 210:100, and 210:300 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	21 ppm 30 ppm	70 ppm 100 ppm	210 ppm 100 ppm	210 ppm 300 ppm
Liver: Hepatocellular Carcinoma					
Overall rate	13/50 (26%)	13/50 (26%)	18/50 (36%)	18/49 (37%)	20/50 (40%)
Adjusted rate	28.8%	28.4%	41.8%	41.0%	41.5%
Terminal rate	8/39 (21%)	7/39 (18%)	9/33 (27%)	11/36 (31%)	16/44 (36%)
First incidence (days)	463	495	481	585	564
Life table test	P=0.125	P=0.569	P=0.126	P=0.167	P=0.205
Logistic regression test	P=0.017	P=0.507N	P=0.255	P=0.169	P=0.045
Cochran-Armitage test	P=0.041				
Fisher exact test		P=0.590N	P=0.194	P=0.175	P=0.101
Liver: Hepatoblastoma or Hepatocellular Carcinoma					
Overall rate	13/50 (26%)	13/50 (26%)	18/50 (36%)	18/49 (37%)	20/50 (40%)
Adjusted rate	28.8%	28.4%	41.8%	41.0%	41.5%
Terminal rate	8/39 (21%)	7/39 (18%)	9/33 (27%)	11/36 (31%)	16/44 (36%)
First incidence (days)	463	495	481	585	564
Life table test	P=0.125	P=0.569	P=0.126	P=0.167	P=0.205
Logistic regression test	P=0.017	P=0.507N	P=0.255	P=0.169	P=0.045
Cochran-Armitage test	P=0.041				
Fisher exact test		P=0.590N	P=0.194	P=0.175	P=0.101
Liver: Hepatoblastoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma					
Overall rate	29/50 (58%)	25/50 (50%)	31/50 (62%)	35/49 (71%)	41/50 (82%)
Adjusted rate	60.1%	53.0%	68.3%	79.2%	82.0%
Terminal rate	20/39 (51%)	17/39 (44%)	19/33 (58%)	27/36 (75%)	35/44 (80%)
First incidence (days)	463	495	481	585	564
Life table test	P=0.021	P=0.330N	P=0.208	P=0.110	P=0.111
Logistic regression test	P<0.001	P=0.228N	P=0.475	P=0.118	P=0.003
Cochran-Armitage test	P<0.001				
Fisher exact test		P=0.274N	P=0.419	P=0.117	P=0.008
Lung: Alveolar/bronchiolar Adenoma					
Overall rate	6/50 (12%)	5/19 (26%)[e]	3/20 (15%)[e]	5/21 (24%)[e]	7/50 (14%)
Adjusted rate	14.7%				15.9%
Terminal rate	5/39 (13%)				7/44 (16%)
First incidence (days)	639				734 (T)
Life table test					P=0.587
Logistic regression test					P=0.550
Cochran-Armitage test					
Fisher exact test					P=0.500
Lung: Alveolar/bronchiolar Carcinoma					
Overall rate	4/50 (8%)	4/19 (21%)[e]	2/20 (10%)[e]	4/21 (19%)[e]	4/50 (8%)
Adjusted rate	10.3%				9.1%
Terminal rate	4/39 (10%)				4/44 (9%)
First incidence (days)	734 (T)				734 (T)
Life table test					P=0.576N
Logistic regression test					P=0.576N
Cochran-Armitage test					
Fisher exact test					P=0.643N

TABLE C2c
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 21:30, 70:100, 210:100, and 210:300 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	21 ppm 30 ppm	70 ppm 100 ppm	210 ppm 100 ppm	210 ppm 300 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate	10/50 (20%)	8/19 (42%)[e]	5/20 (25%)[e]	7/21 (33%)[e]	11/50 (22%)
Adjusted rate	24.7%				25.0%
Terminal rate	9/39 (23%)				11/44 (25%)
First incidence (days)	639				734 (T)
Life table test					P=0.576N
Logistic regression test					P=0.595
Cochran-Armitage test					
Fisher exact test					P=0.500
Pituitary Gland (Pars Distalis): Carcinoma					
Overall rate	0/45 (0%)	1/4 (25%)[e]	0/14 (0%)[e]	0/10 (0%)[e]	0/34 (0%)[e]
Adjusted rate	0.0%				
Terminal rate	0/34 (0%)				
First incidence (days)	–				
Life table test					
Logistic regression test					
Cochran-Armitage test					
Fisher exact test					
Spleen: Hemangiosarcoma					
Overall rate	4/49 (8%)	0/18 (0%)[e]	0/25 (0%)[e]	3/20 (15%)[e]	2/49 (4%)
djusted rate	9.7%				4.5%
Terminal rate	3/39 (8%)				2/44 (5%)
First incidence (days)	650				734 (T)
Life table test					P=0.290N
Logistic regression test					P=0.333N
Cochran-Armitage test					
Fisher exact test					P=0.339N
Thyroid Gland (Follicular Cell): Adenoma					
Overall rate	0/49 (0%)	1/11 (9%)[e]	0/17 (0%)[e]	0/13 (0%)[e]	0/50 (0%)
Adjusted rate	0.0%				0.0%
Terminal rate	0/39 (0%)				0/44 (0%)
First incidence (days)	–				–
Life table test					–
Logistic regression test					–
Cochran-Armitage test					
Fisher exact test					–
All Organs: Hemangiosarcoma					
Overall rate	8/50 (16%)	1/50 (2%)	1/50 (2%)	5/49 (10%)	3/50 (6%)
Adjusted rate	19.7%	2.6%	3.0%	12.2%	6.6%
Terminal rate	7/39 (18%)	1/39 (3%)	1/33 (3%)	2/36 (6%)	2/44 (5%)
First incidence (days)	650	734 (T)	734 (T)	633	710
Life table test	P=0.290N	P=0.019N	P=0.033N	P=0.327N	P=0.072N
Logistic regression test	P=0.323N	P=0.020N	P=0.024N	P=0.289N	P=0.086N
Cochran-Armitage test	P=0.344N				
Fisher exact test		P=0.015N	P=0.015N	P=0.290N	P=0.100N

TABLE C2c
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 21:30, 70:100, 210:100, and 210:300 ppm Groups (continued)

F_0 Concentration	0 ppm	21 ppm	70 ppm	210 ppm	210 ppm
F_1 Concentration	0 ppm	30 ppm	100 ppm	100 ppm	300 ppm
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, or Mixed)					
Overall rate	12/50 (24%)	6/50 (12%)	11/50 (22%)	7/49 (14%)	6/50 (12%)
Adjusted rate	27.6%	14.9%	29.0%	17.4%	13.3%
Terminal rate	8/39 (21%)	5/39 (13%)	7/33 (21%)	4/36 (11%)	5/44 (11%)
First incidence (days)	609	669	632	632	718
Life table test	P=0.091N	P=0.112N	P=0.531	P=0.209N	P=0.066N
Logistic regression test	P=0.098N	P=0.102N	P=0.534N	P=0.166N	P=0.099N
Cochran-Armitage test	P=0.127N				
Fisher exact test		P=0.096N	P=0.500N	P=0.166N	P=0.096N
All Organs: Benign Neoplasms					
Overall rate	25/50 (50%)	20/50 (40%)	24/50 (48%)	26/49 (53%)	37/50 (74%)
Adjusted rate	56.4%	46.3%	59.0%	64.7%	78.7%
Terminal rate	20/39 (51%)	16/39 (41%)	17/33 (52%)	22/36 (61%)	34/44 (77%)
First incidence (days)	540	574	535	585	678
Life table test	P=0.015	P=0.241N	P=0.388	P=0.361	P=0.077
Logistic regression test	P=0.003	P=0.229N	P=0.545N	P=0.458	P=0.019
Cochran-Armitage test	P=0.001				
Fisher exact test		P=0.211N	P=0.500N	P=0.459	P=0.011
All Organs: Malignant Neoplasms					
Overall rate	29/50 (58%)	25/50 (50%)	34/50 (68%)	29/49 (59%)	27/50 (54%)
Adjusted rate	61.5%	53.0%	70.8%	60.2%	54.0%
Terminal rate	21/39 (54%)	17/39 (44%)	19/33 (58%)	17/36 (47%)	21/44 (48%)
First incidence (days)	463	495	481	585	564
Life table test	P=0.291N	P=0.324N	P=0.095	P=0.441	P=0.241N
Logistic regression test	P=0.443	P=0.243N	P=0.217	P=0.558	P=0.526N
Cochran-Armitage test	P=0.499N				
Fisher exact test		P=0.274N	P=0.204	P=0.534	P=0.420N
All Organs: Benign or Malignant Neoplasms					
Overall rate	43/50 (86%)	33/50 (66%)	45/50 (90%)	42/49 (86%)	45/50 (90%)
Adjusted rate	87.7%	68.6%	91.8%	85.7%	90.0%
Terminal rate	33/39 (85%)	24/39 (62%)	29/33 (88%)	29/36 (81%)	39/44 (89%)
First incidence (days)	463	495	481	585	564
Life table test	P=0.388	P=0.071N	P=0.108	P=0.436	P=0.347N
Logistic regression test	P=0.030	P=0.015N	P=0.340	P=0.577N	P=0.313
Cochran-Armitage test	P=0.033				
Fisher exact test		P=0.017N	P=0.380	P=0.597N	P=0.380

TABLE C2c
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of 0:0, 21:30, 70:100, 210:100, and 210:300 ppm Groups (continued)

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not applicable.
[f] Not applicable; no neoplasms in animal group

TABLE C2d
Statistical Analysis of Liver Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:300 and 210:300 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 300 ppm	210 ppm 300 ppm
Liver: Hemangiosarcoma		
Overall rate[a]	1/49 (2%)	3/50 (6%)
Adjusted rate[b]	2.9%	6.6%
Terminal rate[c]	1/34 (3%)	2/44 (5%)
First incidence (days)	734 (T)	710
Life table test[d]		P=0.401
Logistic regression test[d]		P=0.383
Fisher exact test[d]		P=0.316
Liver: Hepatocellular Adenoma		
Overall rate	22/49 (45%)	31/50 (62%)
Adjusted rate	59.3%	65.9%
Terminal rate	19/34 (56%)	28/44 (64%)
First incidence (days)	612	678
Life table test		P=0.397
Logistic regression test		P=0.294
Fisher exact test		P=0.066
Liver: Hepatocellular Carcinoma		
Overall rate	7/49 (14%)	20/50 (40%)
Adjusted rate	19.1%	41.5%
Terminal rate	5/34 (15%)	16/44 (36%)
First incidence (days)	612	564
Life table test		P=0.030
Logistic regression test		P=0.012
Fisher exact test		P=0.004
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	26/49 (53%)	41/50 (82%)
Adjusted rate	68.3%	82.0%
Terminal rate	22/34 (65%)	35/44 (80%)
First incidence (days)	612	564
Life table test		P=0.134
Logistic regression test		P=0.035
Fisher exact test		P=0.002

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates.

TABLE C2e
Statistical Analysis of Liver Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:100, 70:100, and 210:100 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Liver: Hemangiosarcoma			
Overall rate[a]	2/49 (4%)	1/50 (2%)	4/49 (8%)
Adjusted rate[b]	4.8%	3.0%	9.6%
Terminal rate[c]	1/40 (3%)	1/33 (3%)	1/36 (3%)
First incidence (days)	639	734 (T)	633
Life table test[d]	P=0.197	P=0.544N	P=0.324
Logistic regression test[d]	P=0.182	P=0.496N	P=0.622
Cochran-Armitage test[d]	P=0.196		
Fisher exact test[d]		P=0.492N	P=0.339
Liver: Hepatocellular Adenoma			
Overall rate	19/49 (39%)	20/50 (40%)	23/49 (47%)
Adjusted rate	45.1%	50.1%	58.5%
Terminal rate	17/40 (43%)	14/33 (42%)	20/36 (56%)
First incidence (days)	589	535	585
Life table test P=0.185	P=0.277	P=0.159	
Logistic regression test	P=0.268	P=0.502	P=0.291
Cochran-Armitage test	P=0.232		
Fisher exact test		P=0.532	P=0.270
Liver: Hepatocellular Carcinoma			
Overall rate	14/49 (29%)	18/50 (36%)	18/49 (37%)
Adjusted rate	33.2%	41.8%	41.0%
Terminal rate	12/40 (30%)	9/33 (27%)	11/36 (31%)
First incidence (days)	590	481	585
Life table test	P=0.264	P=0.151	P=0.202
Logistic regression test	P=0.266	P=0.280	P=0.264
Cochran-Armitage test	P=0.276		
Fisher exact test		P=0.283	P=0.259
Liver: Hepatoblastoma or Hepatocellular Carcinoma			
Overall rate	15/49 (31%)	18/50 (36%)	18/49 (37%)
Adjusted rate	35.6%	41.8%	41.0%
Terminal rate	13/40 (33%)	9/33 (27%)	11/36 (31%)
First incidence (days)	590	481	585
Life table test	P=0.318	P=0.199	P=0.261
Logistic regression test	P=0.331	P=0.357	P=0.343
Cochran-Armitage test	P=0.340		
Fisher exact test		P=0.361	P=0.335
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	29/49 (59%)	31/50 (62%)	35/49 (71%)
Adjusted rate	65.8%	68.3%	79.2%
Terminal rate	25/40 (63%)	19/33 (58%)	27/36 (75%)
First incidence (days)	589	481	585
Life table test	P=0.115	P=0.173	P=0.075
Logistic regression test	P=0.141	P=0.446	P=0.178
Cochran-Armitage test	P=0.119		
Fisher exact test		P=0.468	P=0.144

TABLE C2e
Statistical Analysis of Liver Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:100, 70:100, and 210:100 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Liver: Hepatoblastoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma			
Overall rate	29/49 (59%)	31/50 (62%)	35/49 (71%)
Adjusted rate	65.8%	68.3%	79.2%
Terminal rate	25/40 (63%)	19/33 (58%)	27/36 (75%)
First incidence (days)	589	481	585
Life table test	P=0.115	P=0.173	P=0.075
Logistic regression test	P=0.141	P=0.446	P=0.178
Cochran-Armitage test	P=0.119		
Fisher exact test		P=0.468	P=0.144

[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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.

TABLE C2f
Statistical Analysis of Liver Neoplasms in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 210:0, 210:100, and 210:300 ppm Groups

F_0 Concentration F_1 Concentration	210 ppm 0 ppm	210 ppm 100 ppm	210 ppm 300 ppm
Liver: Hemangiosarcoma			
Overall rate[a]	3/50 (6%)	4/49 (8%)	3/50 (6%)
Adjusted rate[b]	7.6%	9.6%	6.6%
Terminal rate[c]	2/37 (5%)	1/36 (3%)	2/44 (5%)
First incidence (days)	642	633	710
Life table test[d]	P=0.477N	P=0.494	P=0.585N
Logistic regression test[d]	P=0.566	P=0.456	P=0.658
Cochran-Armitage test[d]	P=0.567N		
Fisher exact test[d]		P=0.489	P=0.661N
Liver: Hepatocellular Adenoma			
Overall rate	23/50 (46%)	23/49 (47%)	31/50 (62%)
Adjusted rate	55.8%	58.5%	65.9%
Terminal rate	19/37 (51%)	20/36 (56%)	28/44 (64%)
First incidence (days)	633	585	678
Life table test P=0.278	P=0.539	P=0.318	
Logistic regression test	P=0.127	P=0.575	P=0.167
Cochran-Armitage test	P=0.056		
Fisher exact test		P=0.543	P=0.080
Liver: Hepatocellular Carcinoma			
Overall rate	14/50 (28%)	18/49 (37%)	20/50 (40%)
Adjusted rate	32.6%	41.0%	41.5%
Terminal rate	9/37 (24%)	11/36 (31%)	16/44 (36%)
First incidence (days)	575	585	564
Life table test	P=0.354	P=0.262	P=0.319
Logistic regression test	P=0.083	P=0.214	P=0.099
Cochran-Armitage test	P=0.150		
Fisher exact test		P=0.238	P=0.146
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	33/50 (66%)	35/49 (71%)	41/50 (82%)
Adjusted rate	71.6%	79.2%	82.0%
Terminal rate	24/37 (65%)	27/36 (75%)	35/44 (80%)
First incidence (days)	575	585	564
Life table test	P=0.437	P=0.382	P=0.424
Logistic regression test	P=0.055	P=0.372	P=0.064
Cochran-Armitage test	P=0.046		
Fisher exact test		P=0.358	P=0.055

[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

TABLE C3
Historical Incidence of Hepatocellular Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
2,4-Dichlorophenol	4/50	7/50	10/50
5,5-Diphenylhydantoin	19/50	13/50	29/50
Ethylene thiourea	11/49	13/49	20/49
Polybrominated biphenyls (Firemaster FF-1®)	9/50	8/50	16/50
Manganese sulfate monohydrate	30/50	9/50	34/50
Pentachlorophenol (Dowicide EC-7)	5/35	1/35	6/35
Pentachlorophenol (technical grade)	5/32	2/32	7/32
Triamterene	17/50	5/50	20/50
Triamterene	21/50	9/50	25/50
Overall Historical Incidence			
Total	226/1,114 (20.3%)	169/1,114 (15.2%)	363/1,114 (32.6%)
Standard deviation	13.2%	7.1%	13.6%
Range	4%–60%	3%–27%	10%–68%

[a] Data as of 17 December 1991

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 30 ppm	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	6	8	2	3	10	7
Natural deaths	5	5	9	6	7	6
Survivors						
Died last week of study	1	1	1	1		
Terminal sacrifice	38	36	38	39	33	36
Missing				1		1
Animals examined microscopically	50	50	50	49	50	49
Alimentary System						
Gallbladder	(46)	(11)	(8)	(5)	(10)	(11)
Inflammation, suppurative			1 (13%)			
Intestine large, cecum	(49)	(13)	(11)	(7)	(16)	(12)
Parasite metazoan	1 (2%)					
Intestine large, colon	(49)	(13)	(12)	(7)	(15)	(13)
Parasite metazoan	1 (2%)	1 (8%)				
Intestine large, rectum	(47)	(13)	(11)	(8)	(15)	(12)
Parasite metazoan	1 (2%)				1 (7%)	
Prolapse				1 (13%)		
Intestine small, duodenum	(48)	(12)	(9)	(7)	(15)	(10)
Hyperplasia, atypical					1 (7%)	
Inflammation, acute, necrotizing					1 (7%)	
Inflammation, chronic active				1 (14%)		
Intestine small, jejunum	(50)	(12)	(17)	(8)	(16)	(10)
Inflammation, acute, necrotizing	2 (4%)		1 (6%)		1 (6%)	
Inflammation, chronic active			1 (6%)			
Liver	(50)	(50)	(50)	(49)	(50)	(49)
Angiectasis		1 (2%)				
Basophilic focus	4 (8%)	3 (6%)	5 (10%)	6 (12%)	1 (2%)	
Clear cell focus	7 (14%)	6 (12%)	2 (4%)	5 (10%)	4 (8%)	3 (6%)
Clear cell focus, multiple				1 (2%)		
Cyst	1 (2%)		1 (2%)	2 (4%)	1 (2%)	
Degeneration, cystic		3 (6%)	2 (4%)	9 (18%)	7 (14%)	9 (18%)
Eosinophilic focus	3 (6%)	2 (4%)		5 (10%)	3 (6%)	3 (6%)
Fatty change	5 (10%)	2 (4%)	7 (14%)	2 (4%)	4 (8%)	
Fibrosis, focal						1 (2%)
Hematopoietic cell proliferation	2 (4%)		1 (2%)			
Hepatodiaphragmatic nodule			1 (2%)			1 (2%)
Infarct, multifocal	4 (8%)	8 (16%)	5 (10%)	6 (12%)	7 (14%)	11 (22%)
Inflammation, chronic			1 (2%)			
Mineralization			1 (2%)			
Mixed cell focus	3 (6%)	1 (2%)	1 (2%)	1 (2%)		1 (2%)
Necrosis	2 (4%)					
Necrosis, multifocal				1 (2%)		
Regeneration, diffuse	1 (2%)					
Bile duct, hyperplasia	2 (4%)				1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 300 ppm	210 ppm 300 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	9	4
Natural deaths	7	2
Survivors		
Terminal sacrifice	34	44
Animals examined microscopically	50	50
Alimentary System		
Intestine large, colon	(50)	(48)
Inflammation, acute, necrotizing	1 (2%)	
Intestine large, rectum	(48)	(49)
Parasite metazoan	1 (2%)	2 (4%)
Intestine small, ileum	(46)	(49)
Inflammation, acute, necrotizing	1 (2%)	1 (2%)
Intestine small, jejunum	(48)	(48)
Inflammation, chronic active		1 (2%)
Parasite metazoan		1 (2%)
Liver	(49)	(50)
Basophilic focus	3 (6%)	1 (2%)
Clear cell focus	20 (41%)	26 (52%)
Degeneration, cystic	29 (59%)	39 (78%)
Eosinophilic focus	2 (4%)	5 (10%)
Hematopoietic cell proliferation	1 (2%)	
Infarct, multifocal	2 (4%)	3 (6%)
Inflammation, chronic	1 (2%)	1 (2%)
Mixed cell focus		3 (6%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 30 ppm	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Alimentary System (continued)						
Liver (continued)	(50)	(50)	(50)	(49)	(50)	(49)
Centrilobular, congestion, chronic			1 (2%)			
Centrilobular, hepatocyte hypertrophy[b]		16 (32%)	5 (10%)	19 (39%)	20 (40%)	33 (67%)
Mesentery	(3)	(2)	(2)		(3)	
Inflammation, suppurative	1 (33%)				2 (67%)	
Artery, inflammation, chronic active	1 (33%)		1 (50%)			
Fat, necrosis		1 (50%)				
Pancreas	(50)	(13)	(11)	(8)	(17)	(13)
Acinus, atrophy	4 (8%)	1 (8%)	1 (9%)			1 (8%)
Acinus, focal cellular change	1 (2%)	1 (8%)				
Artery, inflammation, chronic active						1 (8%)
Duct, dilatation						1 (8%)
Stomach, forestomach	(50)	(16)	(11)	(9)	(15)	(18)
Diverticulum						1 (6%)
Hyperplasia, squamous				1 (11%)		4 (22%)
Epithelium, hyperplasia	1 (2%)		2 (18%)			2 (11%)
Stomach, glandular	(50)	(15)	(9)	(9)	(13)	(16)
Inflammation, acute, necrotizing	1 (2%)					
Mineralization		1 (7%)				
Tooth	(36)	(1)	(5)	(2)	(2)	(1)
Incisor, upper, dysplasia	36 (100%)	1 (100%)	5 (100%)	2 (100%)	2 (100%)	1 (100%)
Cardiovascular System						
Heart	(50)	(14)	(12)	(10)	(17)	(13)
Bacterium				1 (10%)		
Cardiomyopathy, chronic active			1 (8%)	1 (10%)		
Atrium, thrombosis			1 (8%)			
Valve, inflammation, suppurative				1 (10%)		
Endocrine System						
Adrenal gland, cortex	(50)	(14)	(11)	(9)	(17)	(12)
Hyperplasia	4 (8%)	1 (7%)	1 (9%)		1 (6%)	
Hypertrophy	17 (34%)	1 (7%)	2 (18%)			4 (33%)
Necrosis			1 (9%)			
Adrenal gland, medulla	(50)	(14)	(11)	(9)	(17)	(11)
Hyperplasia	5 (10%)					
Necrosis			1 (9%)			
Islets, pancreatic	(50)	(13)	(10)	(8)	(17)	(12)
Hyperplasia	1 (2%)				1 (6%)	1 (8%)
Pituitary gland	(45)	(11)	(4)	(7)	(14)	(10)
Pars distalis, hyperplasia	2 (4%)					
Thyroid gland	(49)	(14)	(11)	(9)	(17)	(13)
Follicle, cyst multilocular	1 (2%)	1 (7%)				
Follicular cell, hyperplasia	2 (4%)					
General Body System						
None						

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 300 ppm	210 ppm 300 ppm
Alimentary System (continued)		
Liver (continued)	(49)	(50)
Centrilobular, hepatocyte hypertrophy	37 (76%)	46 (92%)
Mesentery	(4)	(3)
Artery, inflammation, chronic active		1 (33%)
Pancreas	(49)	(49)
Acinus, atrophy		6 (12%)
Acinus, depletion secretory		1 (2%)
Acinus, focal cellular change	2 (4%)	
Salivary glands	(49)	(50)
Inflammation, granulomatous	1 (2%)	
Necrosis	1 (2%)	
Acinus, atrophy	1 (2%)	
Stomach, forestomach	(48)	(49)
Ulcer		1 (2%)
Epithelium, hyperplasia	2 (4%)	1 (2%)
Stomach, glandular	(47)	(49)
Inflammation, acute, necrotizing	4 (9%)	
Tooth	(39)	(44)
Incisor, upper, dysplasia	38 (97%)	44 (100%)
Peridontal tissue, inflammation, suppurative	1 (3%)	1 (2%)
Cardiovascular System		
Heart	(49)	(50)
Artery, inflammation, chronic active		2 (4%)
Endocrine System		
Adrenal gland, cortex	(49)	(49)
Hyperplasia	4 (8%)	2 (4%)
Hypertrophy	7 (14%)	15 (31%)
Islets, pancreatic	(49)	(49)
Hyperplasia		1 (2%)
Thyroid gland	(49)	(50)
Inflammation, chronic active	1 (2%)	
Follicle, cyst multilocular	1 (2%)	
Follicular cell, hyperplasia	6 (12%)	4 (8%)
General Body System		
None		

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 30 ppm	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Genital System						
Preputial gland	(5)	(2)	(4)	(8)	(3)	(2)
Inflammation, chronic active	1 (20%)	1 (50%)	1 (25%)	3 (38%)	2 (67%)	
Duct, dilatation	4 (80%)	1 (50%)	4 (100%)	4 (50%)	1 (33%)	2 (100%)
Prostate	(50)	(14)	(12)	(9)	(17)	(13)
Inflammation, suppurative				2 (22%)		
Artery, inflammation, chronic active	1 (2%)					
Seminal vesicle		(1)	(1)	(1)	(2)	
Inflammation, chronic active				1 (100%)		
Testes	(50)	(14)	(12)	(9)	(17)	(13)
Degeneration					1 (6%)	1 (8%)
Inflammation, acute, necrotizing				1 (11%)		
Hematopoietic System						
Lymph node	(50)	(30)	(32)	(23)	(31)	(27)
Deep cervical, edema						1 (4%)
Lumbar, edema				1 (4%)		
Mandibular, angiectasis			1 (3%)		1 (3%)	1 (4%)
Mandibular, edema		1 (3%)	1 (3%)		2 (6%)	
Mandibular, hyperplasia, lymphoid			2 (6%)		2 (6%)	1 (4%)
Renal, edema				1 (4%)		
Lymph node, mesenteric	(22)	(22)	(20)	(15)	(20)	(16)
Angiectasis	15 (68%)	12 (55%)	15 (75%)	11 (73%)	9 (45%)	11 (69%)
Edema		2 (9%)				1 (6%)
Hyperplasia, lymphoid	1 (5%)		1 (5%)		2 (10%)	1 (6%)
Spleen	(49)	(21)	(18)	(17)	(25)	(20)
Angiectasis, focal	1 (2%)					
Hematopoietic cell proliferation	4 (8%)	1 (5%)	3 (17%)	1 (6%)	4 (16%)	3 (15%)
Hyperplasia, lymphoid				3 (18%)		1 (5%)
Integumentary System						
Skin	(50)	(15)	(13)	(9)	(20)	(14)
Cyst epithelial inclusion			1 (8%)			
Inflammation, acute				2 (22%)		
Inflammation, chronic active	2 (4%)					
Subcutaneous tissue, fibrosis					1 (5%)	
Musculoskeletal System						
Bone	(50)	(30)	(40)	(35)	(37)	(30)
Femur, fibrosis, focal	1 (2%)					
Tarsal, hyperostosis	28 (56%)	16 (53%)	31 (78%)	27 (77%)	22 (59%)	19 (63%)
Tarsal, inflammation, chronic active			1 (3%)			
Skeletal muscle	(1)	(1)	(1)			
Artery, inflammation, chronic active	1 (100%)					

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 300 ppm	210 ppm 300 ppm
Genital System		
Epididymis	(50)	(49)
Inflammation, chronic active		1 (2%)
Preputial gland	(8)	(4)
Inflammation, chronic active	3 (38%)	
Duct, dilatation	7 (88%)	4 (100%)
Prostate	(50)	(50)
Inflammation, suppurative	3 (6%)	
Hematopoietic System		
Bone marrow	(50)	(50)
Femoral, thrombosis		1 (2%)
Lymph node	(47)	(50)
Mandibular, angiectasis		1 (2%)
Mandibular, edema		1 (2%)
Mediastinal, angiectasis	1 (2%)	
Lymph node, mesenteric	(24)	(18)
Angiectasis	13 (54%)	13 (72%)
Spleen	(47)	(49)
Cyst	1 (2%)	
Depletion lymphoid		1 (2%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)
Thymus	(41)	(43)
Atrophy	1 (2%)	
Integumentary System		
Skin	(50)	(50)
Inflammation, acute		1 (2%)
Inflammation, chronic active	2 (4%)	
Musculoskeletal System		
Bone	(50)	(50)
Femur, hyperostosis	1 (2%)	
Tarsal, hyperostosis	13 (26%)	9 (18%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 30 ppm	0 ppm 100 ppm	70 ppm 100 ppm	210 ppm 100 ppm
Nervous System						
Brain	(50)	(14)	(12)	(10)	(18)	(13)
Compression			1 (8%)			
Hemorrhage					1 (6%)	1 (8%)
Hydrocephalus					1 (6%)	
Necrosis					1 (6%)	
Artery, inflammation, chronic active	1 (2%)		1 (8%)			
Meninges, infiltration cellular, lymphocyte	1 (2%)					
Respiratory System						
Lung	(50)	(19)	(19)	(22)	(20)	(21)
Inflammation, chronic active	2 (4%)		3 (16%)			3 (14%)
Metaplasia, focal, squamous	1 (2%)					
Alveolar epithelium, hyperplasia	11 (22%)	1 (5%)		2 (9%)	1 (5%)	2 (10%)
Alveolus, infiltration cellular, histiocyte	1 (2%)					
Special Senses System						
Eye	(2)	(3)		(3)		(2)
Degeneration	1 (50%)	1 (33%)		3 (100%)		2 (100%)
Cornea, inflammation, chronic active	1 (50%)	1 (33%)				
Harderian gland	(9)	(2)	(2)	(4)	(3)	(2)
Hyperplasia	3 (33%)					
Inflammation, suppurative	1 (11%)					1 (50%)
Urinary System						
Kidney	(50)	(16)	(12)	(9)	(18)	(13)
Cyst	1 (2%)					
Hydronephrosis	1 (2%)					
Infiltration cellular, lymphocyte	1 (2%)					
Metaplasia, osseous	1 (2%)					
Nephropathy, chronic	36 (72%)	4 (25%)	6 (50%)	1 (11%)	9 (50%)	4 (31%)
Artery, inflammation, chronic active	1 (2%)		1 (8%)			
Pelvis, inflammation, suppurative	1 (2%)			2 (22%)		
Renal tubule, hyperplasia				1 (11%)		
Urinary bladder	(49)	(13)	(12)	(8)	(18)	(13)
Inflammation, chronic active	1 (2%)			2 (25%)	2 (11%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration	0 ppm	210 ppm
F_1 Concentration	300 ppm	300 ppm
Nervous System		
None		
Respiratory System		
Lung	(49)	(50)
Inflammation, acute		2 (4%)
Inflammation, chronic active	2 (4%)	1 (2%)
Alveolar epithelium, hyperplasia	6 (12%)	1 (2%)
Artery, mediastinum, inflammation, chronic active		1 (2%)
Nose	(50)	(50)
Mucosa, inflammation, suppurative	1 (2%)	
Nasolacrimal duct, inflammation, suppurative		1 (2%)
Special Senses System		
Eye	(1)	(2)
Degeneration	1 (100%)	2 (100%)
Harderian gland	(5)	(4)
Hyperplasia	1 (20%)	1 (25%)
Urinary System		
Kidney	(50)	(50)
Embolus bacterial	1 (2%)	
Nephropathy, chronic	35 (70%)	39 (78%)
Artery, inflammation, chronic active		1 (2%)
Pelvis, inflammation, suppurative	2 (4%)	
Urinary bladder	(50)	(50)
Concretion	1 (2%)	
Inflammation, chronic active	5 (10%)	

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report because it is more widely used and understood.

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF 5,5-DIPHENYLHYDANTOIN

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 60 ppm	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	8	11	9	8	6	6
Natural deaths	6	5	6	4	6	7
Survivors						
Terminal sacrifice	36	34	35	38	38	37
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Gallbladder	(45)	(14)	(14)	(11)	(11)	(12)
Intestine large, cecum	(46)	(15)	(20)	(11)	(10)	(13)
Leiomyosarcoma						1 (8%)
Intestine large, colon	(48)	(16)	(15)	(11)	(12)	(13)
Leiomyoma	1 (2%)					
Leiomyosarcoma					1 (8%)	
Intestine small, ileum	(46)	(15)	(14)	(11)	(11)	(14)
Intestine small, jejunum	(48)	(16)	(14)	(11)	(12)	(15)
Adenocarcinoma					1 (8%)	
Liver	(48)	(49)	(50)	(49)	(50)	(50)
Hemangiosarcoma		2 (4%)	2 (4%)			
Hemangiosarcoma, multiple		1 (2%)				
Hepatocellular carcinoma		1 (2%)	4 (8%)	1 (2%)	3 (6%)	4 (8%)
Hepatocellular adenoma	5 (10%)	8 (16%)	10 (20%)	8 (16%)	20 (40%)	7 (14%)
Hepatocellular adenoma, multiple		3 (6%)	1 (2%)	5 (10%)	5 (10%)	5 (10%)
Mesentery	(8)	(2)	(5)	(2)	(4)	(4)
Pancreas	(47)	(15)	(15)	(11)	(12)	(12)
Salivary glands	(49)	(15)	(14)	(11)	(12)	(14)
Stomach, forestomach	(48)	(20)	(20)	(16)	(12)	(20)
Squamous cell papilloma	2 (4%)	3 (15%)	3 (15%)			2 (10%)
Squamous cell papilloma, multiple						1 (5%)
Stomach, glandular	(48)	(16)	(16)	(13)	(11)	(15)
Tongue			(1)			
Squamous cell carcinoma			1 (100%)			
Tooth	(5)			(1)		(1)
Cardiovascular System						
Heart	(50)	(16)	(15)	(13)	(12)	(13)
Endocrine System						
Adrenal gland, cortex	(48)	(16)	(14)	(11)	(12)	(13)
Adrenal gland, medulla	(48)	(16)	(14)	(11)	(12)	(13)
Pheochromocytoma malignant	1 (2%)					
Pheochromocytoma complex	1 (2%)					
Pheochromocytoma benign	2 (4%)	2 (13%)	1 (7%)			1 (8%)
Islets, pancreatic	(47)	(15)	(14)	(11)	(11)	(12)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	8	6
Natural deaths	5	4
Survivors		
Terminal sacrifice	37	40
Animals examined microscopically	50	50
Alimentary System		
Gallbladder	(45)	(45)
Intestine large, rectum	(47)	(50)
Intestine small, duodenum	(48)	(49)
Intestine small, jejunum	(48)	(48)
Liver	(50)	(50)
Hepatoblastoma	1 (2%)	
Hepatocellular carcinoma	9 (18%)	10 (20%)
Hepatocellular carcinoma, multiple	2 (4%)	
Hepatocellular adenoma	16 (32%)	19 (38%)
Hepatocellular adenoma, multiple	6 (12%)	7 (14%)
Mesentery	(6)	(6)
Pancreas	(48)	(49)
Salivary glands	(49)	(50)
Stomach, forestomach	(48)	(49)
Mast cell tumor NOS		1 (2%)
Stomach, glandular	(48)	(49)
Mast cell tumor NOS		1 (2%)
Tooth	(9)	(12)
Cardiovascular System		
Heart	(50)	(50)
Endocrine System		
Adrenal gland, cortex	(48)	(50)
Adrenal gland, medulla	(48)	(50)
Pheochromocytoma malignant	1 (2%)	
Pheochromocytoma benign		2 (4%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 60 ppm	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Endocrine System (continued)						
Pituitary gland	(44)	(19)	(17)	(15)	(14)	(17)
Pars distalis, adenoma	6 (14%)	8 (42%)	3 (18%)	8 (53%)	3 (21%)	2 (12%)
Pars distalis, carcinoma	1 (2%)					
Pars intermedia, adenoma		1 (5%)				
Thyroid gland	(47)	(16)	(15)	(11)	(12)	(14)
C-cell, carcinoma						1 (7%)
Follicular cell, adenoma	4 (9%)					
General Body System						
None						
Genital System						
Ovary	(49)	(28)	(36)	(30)	(25)	(28)
Choriocarcinoma		1 (4%)				
Cystadenoma	1 (2%)		1 (3%)	1 (3%)	1 (4%)	3 (11%)
Granulosa cell tumor benign	1 (2%)					
Hemangioma						1 (4%)
Luteoma	1 (2%)					
Periovarian tissue, hemangiosarcoma	1 (2%)					
Uterus	(49)	(36)	(31)	(32)	(33)	(33)
Hemangiosarcoma		1 (3%)				
Polyp stromal	1 (2%)				1 (3%)	
Sarcoma stromal		1 (3%)		2 (6%)		
Sarcoma stromal, multiple	1 (2%)					
Hematopoietic System						
Blood	(2)					
Bone marrow	(48)	(16)	(15)	(11)	(12)	(13)
Femoral, hemangiosarcoma	1 (2%)	2 (13%)				
Lymph node	(50)	(18)	(16)	(20)	(15)	(20)
Mandibular, adenocarcinoma, metastatic, harderian gland				1 (5%)		
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung				1 (5%)		
Mediastinal, osteosarcoma, metastatic, uncertain primary site				1 (5%)		
Mediastinal, mandibular, sarcoma, metastatic, eye			1 (6%)			
Lymph node, mesenteric	(12)	(7)	(5)	(9)	(4)	(6)
Hemangiosarcoma		1 (14%)				
Spleen	(48)	(23)	(27)	(27)	(23)	(23)
Hemangiosarcoma	3 (6%)	4 (17%)				
Thymus	(42)	(12)	(11)	(10)	(11)	(9)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Endocrine System (continued)		
Pituitary gland	(41)	(44)
Pars distalis, adenoma	3 (7%)	3 (7%)
Thyroid gland	(50)	(49)
Follicular cell, adenoma	2 (4%)	4 (8%)
Follicular cell, adenoma, multiple	2 (4%)	
General Body System		
None		
Genital System		
Ovary	(49)	(50)
Cystadenoma	2 (4%)	1 (2%)
Luteoma	1 (2%)	
Teratoma		1 (2%)
Uterus	(50)	(50)
Adenocarcinoma	1 (2%)	
Polyp stromal		1 (2%)
Vagina	(1)	
Hematopoietic System		
Blood	(1)	(3)
Bone marrow	(50)	(49)
Lymph node	(49)	(48)
Lymph node, mesenteric	(6)	(6)
Spleen	(48)	(50)
Thymus	(45)	(42)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 60 ppm	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Integumentary System						
Mammary gland	(46)	(15)	(12)	(5)	(9)	(13)
Adenocarcinoma	1 (2%)	1 (7%)	2 (17%)			2 (15%)
Skin	(49)	(16)	(17)	(13)	(12)	(13)
Squamous cell carcinoma				1 (8%)		
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (6%)	1 (6%)	1 (8%)	1 (8%)	
Subcutaneous tissue, hemangiosarcoma		3 (19%)				
Subcutaneous tissue, myxoma	1 (2%)					
Subcutaneous tissue, myxosarcoma			1 (6%)			
Subcutaneous tissue, sarcoma		1 (6%)				
Musculoskeletal System						
Bone	(50)	(16)	(16)	(12)	(12)	(13)
Femur, osteoma			1 (6%)			
Skeletal muscle	(1)	(1)				
Nervous System						
Brain	(50)	(16)	(15)	(12)	(13)	(13)
Adenocarcinoma, metastatic, harderian gland					1 (8%)	
Carcinoma, metastatic, pituitary gland	1 (2%)					
Spinal cord	(1)	(1)				
Respiratory System						
Lung	(50)	(20)	(20)	(13)	(13)	(15)
Adenocarcinoma, metastatic, harderian gland		1 (5%)		1 (8%)		
Alveolar/bronchiolar adenoma	5 (10%)	2 (10%)	1 (5%)	2 (15%)		2 (13%)
Alveolar/bronchiolar carcinoma		1 (5%)	1 (5%)	1 (8%)	1 (8%)	1 (7%)
Hepatocellular carcinoma, metastatic, liver		1 (5%)	2 (10%)	1 (8%)		
Osteosarcoma, metastatic, uncertain primary site			1 (5%)	1 (8%)		
Pheochromocytoma malignant, metastatic, adrenal gland	1 (2%)					
Sarcoma, metastatic, eye			1 (5%)			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung				1 (8%)		
Nose	(50)	(16)	(15)	(12)	(12)	(13)
Adenocarcinoma, metastatic, harderian gland		1 (6%)		1 (8%)		
Sarcoma, metastatic, eye			1 (7%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Integumentary System		
Mammary gland	(40)	(39)
Skin	(50)	(49)
Subcutaneous tissue, fibrosarcoma	1 (2%)	
Musculoskeletal System		
Skeletal muscle	(1)	
Nervous System		
Brain	(49)	(50)
Respiratory System		
Lung	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	2 (4%)
Osteosarcoma, metastatic, uncertain primary site		1 (2%)
Mediastinum, hepatocellular carcinoma, metastatic, liver	1 (2%)	
Trachea	(50)	(49)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 60 ppm	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Special Senses System						
Eye	(2)	(2)	(1)	(3)	(1)	(2)
Adenocarcinoma, metastatic, harderian gland				1 (33%)		
Sarcoma			1 (100%)			
Harderian gland	(8)	(4)	(4)	(4)	(4)	(3)
Adenocarcinoma	1 (13%)	3 (75%)		2 (50%)	1 (25%)	1 (33%)
Adenoma	3 (38%)	1 (25%)	2 (50%)	2 (50%)	3 (75%)	1 (33%)
Urinary System						
Kidney	(48)	(16)	(16)	(12)	(14)	(13)
Osteosarcoma, metastatic, uncertain primary site			1 (8%)			
Urinary bladder	(48)	(15)	(15)	(11)	(11)	(13)
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)	(50)
Lymphoma malignant histiocytic	5 (10%)	3 (6%)	6 (12%)		2 (4%)	4 (8%)
Lymphoma malignant lymphocytic	9 (18%)	2 (4%)		1 (2%)	2 (4%)	
Lymphoma malignant mixed	8 (16%)	8 (16%)	3 (6%)	12 (24%)	10 (20%)	11 (22%)
Lymphoma malignant undifferentiated cell	1 (2%)		2 (4%)			
Neoplasm Summary						
Total animals with primary neoplasms[c]	40	36	27	36	39	36
Total primary neoplasms	68	65	47	47	55	50
Total animals with benign neoplasms	23	22	18	24	29	22
Total benign neoplasms	33	28	23	26	33	25
Total animals with malignant neoplasms	28	28	21	20	21	23
Total malignant neoplasms	35	37	24	21	22	25
Total animals with metastatic neoplasms	2	2	4	4	1	
Total metastatic neoplasms	2	3	6	10	1	
Total animals with malignant neoplasms of uncertain primary site			1	1		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Special Senses System		
Harderian gland	(2)	
Adenoma	1 (50%)	
Urinary System		
Kidney	(50)	(50)
Urinary bladder	(49)	(49)
Systemic Lesions		
Multiple organs	(50)	(50)
Lymphoma malignant histiocytic	6 (12%)	4 (8%)
Lymphoma malignant lymphocytic	5 (10%)	3 (6%)
Lymphoma malignant mixed	4 (8%)	5 (10%)
Lymphoma malignant undifferentiated cell		1 (2%)
Neoplasm Summary		
Total animals with primary neoplasms	42	46
Total primary neoplasms	65	64
Total animals with benign neoplasms	28	33
Total benign neoplasms	35	39
Total animals with malignant neoplasms	26	21
Total malignant neoplasms	30	23
Total animals with metastatic neoplasms	3	3
Total metastatic neoplasms	3	
Total animals with malignant neoplasms uncertain primary site		1
Total animals with neoplasms uncertain benign or malignant		1
Total uncertain neoplasms		2

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 0:200, and 0:600 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 200 ppm	0 ppm 600 ppm
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma			
Overall rate[a]	4/48 (8%)	0/11 (0%)[e]	1/48 (2%)
Adjusted rate[b]	10.6%		2.8%
Terminal rate[c]	3/36 (8%)		1/36 (3%)
First incidence (days)	670		736 (T)
Life table test[d]			P=0.179N
Logistic regression test[d]			P=0.174N
Cochran-Armitage test[d]			
Fisher exact test[d]			P=0.181N
Harderian Gland: Adenoma			
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	8.1%	5.3%	2.7%
Terminal rate	2/36 (6%)	2/38 (5%)	1/37 (3%)
First incidence (days)	726	736 (T)	736 (T)
Life table test	P=0.245N	P=0.476N	P=0.295N
Logistic regression test	P=0.234N	P=0.471N	P=0.283N
Cochran-Armitage test	P=0.252N		
Fisher exact test		P=0.500N	P=0.309N
Harderian Gland: Adenoma or Carcinoma			
Overall rate	4/50 (8%)	4/50 (8%)	1/50 (2%)
Adjusted rate	10.8%	9.9%	2.7%
Terminal rate	3/36 (8%)	3/38 (8%)	1/37 (3%)
First incidence (days)	726	662	736 (T)
Life table test	P=0.129N	P=0.609N	P=0.172N
Logistic regression test	P=0.124N	P=0.623N	P=0.160N
Cochran-Armitage test	P=0.135N		
Fisher exact test		P=0.643N	P=0.181N
Liver: Hepatocellular Adenoma			
Overall rate	5/48 (10%)	13/49 (27%)	22/50 (44%)
Adjusted rate	13.3%	32.3%	50.9%
Terminal rate	4/36 (11%)	11/38 (29%)	16/37 (43%)
First incidence (days)	670	675	664
Life table test	P<0.001	P=0.049	P<0.001
Logistic regression test	P<0.001	P=0.042	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.036	P<0.001
Liver: Hepatocellular Carcinoma			
Overall rate	0/48 (0%)	1/49 (2%)	11/50 (22%)
Adjusted rate	0.0%	2.6%	26.6%
Terminal rate	0/36 (0%)	1/38 (3%)	8/37 (22%)
First incidence (days)	–[f]	736 (T)	570
Life table test	P<0.001	P=0.511	P=0.001
Logistic regression test	P<0.001	P=0.511	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.505	P<0.001

TABLE D2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 0:200, and 0:600 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 200 ppm	0 ppm 600 ppm
Liver: Hepatoblastoma or Hepatocellular Carcinoma			
Overall rate	0/48 (0%)	1/49 (2%)	12/50 (24%)
Adjusted rate	0.0%	2.6%	29.1%
Terminal rate	0/36 (0%)	1/38 (3%)	9/37 (24%)
First incidence (days)	–	736 (T)	570
Life table test	P<0.001	P=0.511	P<0.001
Logistic regression test	P<0.001	P=0.511	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.505	P<0.001
Liver: Hepatoblastoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma			
Overall rate	5/48 (10%)	14/49 (29%)	30/50 (60%)
Adjusted rate	13.3%	34.8%	66.4%
Terminal rate	4/36 (11%)	12/38 (32%)	22/37 (59%)
First incidence (days)	670	675	570
Life table test	P<0.001	P=0.031	P<0.001
Logistic regression test	P<0.001	P=0.026	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.022	P<0.001
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	5/50 (10%)	2/13 (15%)[e]	2/50 (4%)
Adjusted rate	13.9%		5.4%
Terminal rate	5/36 (14%)		2/37 (5%)
First incidence (days)	736 (T)		736 (T)
Life table test			P=0.204N
Logistic regression test			P=0.204N
Cochran-Armitage test			
Fisher exact test			P=0.218N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	5/50 (10%)	3/13 (23%)[e]	2/50 (4%)
Adjusted rate	13.9%		5.4%
Terminal rate	5/36 (14%)		2/37 (5%)
First incidence (days)	736 (T)		736 (T)
Life table test			P=0.204N
Logistic regression test			P=0.204N
Cochran-Armitage test			
Fisher exact test			P=0.218N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	6/44 (14%)	8/15 (53%)[e]	3/41 (7%)
Adjusted rate	18.8%		10.0%
Terminal rate	6/32 (19%)		3/30 (10%)
First incidence (days)	736 (T)		736 (T)
Life table test			P=0.270N
Logistic regression test			P=0.270N
Cochran-Armitage test			
Fisher exact test			P=0.278N

TABLE D2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 0:200, and 0:600 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 200 ppm	0 ppm 600 ppm
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rate	7/44 (16%)	8/15 (53%)[e]	3/41 (7%)
Adjusted rate	20.7%		10.0%
Terminal rate	6/32 (19%)		3/30 (10%)
First incidence (days)	655		736 (T)
Life table test			P=0.178N
Logistic regression test			P=0.170N
Cochran-Armitage test			
Fisher exact test			P=0.187N
Spleen: Hemangiosarcoma			
Overall rate	3/48 (6%)	0/27 (0%)[e]	0/48 (0%)
Adjusted rate	7.9%		0.0%
Terminal rate	2/36 (6%)		0/37 (0%)
First incidence (days)	670		–
Life table test			P=0.117N
Logistic regression test			P=0.119N
Cochran-Armitage test			
Fisher exact test			P=0.121N
Thyroid Gland (Follicular Cell): Adenoma			
Overall rate	4/47 (9%)	0/11 (0%)[e]	4/50 (8%)
Adjusted rate	11.1%		10.8%
Terminal rate	4/36 (11%)		4/37 (11%)
First incidence (days)	736 (T)		736 (T)
Life table test			P=0.630N
Logistic regression test			P=0.630N
Cochran-Armitage test			
Fisher exact test			P=0.607N
All Organs: Hemangiosarcoma			
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	7.9%	0.0%	0.0%
Terminal rate	2/36 (6%)	0/38 (0%)	0/37 (0%)
First incidence (days)	670	–	–
Life table test	P=0.078N	P=0.113N	P=0.117N
Logistic regression test	P=0.078N	P=0.117N	P=0.116N
Cochran-Armitage test	P=0.080N		
Fisher exact test		P=0.121N	P=0.121N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rate	22/50 (44%)	13/50 (26%)	15/50 (30%)
Adjusted rate	49.1%	30.6%	34.4%
Terminal rate	14/36 (39%)	9/38 (24%)	9/37 (24%)
First incidence (days)	128	574	568
Life table test	P=0.149N	P=0.047N	P=0.115N
Logistic regression test	P=0.159N	P=0.053N	P=0.126N
Cochran-Armitage test	P=0.144N		
Fisher exact test		P=0.046N	P=0.107N

TABLE D2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 0:200, and 0:600 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 200 ppm	0 ppm 600 ppm
All Organs: Benign Neoplasms			
Overall rate	23/50 (46%)	24/50 (48%)	28/50 (56%)
Adjusted rate	60.4%	56.9%	64.9%
Terminal rate	21/36 (58%)	20/38 (53%)	22/37 (59%)
First incidence (days)	670	636	664
Life table test	P=0.219	P=0.551N	P=0.271
Logistic regression test	P=0.226	P=0.570N	P=0.281
Cochran-Armitage test	P=0.180		
Fisher exact test		P=0.500	P=0.212
All Organs: Malignant Neoplasms			
Overall rate	28/50 (56%)	21/50 (42%)	26/50 (52%)
Adjusted rate	58.1%	45.1%	54.8%
Terminal rate	16/36 (44%)	13/38 (34%)	16/37 (43%)
First incidence (days)	128	465	423
Life table test	P=0.459N	P=0.120N	P=0.385N
Logistic regression test	P=0.543	P=0.136N	P=0.492N
Cochran-Armitage test	P=0.500N		
Fisher exact test		P=0.115N	P=0.421N
All Organs: Benign or Malignant Neoplasms			
Overall rate	40/50 (80%)	36/50 (72%)	42/50 (84%)
Adjusted rate	83.2%	73.5%	84.0%
Terminal rate	28/36 (78%)	25/38 (66%)	29/37 (78%)
First incidence (days)	128	465	423
Life table test	P=0.410	P=0.207N	P=0.522
Logistic regression test	P=0.264	P=0.245N	P=0.377
Cochran-Armitage test	P=0.279		
Fisher exact test		P=0.241N	P=0.398

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not applicable.
[f] Not applicable; no neoplasms in animal group

TABLE D2b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 210:0 ppm Groups

F_0 Concentration	0 ppm	210 ppm
F_1 Concentration	0 ppm	0 ppm
Adrenal Medulla: Benign Pheochromocytoma		
Overall rate[a]	2/48 (4%)	2/16 (13%)
Adjusted rate[b]	5.6%	0.0%
Terminal rate[c]	2/36 (6%)	0/0
First incidence (days)	736 (T)	607
Life table test[d]		P=0.221
Logistic regression test[d]		P=0.133
Fisher exact test[d]		P=0.258
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma		
Overall rate	4/48 (8%)	2/16 (13%)
Adjusted rate	10.6%	0.0%
Terminal rate	3/36 (8%)	0/0
First incidence (days)	670	607
Life table test		P=0.472
Logistic regression test		P=0.319
Fisher exact test		P=0.471
Bone Marrow: Hemangiosarcoma		
Overall rate	1/48 (2%)	2/16 (13%)
Adjusted rate	2.4%	0.0%
Terminal rate	0/36 (0%)	0/0
First incidence (days)	670	607
Life table test		P=0.475
Logistic regression test		P=0.181
Fisher exact test		P=0.152
Harderian Gland: Adenoma		
Overall rate	3/50 (6%)	1/50 (2%)
Adjusted rate	8.1%	2.9%
Terminal rate	2/36 (6%)	1/34 (3%)
First incidence (days)	726	736 (T)
Life table test		P=0.330N
Logistic regression test		P=0.332N
Fisher exact test		P=0.309N
Harderian Gland: Carcinoma		
Overall rate	1/50 (2%)	3/50 (6%)
Adjusted rate	2.8%	7.8%
Terminal rate	1/36 (3%)	2/34 (6%)
First incidence (days)	736 (T)	491
Life table test		P=0.291
Logistic regression test		P=0.306
Fisher exact test		P=0.309

TABLE D2b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 210:0 ppm Groups (continued)

F_0 Concentration	0 ppm	210 ppm
F_1 Concentration	0 ppm	0 ppm
Harderian Gland: Adenoma or Carcinoma		
Overall rate	4/50 (8%)	4/50 (8%)
Adjusted rate	10.8%	10.7%
Terminal rate	3/36 (8%)	3/34 (9%)
First incidence (days)	726	491
Life table test		P=0.613
Logistic regression test		P=0.637
Fisher exact test		P=0.643N
Liver: Hemangiosarcoma		
Overall rate	0/48 (0%)	3/49 (6%)
Adjusted rate	0.0%	8.7%
Terminal rate	0/36 (0%)	2/33 (6%)
First incidence (days)	-[e]	682
Life table test		P=0.110
Logistic regression test		P=0.110
Fisher exact test		P=0.125
Liver: Hepatocellular Adenoma		
Overall rate	5/48 (10%)	11/49 (22%)
Adjusted rate	13.3%	29.9%
Terminal rate	4/36 (11%)	8/33 (24%)
First incidence (days)	670	607
Life table test		P=0.066
Logistic regression test		P=0.070
Fisher exact test		P=0.092
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	5/48 (10%)	12/49 (24%)
Adjusted rate	13.3%	31.3%
Terminal rate	4/36 (11%)	8/33 (24%)
First incidence (days)	670	486
Life table test		P=0.043
Logistic regression test		P=0.055
Fisher exact test		P=0.059
Lung: Alveolar/bronchiolar Adenoma		
Overall rate	5/50 (10%)	2/20 (10%)
Adjusted rate	13.9%	50.0%
Terminal rate	5/36 (14%)	2/4 (50%)
First incidence (days)	736 (T)	736 (T)
Life table test		P=0.137
Logistic regression test		P=0.134
Fisher exact test		P=0.684N

TABLE D2b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 210:0 ppm Groups (continued)

F_0 Concentration	0 ppm	210 ppm
F_1 Concentration	0 ppm	0 ppm
Lung: Alveolar/bronchiolar Carcinoma		
Overall rate	0/50 (0%)	1/20 (5%)
Adjusted rate	0.0%	25.0%
Terminal rate	0/36 (0%)	1/4 (25%)
First incidence (days)	–	736 (T)
Life table test		P=0.091
Logistic regression test		P=0.091
Fisher exact test		P=0.286
Lung: Alveolar/bronchiolar Adenoma or Carcinoma		
Overall rate	5/50 (10%)	3/20 (15%)
Adjusted rate	13.9%	75.0%
Terminal rate	5/36 (14%)	3/4 (75%)
First incidence (days)	736 (T)	736 (T)
Life table test		P=0.013
Logistic regression test		P=0.013
Fisher exact test		P=0.412
Pituitary Gland (Pars Distalis): Adenoma		
Overall rate	6/44 (14%)	8/19 (42%)
Adjusted rate	18.8%	84.6%
Terminal rate	6/32 (19%)	5/6 (83%)
First incidence (days)	736 (T)	530
Life table test		P<0.001
Logistic regression test		P<0.001
Fisher exact test		P=0.017
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma		
Overall rate	7/44 (16%)	8/19 (42%)
Adjusted rate	20.7%	84.6%
Terminal rate	6/32 (19%)	5/6 (83%)
First incidence (days)	655	530
Life table test		P=0.004
Logistic regression test		P=0.002
Fisher exact test		P=0.030
Skin (Subcutaneous Tissue): Hemangiosarcoma		
Overall rate	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	7.1%
Terminal rate	0/36 (0%)	0/34 (0%)
First incidence (days)	–	579
Life table test		P=0.113
Logistic regression test		P=0.110
Fisher exact test		P=0.121

TABLE D2b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 210:0 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm
Spleen: Hemangiosarcoma		
Overall rate	3/48 (6%)	4/23 (17%)
Adjusted rate	7.9%	20.4%
Terminal rate	2/36 (6%)	1/7 (14%)
First incidence (days)	670	579
Life table test		P=0.180
Logistic regression test		P=0.142
Fisher exact test		P=0.148
Stomach (Forestomach): Squamous Cell Papilloma		
Overall rate	2/50 (4%)	3/50 (6%)
Adjusted rate	5.6%	8.8%
Terminal rate	2/36 (6%)	3/34 (9%)
First incidence (days)	736 (T)	736 (T)
Life table test		P=0.474
Logistic regression test		P=0.474
Fisher exact test		P=0.500
Thyroid Gland (Follicular Cell): Adenoma		
Overall rate	4/47 (9%)	0/16 (0%)
Adjusted rate	11.1%	0.0%
Terminal rate	4/36 (11%)	0/0
First incidence (days)	736 (T)	–
Life table test		–
Logistic regression test		–
Fisher exact test		P=0.299N
All Organs: Hemangiosarcoma		
Overall rate	3/50 (6%)	7/50 (14%)
Adjusted rate	7.9%	18.1%
Terminal rate	2/36 (6%)	4/34 (12%)
First incidence (days)	670	579
Life table test		P=0.143
Logistic regression test		P=0.157
Fisher exact test		P=0.159
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)		
Overall rate	22/50 (44%)	12/50 (24%)
Adjusted rate	49.1%	29.1%
Terminal rate	14/36 (39%)	6/34 (18%)
First incidence (days)	128	580
Life table test		P=0.070N
Logistic regression test		P=0.026N
Fisher exact test		P=0.028N

TABLE D2b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0 and 210:0 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm
All Organs: Benign Neoplasms		
Overall rate	23/50 (46%)	22/50 (44%)
Adjusted rate	60.4%	53.3%
Terminal rate	21/36 (58%)	15/34 (44%)
First incidence (days)	670	530
Life table test		P=0.553
Logistic regression test		P=0.573
Fisher exact test		P=0.500N
All Organs: Malignant Neoplasms		
Overall rate	29/50 (58%)	28/50 (56%)
Adjusted rate	60.2%	58.0%
Terminal rate	17/36 (47%)	14/34 (41%)
First incidence (days)	128	364
Life table test		P=0.517
Logistic regression test		P=0.358N
Fisher exact test		P=0.500N
All Organs: Benign or Malignant Neoplasms		
Overall rate	40/50 (80%)	36/50 (72%)
Adjusted rate	83.2%	73.3%
Terminal rate	28/36 (78%)	21/34 (62%)
First incidence (days)	128	364
Life table test		P=0.454N
Logistic regression test		P=0.201N
Fisher exact test		P=0.241N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE D2c
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 21:60, 70:200, 210:200, and 210:600 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	21 ppm 60 ppm	70 ppm 200 ppm	210 ppm 200 ppm	210 ppm 600 ppm
Adrenal Medulla: Benign Pheochromocytoma					
Overall rate[a]	2/48 (4%)	1/14 (7%)[e]	0/12 (0%)[e]	1/13 (8%)[e]	2/50 (4%)
Adjusted rate[b]	5.6%				5.0%
Terminal rate[c]	2/36 (6%)				2/40 (5%)
First incidence (days)	736 (T)				736 (T)
Life table test[d]					P=0.657N
Logistic regression test[d]					P=0.657N
Cochran-Armitage test[d]					
Fisher exact test[d]					P=0.676N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma					
Overall rate	4/48 (8%)	1/14 (7%)[e]	0/12 (0%)[e]	1/13 (8%)[e]	2/50 (4%)
Adjusted rate	10.6%				5.0%
Terminal rate	3/36 (8%)				2/40 (5%)
First incidence (days)	670				736 (T)
Life table test					P=0.292N
Logistic regression test					P=0.301N
Cochran-Armitage test					
Fisher exact test					P=0.319N
Harderian Gland: Adenoma					
Overall rate	3/50 (6%)	2/50 (4%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	8.1%	5.7%	6.8%	2.7%	0.0%
Terminal rate	2/36 (6%)	2/35 (6%)	1/38 (3%)	1/37 (3%)	0/40 (0%)
First incidence (days)	726	736 (T)	625	736 (T)	–[f]
Life table test	P=0.045N	P=0.515N	P=0.633N	P=0.297N	P=0.105N
Logistic regression test	P=0.050N	P=0.520N	P=0.662	P=0.289N	P=0.102N
Cochran-Armitage test	P=0.054N				
Fisher exact test		P=0.500N	P=0.661N	P=0.309N	P=0.121N
Harderian Gland: Adenoma or Carcinoma					
Overall rate	4/50 (8%)	2/50 (4%)	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted rate	10.8%	5.7%	9.3%	5.4%	0.0%
Terminal rate	3/36 (8%)	2/35 (6%)	2/38 (5%)	2/37 (5%)	0/40 (0%)
First incidence (days)	726	736 (T)	625	736 (T)	–
Life table test	P=0.043N	P=0.353N	P=0.609N	P=0.326N	P=0.052N
Logistic regression test	P=0.047N	P=0.356N	P=0.638N	P=0.315N	P=0.049N
Cochran-Armitage test	P=0.054N				
Fisher exact test		P=0.339N	P=0.643N	P=0.339N	P=0.059N
Liver: Hepatocellular Adenoma					
Overall rate	5/48 (10%)	11/50 (22%)	25/50 (50%)	12/50 (24%)	26/50 (52%)
Adjusted rate	13.3%	30.2%	60.9%	31.4%	54.0%
Terminal rate	4/36 (11%)	10/35 (29%)	22/38 (58%)	11/37 (30%)	18/40 (45%)
First incidence (days)	670	587	660	670	503
Life table test	P<0.001	P=0.075	P<0.001	P=0.062	P<0.001
Logistic regression test	P<0.001	P=0.075	P<0.001	P=0.064	P<0.001
Cochran-Armitage test	P<0.001				
Fisher exact test		P=0.100	P<0.001	P=0.065	P<0.001

TABLE D2c
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 21:60, 70:200, 210:200, and 210:600 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	21 ppm 60 ppm	70 ppm 200 ppm	210 ppm 200 ppm	210 ppm 600 ppm
Liver: Hepatocellular Carcinoma					
Overall rate	0/48 (0%)	4/50 (8%)	3/50 (6%)	4/50 (8%)	10/50 (20%)
Adjusted rate	0.0%	9.6%	7.9%	10.1%	24.2%
Terminal rate	0/36 (0%)	1/35 (3%)	3/38 (8%)	2/37 (5%)	9/40 (23%)
First incidence (days)	–	587	736 (T)	675	670
Life table test	P=0.002	P=0.060	P=0.131	P=0.072	P=0.002
Logistic regression test	P=0.001	P=0.067	P=0.131	P=0.069	P=0.002
Cochran-Armitage test	P<0.001				
Fisher exact test		P=0.064	P=0.129	P=0.064	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	5/48 (10%)	13/50 (26%)	26/50 (52%)	16/50 (32%)	34/50 (68%)
Adjusted rate	13.3%	33.5%	63.4%	39.8%	69.3%
Terminal rate	4/36 (11%)	10/35 (29%)	23/38 (61%)	13/37 (35%)	25/40 63%)
First incidence (days)	670	587	660	670	503
Life table test	P<0.001	P=0.031	P<0.001	P=0.010	P<0.001
Logistic regression test	P<0.001	P=0.035	P<0.001	P=0.009	P<0.001
Cochran-Armitage test	P<0.001				
Fisher exact test		P=0.041	P<0.001	P=0.008	P<0.001
Lung: Alveolar/bronchiolar Adenoma					
Overall rate	5/50 (10%)	1/20 (5%)[e]	0/13 (0%)[e]	2/15 (13%)[e]	1/50 (2%)
Adjusted rate	13.9%				2.5%
Terminal rate	5/36 (14%)				1/40 (3%)
First incidence (days)	736 (T)				736 (T)
Life table test					P=0.080N
Logistic regression test					P=0.080N
Cochran-Armitage test					
Fisher exact test					P=0.102N
Lung: Alveolar/bronchiolar Carcinoma					
Overall rate	0/50 (0%)	1/20 (5%)[e]	1/13 (8%)e	1/15 (7%)[e]	0/50 (0%)
Adjusted rate	0.0%				0.0%
Terminal rate	0/36 (0%)				0/40 (0%)
First incidence (days)	–				–
Life table test					–
Logistic regression test					–
Cochran-Armitage test					
Fisher exact test					–
Lung: Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate	5/50 (10%)	2/20 (10%)[e]	1/13 (8%)e	3/15 (20%)[e]	1/50 (2%)
Adjusted rate	13.9%				2.5%
Terminal rate	5/36 (14%)				1/40 (3%)
First incidence (days)	736 (T)				736 (T)
Life table test					P=0.080N
Logistic regression test					P=0.080N
Cochran-Armitage test					
Fisher exact test					P=0.102N

TABLE D2c
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 21:60, 70:200, 210:200, and 210:600 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	21 ppm 60 ppm	70 ppm 200 ppm	210 ppm 200 ppm	210 ppm 600 ppm
Ovary: Cystadenoma					
Overall rate	1/49 (2%)	1/36 (3%)[e]	1/25 (4%)[e]	3/28 (11%)[e]	1/50 (2%)
Adjusted rate	2.8%				2.5%
Terminal rate	1/36 (3%)				1/40 (3%)
First incidence (days)	736 (T)				736 (T)
Life table test					P=0.738N
Logistic regression test					P=0.738N
Cochran-Armitage test					
Fisher exact test					P=0.747N
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	6/44 (14%)	3/17 (18%)[e]	3/14 (21%)[e]	2/17 (12%)[e]	3/44 (7%)
Adjusted rate	18.8%				8.3%
Terminal rate	6/32 (19%)				3/36 (8%)
First incidence (days)	736 (T)				736 (T)
Life table test					P=0.184N
Logistic regression test					P=0.184N
Cochran-Armitage test					
Fisher exact test					P=0.242N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma					
Overall rate	7/44 (16%)	3/17 (18%)[e]	3/14 (21%)[e]	2/17 (12%)[e]	3/44 (7%)
Adjusted rate	20.7%				8.3%
Terminal rate	6/32 (19%)				3/36 (8%)
First incidence (days)	655				736 (T)
Life table test					P=0.116N
Logistic regression test					P=0.134N
Cochran-Armitage test					
Fisher exact test					P=0.157N
Spleen: Hemangiosarcoma					
Overall rate	3/48 (6%)	0/27 (0%)[e]	0/23 (0%)[e]	0/23 (0%)[e]	0/50 (0%)
Adjusted rate	7.9%				0.0%
Terminal rate	2/36 (6%)				0/40 (0%)
First incidence (days)	670				–
Life table test					P=0.106N
Logistic regression test					P=0.111N
Cochran-Armitage test					
Fisher exact test					P=0.114N
Stomach (Forestomach): Squamous Cell Papilloma					
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	5.6%	8.0%	0.0%	8.1%	0.0%
Terminal rate	2/36 (6%)	2/35 (6%)	0/38 (0%)	3/37 (8%)	0/40 (0%)
First incidence (days)	736 (T)	628	–	736 (T)	–
Life table test	P=0.166N	P=0.484	P=0.226N	P=0.513	P=0.215N
Logistic regression test	P=0.173N	P=0.489	P=0.226N	P=0.513	P=0.215N
Cochran-Armitage test	P=0.193N				
Fisher exact test		P=0.500	P=0.247N	P=0.500	P=0.247N

TABLE D2c
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 21:60, 70:200, 210:200, and 210:600 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	21 ppm 60 ppm	70 ppm 200 ppm	210 ppm 200 ppm	210 ppm 600 ppm
Thyroid Gland (Follicular Cell): Adenoma					
Overall rate	4/47 (9%)	0/15 (0%)[e]	0/12 (0%)[e]	0/14 (0%)[e]	4/49 (8%)
Adjusted rate	11.1%				9.9%
Terminal rate	4/36 (11%)				3/39 (8%)
First incidence (days)	736 (T)				730
Life table test					P=0.595N
Logistic regression test					P=0.589
Cochran-Armitage test					
Fisher exact test					P=0.619N
All Organs: Hemangiosarcoma					
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	7.9%	5.2%	0.0%	0.0%	0.0%
Terminal rate	2/36 (6%)	1/35 (3%)	0/38 (0%)	0/37 (0%)	0/40 (0%)
First incidence (days)	670	633	–	–	–
Life table test	P=0.015N	P=0.519N	P=0.114N	P=0.118N	P=0.106N
Logistic regression test	P=0.017N	P=0.503N	P=0.117N	P=0.117N	P=0.115N
Cochran-Armitage test	P=0.017N				
Fisher exact test		P=0.500N	P=0.121N	P=0.121N	P=0.121N
All Organs: Hemangioma or Hemangiosarcoma					
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	7.9%	5.2%	0.0%	2.0%	0.0%
Terminal rate	2/36 (6%)	1/35 (3%)	0/38 (0%)	0/37 (0%)	0/40 (0%)
First incidence (days)	670	633	–	525	–
Life table test	P=0.042N	P=0.519N	P=0.114N	P=0.299N	P=0.106N
Logistic regression test	P=0.056N	P=0.503N	P=0.117N	P=0.331N	P=0.115N
Cochran-Armitage test	P=0.048N				
Fisher exact test		P=0.500N	P=0.121N	P=0.309N	P=0.121N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)					
Overall rate	22/50 (44%)	11/50 (22%)	14/50 (28%)	15/50 (30%)	13/50 (26%)
Adjusted rate	49.1%	27.8%	31.1%	37.1%	29.9%
Terminal rate	14/36 (39%)	7/35 (20%)	8/38 (21%)	12/37 (32%)	10/40 (25%)
First incidence (days)	128	544	185	635	637
Life table test	P=0.107N	P=0.036N	P=0.073N	P=0.108N	P=0.034N
Logistic regression test	P=0.201N	P=0.016N	P=0.093N	P=0.115N	P=0.064N
Cochran-Armitage test	P=0.169N				
Fisher exact test		P=0.016N	P=0.072N	P=0.107N	P=0.046N
All Organs: Benign Neoplasms					
Overall rate	23/50 (46%)	19/50 (38%)	29/50 (58%)	22/50 (44%)	34/50 (68%)
Adjusted rate	60.4%	48.4%	67.3%	53.4%	70.7%
Terminal rate	21/36 (58%)	15/35 (43%)	24/38 (63%)	18/37 (49%)	26/40 (65%)
First incidence (days)	670	587	625	525	503
Life table test	P=0.058	P=0.316N	P=0.239	P=0.448N	P=0.091
Logistic regression test	P=0.026	P=0.337N	P=0.228	P=0.438N	P=0.038
Cochran-Armitage test	P=0.011				
Fisher exact test		P=0.272N	P=0.158	P=0.500N	P=0.021

TABLE D2c
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:0, 21:60, 70:200, 210:200, and 210:600 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	21 ppm 60 ppm	70 ppm 200 ppm	210 ppm 200 ppm	210 ppm 600 ppm
All Organs: Malignant Neoplasms					
Overall rate	29/50 (58%)	23/50 (46%)	21/50 (42%)	23/50 (46%)	23/50 (46%)
Adjusted rate	60.2%	50.8%	46.1%	52.1%	50.9%
Terminal rate	17/36 (47%)	13/35 (37%)	14/38 (37%)	16/37 (43%)	18/40 (45%)
First incidence (days)	128	461	185	635	637
Life table test	P=0.132N	P=0.265N	P=0.088N	P=0.169N	P=0.108N
Logistic regression test	P=0.278N	P=0.144N	P=0.105N	P=0.170N	P=0.196N
Cochran-Armitage test	P=0.228N				
Fisher exact test		P=0.158N	P=0.081N	P=0.158N	P=0.158N
All Organs: Benign or Malignant Neoplasms					
Overall rate	40/50 (80%)	29/50 (58%)	39/50 (78%)	36/50 (72%)	47/50 (94%)
Adjusted rate	83.2%	64.2%	82.9%	78.2%	94.0%
Terminal rate	28/36 (78%)	19/35 (54%)	30/38 (79%)	27/37 (73%)	37/40 (93%)
First incidence (days)	128	461	185	525	503
Life table test	P=0.134	P=0.079N	P=0.361N	P=0.239N	P=0.374
Logistic regression test	P=0.005	P=0.015N	P=0.507N	P=0.223N	P=0.036
Cochran-Armitage test	P=0.004				
Fisher exact test		P=0.015N	P=0.500N	P=0.241N	P=0.036

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a exposure group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not applicable.
[f] Not applicable; no neoplasms in animal group

TABLE D2d
Statistical Analysis of Liver Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:600 and 210:600 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Liver: Hepatocellular Adenoma		
Overall rate[a]	22/50 (44%)	26/50 (52%)
Adjusted rate[b]	50.9%	54.0%
Terminal rate[c]	16/37 (43%)	18/40 (45%)
First incidence (days)	664	503
Life table test[d]		P=0.410
Logistic regression test[d]		P=0.276
Fisher exact test[d]		P=0.274
Liver: Hepatocellular Carcinoma		
Overall rate	11/50 (22%)	10/50 (20%)
Adjusted rate	26.6%	24.2%
Terminal rate	8/37 (22%)	9/40 (23%)
First incidence (days)	570	670
Life table test		P=0.431N
Logistic regression test		P=0.502N
Fisher exact test		P=0.500N
Liver: Hepatoblastoma or Hepatocellular Carcinoma		
Overall rate	12/50 (24%)	10/50 (20%)
Adjusted rate	29.1%	24.2%
Terminal rate	9/37 (24%)	9/40 (23%)
First incidence (days)	570	670
Life table test		P=0.338N
Logistic regression test		P=0.404N
Fisher exact test		P=0.405N
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	30/50 (60%)	34/50 (68%)
Adjusted rate	66.4%	69.3%
Terminal rate	22/37 (59%)	25/40 (63%)
First incidence (days)	570	503
Life table test		P=0.450
Logistic regression test		P=0.264
Fisher exact test		P=0.266
Liver: Hepatoblastoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma		
Overall rate	30/50 (60%)	34/50 (68%)
Adjusted rate	66.4%	69.3%
Terminal rate	22/37 (59%)	25/40 (63%)
First incidence (days)	570	503
Life table test		P=0.450
Logistic regression test		P=0.264
Fisher exact test		P=0.266

[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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 Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

TABLE D2e
Statistical Analysis of Liver Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 0:200, 70:200, and 210:200 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Liver: Hepatocellular Adenoma			
Overall rate[a]	13/49 (27%)	25/50 (50%)	12/50 (24%)
Adjusted rate[b]	32.3%	60.9%	31.4%
Terminal rate[c]	11/38 (29%)	22/38 (58%)	11/37 (30%)
First incidence (days)	675	660	670
Life table test[d]	P=0.259N	P=0.012	P=0.534N
Logistic regression test[d]	P=0.240N	P=0.010	P=0.516N
Cochran-Armitage test[d]	P=0.208N		
Fisher exact test[d]		P=0.014	P=0.477N
Liver: Hepatocellular Carcinoma			
Overall rate	1/49 (2%)	3/50 (6%)	4/50 (8%)
Adjusted rate	2.6%	7.9%	10.1%
Terminal rate	1/38 (3%)	3/38 (8%)	2/37 (5%)
First incidence (days)	736 (T)	736 (T)	675
Life table test	P=0.166	P=0.305	P=0.175
Logistic regression test	P=0.170	P=0.305	P=0.180
Cochran-Armitage test	P=0.179		
Fisher exact test		P=0.316	P=0.187
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	14/49 (29%)	26/50 (52%)	16/50 (32%)
Adjusted rate	34.8%	63.4%	39.8%
Terminal rate	12/38 (32%)	23/38 (61%)	13/37 (35%)
First incidence (days)	675	660	670
Life table test	P=0.529N	P=0.013	P=0.379
Logistic regression test	P=0.510N	P=0.010	P=0.399
Cochran-Armitage test	P=0.455N		
Fisher exact test		P=0.015	P=0.440

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

TABLE D2f
Statistical Analysis of Liver Neoplasms in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin: Comparison of the 210:0, 210:200, and 210:600 ppm Groups

F_0 Concentration F_1 Concentration	210 ppm 0 ppm	210 ppm 200 ppm	210 ppm 600 ppm
Liver: Hemangiosarcoma			
Overall rate[a]	3/49 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate[b]	8.7%	0.0%	0.0%
Terminal rate[c]	2/33 (6%)	0/37 (0%)	0/40 (0%)
First incidence (days)	682	-[e]	-
Life table test[d]	P=0.065N	P=0.104N	P=0.091N
Logistic regression test[d]	P=0.069N	P=0.103N	P=0.098N
Cochran-Armitage test[d]	P=0.078N		
Fisher exact test[d]		P=0.117N	P=0.117N
Liver: Hepatocellular Adenoma			
Overall rate	11/49 (22%)	12/50 (24%)	26/50 (52%)
Adjusted rate	29.9%	31.4%	54.0%
Terminal rate	8/33 (24%)	11/37 (30%)	18/40 (45%)
First incidence (days)	607	670	503
Life table test P=0.006	P=0.568N	P=0.022	
Logistic regression test	P=0.001	P=0.571N	P=0.003
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.522	P=0.002
Liver: Hepatocellular Carcinoma			
Overall rate	1/49 (2%)	4/50 (8%)	10/50 (20%)
Adjusted rate	2.0%	10.1%	24.2%
Terminal rate	0/33 (0%)	2/37 (5%)	9/40 (23%)
First incidence (days)	486	675	670
Life table test	P=0.006	P=0.216	P=0.013
Logistic regression test	P=0.003	P=0.152	P=0.006
Cochran-Armitage test	P=0.002		
Fisher exact test		P=0.187	P=0.004
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	12/49 (24%)	16/50 (32%)	34/50 (68%)
Adjusted rate	31.3%	39.8%	69.3%
Terminal rate	8/33 (24%)	13/37 (35%)	25/40 (63%)
First incidence (days)	486	670	503
Life table test	P<0.001	P=0.376	P=0.001
Logistic regression test	P<0.001	P=0.329	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.272	P<0.001

[a] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms 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. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE D3
Historical Incidence of Hepatocellular Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
2,4-Dichlorophenol	0/50	2/50	2/50
5,5-Diphenylhydantoin	5/48	0/48	5/48
Ethylene thiourea	2/50	2/50	4/50
Polybrominated biphenyls (Firemaster FF-1®)	4/50	1/50	5/50
Manganese sulfate monohydrate	12/51	3/51	13/51
Pentachlorophenol (Dowicide EC-7)	1/34	0/34	1/34
Pentachlorophenol (technical grade)	3/33	0/33	3/33
Triamterene	10/50	4/50	13/50
Triamterene	7/50	5/50	10/50
Overall Historical Incidence			
Total	110/1,113 (9.9%)	54/1,113 (4.9%)	153/1,113[b] (13.7%)
Standard deviation	7.2%	4.7%	8.6%
Range	0%–28%	0%–20%	3%–34%

[a] Data as of 17 December 1991
[b] Includes one hepatoblastoma

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 60 ppm	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	8	11	9	8	6	6
Natural deaths	6	5	6	4	6	7
Survivors						
Terminal sacrifice	36	34	35	38	38	37
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, cecum	(46)	(15)	(20)	(11)	(10)	(13)
Fibrosis				1 (9%)		
Parasite metazoan					1 (10%)	
Intestine small, duodenum	(47)	(14)	(14)	(10)	(10)	(12)
Inflammation, acute, necrotizing			1 (7%)	2 (20%)	1 (10%)	
Intestine small, ileum	(46)	(15)	(14)	(11)	(11)	(14)
Inflammation, acute, necrotizing				1 (9%)		
Intestine small, jejunum	(48)	(16)	(14)	(11)	(12)	(15)
Hyperplasia, lymphoid	1 (2%)					
Liver	(48)	(49)	(50)	(49)	(50)	(50)
Basophilic focus	1 (2%)	2 (4%)		1 (2%)	2 (4%)	3 (6%)
Clear cell focus	1 (2%)			1 (2%)		
Degeneration, cystic				1 (2%)		1 (2%)
Eosinophilic focus		1 (2%)	3 (6%)	3 (6%)	4 (8%)	5 (10%)
Fatty change	2 (4%)		6 (12%)	23 (47%)	27 (54%)	26 (52%)
Hematopoietic cell proliferation	2 (4%)		2 (4%)	1 (2%)	1 (2%)	
Infarct, multifocal	6 (13%)	5 (10%)	3 (6%)	3 (6%)	2 (4%)	2 (4%)
Infiltration cellular, lymphocyte		1 (2%)				
Inflammation, chronic					1 (2%)	5 (10%)
Mixed cell focus		1 (2%)				
Necrosis, focal			1 (2%)			
Regeneration, diffuse	1 (2%)					
Bile duct, hyperplasia				1 (2%)		
Centrilobular, congestion, chronic						1 (2%)
Centrilobular, hepatocyte hypertrophy[b]			3 (6%)	25 (51%)	25 (50%)	26 (52%)
Hepatocyte, necrosis					1 (2%)	1 (2%)
Mesentery	(8)	(2)	(5)	(2)	(4)	(4)
Inflammation, suppurative			1 (20%)		1 (25%)	1 (25%)
Artery, inflammation, chronic active			1 (20%)			1 (25%)
Fat, necrosis	1 (13%)		2 (40%)		1 (25%)	1 (25%)
Pancreas	(47)	(15)	(15)	(11)	(12)	(12)
Inflammation, chronic			1 (7%)	1 (9%)		
Acinus, atrophy	6 (13%)		2 (13%)	2 (18%)	2 (17%)	
Acinus, focal cellular change	1 (2%)					
Duct, dilatation	1 (2%)		1 (7%)		1 (8%)	
Duct, hyperplasia				1 (9%)		
Salivary glands	(49)	(15)	(14)	(11)	(12)	(14)
Necrosis		1 (7%)				

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	8	6
Natural deaths	5	4
Survivors		
Terminal sacrifice	37	40
Animals examined microscopically	50	50
Alimentary System		
Intestine large, colon	(48)	(49)
Parasite metazoan	1 (2%)	
Intestine small, jejunum	(48)	(48)
Edema		1 (2%)
Inflammation, acute, necrotizing	1 (2%)	1 (2%)
Liver	(50)	(50)
Basophilic focus	4 (8%)	3 (6%)
Clear cell focus	1 (2%)	5 (10%)
Degeneration, cystic	5 (10%)	
Eosinophilic focus	8 (16%)	8 (16%)
Fibrosis, multifocal		1 (2%)
Hematopoietic cell proliferation		1 (2%)
Infarct, multifocal	11 (22%)	6 (12%)
Inflammation, chronic	2 (4%)	
Mixed cell focus		2 (4%)
Vacuolization cytoplasmic		1 (2%)
Centrilobular, hepatocyte hypertrophy	31 (62%)	37 (74%)
Mesentery	(6)	(6)
Artery, inflammation, chronic active		1 (17%)
Pancreas	(48)	(49)
Inflammation, chronic	1 (2%)	
Acinus, atrophy	3 (6%)	5 (10%)
Acinus, depletion secretory	1 (2%)	1 (2%)
Artery, inflammation, chronic active		2 (4%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 60 ppm	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Alimentary System (continued)						
Stomach, forestomach	(48)	(20)	(20)	(16)	(12)	(20)
Cyst			1 (5%)		1 (8%)	
Hyperplasia, squamous				3 (19%)		4 (20%)
Epithelium, hyperplasia			2 (10%)	2 (13%)		2 (10%)
Stomach, glandular	(48)	(16)	(16)	(13)	(11)	(15)
Cyst			1 (6%)			
Artery, inflammation, chronic active						1 (7%)
Tooth	(5)			(1)		(1)
Incisor, upper, dysplasia	3 (60%)			1 (100%)		1 (100%)
Cardiovascular System						
Heart	(50)	(16)	(15)	(13)	(12)	(13)
Bacterium				1 (8%)		1 (8%)
Cardiomyopathy, chronic active	2 (4%)					1 (8%)
Mineralization		1 (6%)				
Artery, inflammation, chronic active			2 (13%)			2 (15%)
Atrium, thrombosis				1 (8%)		
Valve, inflammation, suppurative				1 (8%)		
Endocrine System						
Adrenal gland, cortex	(48)	(16)	(14)	(11)	(12)	(13)
Hematopoietic cell proliferation					1 (8%)	
Hypertrophy	3 (6%)					
Necrosis	1 (2%)					
Corticomedullary junction, degeneration, fatty	5 (10%)				1 (8%)	
Adrenal gland, medulla	(48)	(16)	(14)	(11)	(12)	(13)
Hyperplasia	1 (2%)	1 (6%)		1 (9%)		
Pituitary gland	(44)	(19)	(17)	(15)	(14)	(17)
Pars distalis, hyperplasia	19 (43%)		1 (6%)	2 (13%)	1 (7%)	2 (12%)
Thyroid gland	(47)	(16)	(15)	(11)	(12)	(14)
Inflammation, chronic active	3 (6%)					
Follicular cell, hyperplasia	4 (9%)	1 (6%)		2 (18%)	1 (8%)	1 (7%)
General Body System						
None						

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Alimentary System (continued)		
Stomach, forestomach	(48)	(49)
Diverticulum		1 (2%)
Inflammation, chronic active		1 (2%)
Tooth	(9)	(12)
Incisor, upper, dysplasia	7 (78%)	10 (83%)
Peridontal tissue, inflammation, suppurative		1 (8%)
Heart	(50)	(50)
Artery, inflammation, chronic active		2 (4%)
Endocrine System		
Adrenal gland, cortex	(48)	(50)
Hematopoietic cell proliferation	1 (2%)	1 (2%)
Hyperplasia	1 (2%)	
Hypertrophy	2 (4%)	
Thrombosis	1 (2%)	
Adrenal gland, medulla	(48)	(50)
Hyperplasia		1 (2%)
Pituitary gland	(41)	(44)
Pars distalis, hyperplasia	17 (41%)	17 (39%)
Thyroid gland	(50)	(49)
Inflammation, chronic active		4 (8%)
Follicular cell, hyperplasia	13 (26%)	15 (31%)
General Body System		
None		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 60 ppm	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Genital System						
Clitoral gland	(1)					(1)
Duct, dilatation	1 (100%)					1 (100%)
Ovary	(49)	(28)	(36)	(30)	(25)	(28)
Angiectasis	1 (2%)	1 (4%)		1 (3%)		
Cyst	25 (51%)	17 (61%)	29 (81%)	24 (80%)	15 (60%)	16 (57%)
Fibrosis				1 (3%)		
Inflammation, chronic active				1 (3%)	2 (8%)	6 (21%)
Mineralization	1 (2%)					
Thrombosis	2 (4%)		1 (3%)	1 (3%)	1 (4%)	
Uterus	(49)	(36)	(31)	(32)	(33)	(33)
Dilatation		2 (6%)	1 (3%)		2 (6%)	1 (3%)
Fibrosis				1 (3%)		
Inflammation, chronic active						2 (6%)
Inflammation, suppurative	1 (2%)		2 (6%)	1 (3%)	3 (9%)	2 (6%)
Thrombosis				1 (3%)		
Endometrium, hyperplasia, cystic	15 (31%)	18 (50%)	16 (52%)	25 (78%)	24 (73%)	20 (61%)
Hematopoietic System						
Bone marrow	(48)	(16)	(15)	(11)	(12)	(13)
Myelofibrosis	1 (2%)					
Femoral, myelofibrosis	2 (4%)			1 (9%)		
Lymph node	(50)	(18)	(16)	(20)	(15)	(20)
Lumbar, edema						1 (5%)
Mandibular, edema				1 (5%)		1 (5%)
Mandibular, hyperplasia, lymphoid				1 (5%)		
Mediastinal, inflammation, suppurative					2 (13%)	
Pancreatic, hyperplasia, lymphoid				1 (5%)		
Lymph node, mesenteric	(12)	(7)	(5)	(9)	(4)	(6)
Angiectasis	3 (25%)	2 (29%)	1 (20%)	1 (11%)		
Necrosis		1 (14%)				
Spleen	(48)	(23)	(27)	(27)	(23)	(23)
Hematopoietic cell proliferation	6 (13%)	1 (4%)	5 (19%)	3 (11%)	6 (26%)	4 (17%)
Hyperplasia, lymphoid		2 (9%)	3 (11%)	2 (7%)	3 (13%)	
Necrosis		1 (4%)				
Thymus	(42)	(12)	(11)	(10)	(11)	(9)
Necrosis	1 (2%)					
Integumentary System						
Mammary gland	(46)	(15)	(12)	(5)	(9)	(13)
Hyperplasia, cystic	1 (2%)					
Skin	(49)	(16)	(17)	(13)	(12)	(13)
Inflammation, chronic active	1 (2%)					1 (8%)
Subcutaneous tissue, fibrosis	1 (2%)					
Musculoskeletal System						
None						

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Genital System		
Ovary	(49)	(50)
Angiectasis	2 (4%)	2 (4%)
Cyst	18 (37%)	13 (26%)
Thrombosis	1 (2%)	1 (2%)
Uterus	(50)	(50)
Dilatation	3 (6%)	3 (6%)
Inflammation, suppurative	1 (2%)	2 (4%)
Endometrium, hyperplasia, cystic	8 (16%)	
Hematopoietic System		
Bone marrow	(50)	(49)
Femoral, myelofibrosis	3 (6%)	2 (4%)
Lymph node, mesenteric	(6)	(6)
Angiectasis	1 (17%)	
Hyperplasia, lymphoid	1 (17%)	
Spleen	(48)	(50)
Hematopoietic cell proliferation	2 (4%)	
Integumentary System		
Skin	(50)	(49)
Cyst	1 (2%)	
Mineralization	1 (2%)	
Musculoskeletal System		
None		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	210 ppm 0 ppm	21 ppm 60 ppm	0 ppm 200 ppm	70 ppm 200 ppm	210 ppm 200 ppm
Nervous System						
Brain	(50)	(16)	(15)	(12)	(13)	(13)
Compression	1 (2%)	1 (6%)				
Hemorrhage	2 (4%)					
Necrosis	1 (2%)		1 (7%)			
Artery, inflammation, chronic active			1 (7%)			
Meninges, infiltration cellular, lymphocyte						1 (8%)
Respiratory System						
Lung	(50)	(20)	(20)	(13)	(13)	(15)
Bacterium				1 (8%)		
Hemorrhage, chronic						1 (7%)
Hyperplasia, lymphoid					1 (8%)	
Inflammation, acute				1 (8%)		
Inflammation, chronic active	3 (6%)	2 (10%)			2 (15%)	3 (20%)
Alveolar epithelium, hyperplasia	4 (8%)		1 (5%)			
Nose	(50)	(16)	(15)	(12)	(12)	(13)
Mucosa, inflammation, suppurative	1 (2%)					
Nasolacrimal duct, inflammation, suppurative			1 (7%)			
Special Senses System						
Eye	(2)	(2)	(1)	(3)	(1)	(2)
Degeneration	2 (100%)			3 (100%)	1 (100%)	2 (100%)
Cornea, inflammation, chronic active		2 (100%)				
Harderian gland	(8)	(4)	(4)	(4)	(4)	(3)
Hyperplasia	3 (38%)		1 (25%)			1 (33%)
Inflammation, chronic active	1 (13%)					
Zymbal's gland				(1)		
Dilatation				1 (100%)		
Urinary System						
Kidney	(48)	(16)	(16)	(12)	(14)	(13)
Cyst			2 (13%)			
Nephropathy, chronic	14 (29%)	4 (25%)		1 (8%)	1 (7%)	4 (31%)
Artery, inflammation, chronic active						1 (8%)
Glomerulus, inflammation, membranoproliferative	1 (2%)	1 (6%)				1 (8%)
Pelvis, inflammation, suppurative						1 (8%)
Renal tubule, necrosis	1 (2%)		1 (6%)			1 (8%)
Renal tubule, regeneration			1 (6%)			
Urinary bladder	(48)	(15)	(15)	(11)	(11)	(13)
Artery, inflammation, chronic active				1 (9%)	1 (9%)	1 (8%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 600 ppm	210 ppm 600 ppm
Nervous System		
Brain	(49)	(50)
Hemorrhage		1 (2%)
Respiratory System		
Lung	(50)	(50)
Inflammation, chronic active	4 (8%)	1 (2%)
Alveolar epithelium, hyperplasia	2 (4%)	
Nose	(50)	(50)
Mucosa, inflammation, suppurative		1 (2%)
Nasolacrimal duct, inflammation, suppurative		1 (2%)
Special Senses System		
None		
Urinary System		
Kidney	(50)	(50)
Metaplasia, osseous		1 (2%)
Nephropathy, chronic	17 (34%)	14 (28%)
Urinary bladder	(49)	(49)
Artery, inflammation, chronic active		1 (2%)

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Cytomegaly was the term used by the laboratory pathologist to record centrilobular hepatocyte enlargement that occurred in this study. Based upon the morphology of this change, the term hypertrophy is used in place of cytomegaly throughout this report because it is more widely used and understood.

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983). 5,5-Diphenylhydantoin was sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). It 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. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of 5,5-diphenylhydantoin. In the absence of toxicity, 10,000 μg/plate was selected as the high dose. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that was not dose related, not reproducible, or was not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies was observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by Myhr *et al.* (1985). 5,5-Diphenylhydantoin was supplied as a coded aliquot by Radian Corporation. The high dose of 5,5-diphenylhydantoin was determined by solubility. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in Fischer's medium supplemented with *l*-glutamine, sodium pyruvate, pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed to medium containing THMG (thymidine, hypoxanthine, methotrexate, and glycine) for 1 day, to medium containing THG (thymidine, hypoxanthine, and glycine) for 1 day, and to normal medium for 3 to 5 days. For cloning, the horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with 5,5-diphenylhydantoin continued for 4 hours, at which time the medium plus 5,5-diphenylhydantoin was removed and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant ($TK^{-/-}$) cells; 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. The test was initially performed without S9. If a clearly positive response was not obtained, the test was repeated using freshly prepared S9 from the livers of Aroclor 1254-induced male Fischer 344 rats.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). All data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for 5,5-diphenylhydantoin to be

considered positive, *i.e.*, capable of inducing TFT resistance. A single significant response led to a "questionable" conclusion, and the absence of both a trend and peak response resulted in a "negative" call.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). 5,5-Diphenylhydantoin was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), 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-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of 5,5-diphenylhydantoin; the high dose was limited by toxicity. A single flask per dose was used.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with 5,5-diphenylhydantoin in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing 5,5-diphenylhydantoin was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 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 5,5-diphenylhydantoin, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no 5,5-diphenylhydantoin and incubation proceeded for an additional 26 to 28 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). 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. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with 5,5-diphenylhydantoin for 12 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with 5,5-diphenylhydantoin and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 12 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.

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. One hundred first-division metaphase cells were scored at each dose level. 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).

Chromosomal aberration data are presented as percentage of cells with aberrations. Statistical analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P\leq0.05$) difference for one dose point and a significant trend ($P\leq0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated

the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987).

DROSOPHILA MELANOGASTER TEST PROTOCOL

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described by Woodruff *et al.* (1985). 5,5-Diphenylhydantoin was supplied as a coded aliquot by Radian Corporation. It was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no positive response was obtained, 5,5-diphenylhydantoin was retested by injection into adult male flies.

To administer 5,5-diphenylhydantoin by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament, and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 μL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector which automatically delivered a calibrated volume. Flies were anesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of 5,5-diphenylhydantoin at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection while keeping induced sterility at an acceptable level. Oral exposure was achieved by allowing Canton-S males to feed for 72 hours on a solution of 5,5-diphenylhydantoin in 5% sucrose. In the injection experiments, 24- to 72-hour old Canton-S males were treated with a solution of 5,5-diphenylhydantoin dissolved in saline and allowed to recover for 24 hours. A concurrent saline control group was also included. In the adult exposures, treated males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings were treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male results from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls, using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10%, or if the P value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or (b) the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

MOUSE BONE MARROW SISTER CHROMATID EXCHANGE TEST PROTOCOL

A dose range-finding study was performed; the highest dose was limited to 250 mg/kg. 5,5-Diphenylhydantoin was tested for the induction of SCEs in mouse bone marrow using two protocols. Male B6C3F_1 mice (five animals per dose group) were injected intraperitoneally with 5,5-diphenylhydantoin dissolved in corn oil (injection volume = 0.4 mL). Solvent control mice received equivalent injections of corn oil only. The positive control was dimethylbenzanthracene.

The first protocol had a standard harvest time of 23 hours, and the second protocol had a delayed harvest of 42 hours. The mice were implanted subcutaneously with a BrdU tablet (McFee *et al.*, 1983) 24 hours before harvest (1 hour before 5,5-diphenylhydantoin treatment in the case of the standard protocol). The use of BrdU allowed selection of the appropriate cell population (cells in the second metaphase following 5,5-diphenylhydantoin treatment) for scoring. Two hours before sacrifice, the mice received an intraperitoneal injection of colchicine in saline. The animals were killed by cervical dislocation 23 or 42 hours after treatment. One or both femurs were removed, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). The cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained using fluorescence-plus-Giemsa, and scored.

Twenty-five second-division metaphase cells were scored from each of four animals per treatment. Responses were evaluated as SCEs/cell, and the data were analyzed by a trend test with significance set at $P \leq 0.05$ (Margolin *et al.*, 1986). Individual dose points were compared to the solvent control using a one-tailed *t*-test.

Mouse Bone Marrow Chromosomal Aberrations Test Protocol

A dose range-finding study was performed; the highest dose was limited by induction of cell cycle delay. 5,5-Diphenylhydantoin was tested for induction of Abs in mouse bone marrow using two different protocols. The first protocol used a standard harvest time of 17 hours and the second protocol used a delayed harvest time of 36 hours for the control and 125 mg/kg groups, and 42 hours for the 250 and 500 mg/kg groups.

Male B6C3F$_1$ mice (10 animals per dose group) were injected intraperitoneally with 5,5-diphenylhydantoin dissolved in corn oil (injection volume = 0.4 mL). Solvent control mice received equivalent injections of corn oil only. The positive control was dimethylbenzanthracene. The mice were subcutaneously implanted with a BrdU tablet (McFee *et al.*, 1983) 18 hours before the scheduled harvest. (For the standard protocol, this required BrdU implantation to precede injection with 5,5-diphenylhydantoin by 1 hour.) The use of BrdU allowed selection of the appropriate cell population for scoring. (Abs induced by chemical administration are present in maximum number at the first metaphase following treatment; they decline in number during subsequent nuclear divisions due to cell death.) Two hours before sacrifice, the mice received an intraperitoneal injection of colchicine in saline. The animals were killed by cervical dislocation 17, 36, or 42 hours after 5,5-diphenylhydantoin injection (18 hours after BrdU dosing). One or both femurs were removed and the marrow was flushed out with phosphate-buffered saline (pH 7.0). Cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained and scored.

Fifty first-division metaphase cells were scored from each of eight animals per treatment. Responses were evaluated as the percentage of aberrant metaphase cells, excluding gaps. The data from mice treated with 5,5-diphenylhydantoin were analyzed by a trend test with significance set at $P \leq 0.05$ (Margolin *et al.*, 1986). Positive control data were analyzed by pairwise comparison. Individual dose points were compared to the solvent control using a one-tailed *t*-test.

Mouse Bone Marrow Micronucleus Test Protocols

Intraperitoneal Injection: Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility, toxicity, and the extent of cell cycle delay induced by 5,5-diphenylhydantoin exposure. Male B6C3F$_1$ mice were injected intraperitoneally three times at 24-hour intervals with 5,5-diphenylhydantoin dissolved in corn oil; the total dosing volume was 0.4 mL. Solvent control animals were injected with 0.4 mL of corn oil only. The positive control mice received injections of dimethylbenzanthracene. The mice were killed by cervical dislocation 24 hours after the final injection,

and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five animals per dose group. The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. Data were analyzed by a one-tailed trend test with significance set at $P \leq 0.05$ (Margolin *et al.*, 1986).

Intravenous (Caudal) Injection: Preliminary range finding studies were not performed because this study was an attempt to replicate a published protocol (de Oca-Luna *et al.*, 1984). Male Balb/C mice (five per dose group) were given caudal vein injections of 0.1 to 20.0 mg/kg 5,5-diphenylhydantoin in 0.1 mL of 0.1 normal sodium hydroxide. Two levels (0.5 and 5.0 mg/kg) of mitomycin-C were used as positive controls. Twenty-four hours after injection, the mice were killed and femoral bone marrow smears were prepared. Air-dried slides were fixed and stained. Two thousand PCEs were scored per animal. The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. Data were analyzed by a one-tailed trend test with significance set at $P \leq 0.05$ (Margolin *et al.*, 1986).

RESULTS

5,5-Diphenylhydantoin (100 to 10,000 μg/plate) was not mutagenic in any of four strains of *S. typhimurium* (TA98, TA100, TA1535, and TA1537) when tested in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 in each of two laboratories (Table E1; Haworth *et al.*, 1983). No induction of trifluorothymidine resistance was observed in L5178Y mouse lymphoma cells tested with and without S9 from Aroclor 1254-induced male F344 rat liver (Table E2; Myhr *et al.*, 1985). Concentrations of 5,5-diphenylhydantoin tested in this assay ranged from 15 to 500 μg/mL in the absence of S9, and 18.75 to 350 μg/mL in the presence of S9; relative total growth at the highest concentrations was less than 30%. In cytogenetic tests with CHO cells, high doses of 5,5-diphenylhydantoin (1.6 and 5.0 mg/mL) induced a small, but statistically significant, increase in SCEs in the trial conducted in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Table E3; Galloway *et al.*, 1987); no increase in SCEs was observed in CHO cells treated in the absence of S9, nor were Abs induced in CHO cells treated with 5,5-diphenylhydantoin with or without S9 (Table E4; Galloway *et al.*, 1987). Doses of 5,5-diphenylhydantoin used in the Abs test equaled or exceeded those used in the SCE test. No induction of sex-linked recessive lethal mutations was observed in the germ cells of adult male *Drosophila melanogaster* administered 5,5-diphenylhydantoin by feeding (5,000 ppm) or by injection (100 ppm) (Table E5; Woodruff *et al.*, 1985).

5,5-Diphenylhydantoin was also tested *in vivo* following a single intraperitoneal injection for induction of cytogenetic effects in mouse bone marrow cells (McFee *et al.*, 1992). Weakly positive responses were observed in the SCE test at both the standard (23-hour) and the extended (42-hour) post-treatment sample times (Table E6), but no increase in Abs was observed in samples taken 17, 36, or 42 hours after treatment (Table E7). In the 23-hour exposure, the middle dose of 125 mg/kg produced a significant increase in SCEs, and this was sufficient for the trial to be considered positive. The data from the 42-hour harvest time showed a small, dose-related increase in SCEs, but no individual doses were judged positive. The results of this second trial were considered to be questionable, and the assay was concluded to be equivocal. No significant increase in the frequency of micronucleated PCEs was observed in bone marrow of male $B6C3F_1$ mice treated with 3 intraperitoneal injections of 5,5-diphenylhydantoin (17.5, 35, and 70 mg/kg) dissolved in corn oil (Table E8). Also, no increase was observed in the frequency of micronucleated PCEs in bone marrow of male Balb/C mice administered a single caudal vein injection of 0.1 to 20.0 mg/kg 5,5-diphenylhydantoin dissolved in 0.1 N NaOH (Table E9).

TABLE E1
Mutagenicity of 5,5-Diphenylhydantoin in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SRI, International							
TA100	0.0	86 ± 3.3	110 ± 5.0	103 ± 5.7	113 ± 2.1	122 ± 13.9	94 ± 3.2
	100.0	94 ± 3.3	129 ± 6.6	104 ± 2.6	99 ± 7.8	118 ± 15.1	108 ± 2.4
	333.3	95 ± 4.8	135 ± 4.2	91 ± 0.9	102 ± 2.1	128 ± 10.3	105 ± 6.2
	1,000.0	94 ± 9.8[c]	136 ± 10.2[c]	87 ± 8.0[c]	91 ± 3.8[c]	124 ± 5.8[c]	103 ± 5.0[c]
	3,333.3	87 ± 3.6[c]	149 ± 3.0[c]	104 ± 4.6[c]	92 ± 9.5[c]	111 ± 8.1[c]	105 ± 2.7[c]
	10,000.0	80 ± 2.0[c]	136 ± 4.9[c]	92 ± 6.4[c]	95 ± 5.2[c]	94 ± 3.2[c]	108 ± 8.3[c]
Trial summary		Negative	Equivocal	Negative	Negative	Negative	Negative
Positive control[d]		494 ± 7.3	457 ± 5.0	1,859 ± 47.0	1,380 ± 36.4	1,523 ± 14.2	765 ± 59.6
TA1535	0.0	24 ± 4.8	36 ± 1.5	7 ± 0.9	16 ± 1.5	14 ± 1.9	17 ± 4.6
	100.0	22 ± 1.3	40 ± 2.4	15 ± 0.9	12 ± 3.2	15 ± 1.5	19 ± 3.7
	333.3	24 ± 7.4	48 ± 2.3	12 ± 2.3	16 ± 1.5	14 ± 2.1	17 ± 1.3
	1,000.0	17 ± 2.2[c]	37 ± 2.3[c]	13 ± 3.5[c]	17 ± 1.3[c]	16 ± 2.3[c]	16 ± 1.9[c]
	3,333.3	22 ± 1.5[c]	48 ± 3.8[c]	9 ± 2.0[c]	11 ± 2.7[c]	13 ± 1.9[c]	18 ± 1.5[c]
	10,000.0	23 ± 2.8[c]	52 ± 4.1[c]	15 ± 0.6[c]	9 ± 1.5[c]	11 ± 2.2[c]	14 ± 2.1[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		372 ± 12.7	376 ± 5.7	430 ± 16.8	306 ± 6.4	251 ± 12.8	321 ± 44.2
TA1537	0.0	12 ± 0.3	17 ± 1.5	18 ± 1.8	40 ± 1.7	23 ± 2.3	36 ± 3.7
	100.0	18 ± 1.5	33 ± 7.8	20 ± 3.5	15 ± 1.8	14 ± 2.2	18 ± 3.0
	333.3	14 ± 2.9	29 ± 0.3	18 ± 2.3	23 ± 3.5	17 ± 3.8	18 ± 5.2
	1,000.0	10 ± 1.5[c]	23 ± 1.7[c]	13 ± 0.6[c]	13 ± 0.7[c]	13 ± 2.3[c]	13 ± 1.8[c]
	3,333.3	8 ± 1.3[c]	18 ± 1.0[c]	11 ± 0.6[c]	12 ± 2.6[c]	11 ± 3.8[c]	16 ± 1.2[c]
	10,000.0	8 ± 0.3[c]	12 ± 1.5[c]	13 ± 1.3[c]	5 ± 1.5[c]	10 ± 1.2[c]	12 ± 2.7[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		146 ± 28.6	308 ± 8.0	59 ± 3.9	635 ± 17.2	413 ± 13.5	319 ± 28.4
TA98	0.0	19 ± 4.4	37 ± 3.5	36 ± 3.0	50 ± 2.9	31 ± 4.1	47 ± 5.8
	100.0	20 ± 2.9	41 ± 4.0	34 ± 4.3	50 ± 1.5	34 ± 1.5	56 ± 4.9
	333.3	18 ± 0.9	44 ± 0.6	31 ± 7.2	61 ± 6.6	33 ± 1.5	39 ± 3.0
	1,000.0	14 ± 1.5[c]	40 ± 2.0[c]	27 ± 4.0[c]	54 ± 3.0[c]	32 ± 1.3[c]	46 ± 4.7[c]
	3,333.3	21 ± 2.8[c]	42 ± 2.7[c]	32 ± 3.5[c]	40 ± 1.3[c]	24 ± 3.4[c]	48 ± 2.6[c]
	10,000.0	20 ± 2.3[c]	37 ± 4.0[c]	29 ± 1.3[c]	51 ± 3.2[c]	27 ± 2.3[c]	58 ± 7.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		669 ± 20.2	850 ± 18.0	1,723 ± 57.6	1,170 ± 9.5	1,300 ± 32.9	509 ± 20.5

TABLE E1
Mutagenicity of 5,5-Diphenylhydantoin in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Institute							
TA100	0	106 ± 8.7	94 ± 0.7	114 ± 1.7	101 ± 2.4	118 ± 9.2	98 ± 7.2
	100	105 ± 2.4	87 ± 3.8	110 ± 6.1	103 ± 3.3	94 ± 8.4	97 ± 3.8
	333	103 ± 5.0	89 ± 7.8	108 ± 9.7	92 ± 3.3	101 ± 4.7	108 ± 4.7
	1,000	81 ± 3.3	84 ± 3.8	99 ± 8.7	102 ± 3.2	93 ± 5.0	107 ± 8.4
	3,333	79 ± 2.9[c]	86 ± 3.2[c]	90 ± 3.8[c]	98 ± 0.6[c]	96 ± 12.8[c]	100 ± 3.3[c]
	10,000	73 ± 5.6[c]	63 ± 6.0[c]	105 ± 2.9[c]	84 ± 3.5[c]	94 ± 3.1[c]	95 ± 5.5[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,417 ± 96.6	2,161 ± 64.3	2,384 ± 21.1	838 ± 80.8	1,337 ± 70.0	632 ± 61.1
TA1535	0	15 ± 1.5	20 ± 1.5	10 ± 4.0	11 ± 1.5	9 ± 1.3	11 ± 1.9
	100	14 ± 3.5	24 ± 2.6	8 ± 2.1	11 ± 2.3	11 ± 2.4	8 ± 2.5
	333	14 ± 1.0	23 ± 2.3	9 ± 1.5	11 ± 2.7	7 ± 1.3	9 ± 2.3
	1,000	21 ± 0.9	24 ± 2.8	10 ± 0.9	8 ± 2.9	7 ± 0.9	6 ± 2.1
	3,333	18 ± 0.3[c]	18 ± 0.3[c]	10 ± 0.6[c]	8 ± 2.3[c]	5 ± 1.3[c]	10 ± 0.9[c]
	10,000	15 ± 1.2[c]	12 ± 2.1[c]	5 ± 0.3[c]	9 ± 1.5[e]	6 ± 0.3[c]	9 ± 1.5[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,068 ± 11.4	1,496 ± 75.6	117 ± 13.5	62 ± 12.2	84 ± 2.9	56 ± 7.2
TA1537	0	7 ± 2.0	4 ± 1.2	8 ± 2.1	7 ± 2.1	6 ± 2.3	9 ± 1.3
	100	5 ± 0.3	6 ± 2.5	7 ± 0.7	5 ± 2.0	6 ± 1.5	6 ± 0.6
	333	4 ± 1.5	6 ± 2.2	5 ± 0.6	7 ± 0.3	7 ± 1.2	7 ± 1.2
	1,000	7 ± 2.6	4 ± 0.9	6 ± 0.9	4 ± 1.2	4 ± 0.3	5 ± 0.6
	3,333	5 ± 0.6[c]	4 ± 0.9[c]	5 ± 1.0[c]	4 ± 0.6[c]	7 ± 1.5[c]	5 ± 0.9[c]
	10,000	3 ± 0.9[c]	1 ± 0.7[c]	3 ± 0.7[c]	3 ± 1.2[c]	5 ± 1.7[c]	4 ± 0.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		936 ±151.4	287 ± 6.4	135 ± 18.2	92 ± 10.8	60 ± 4.7	48 ± 10.4
TA98	0	15 ± 1.5	10 ± 2.1	24 ± 2.2	25 ± 2.0	24 ± 3.0	20 ± 5.2
	100	23 ± 2.0	14 ± 0.6	24 ± 1.9	20 ± 1.5	16 ± 3.4	27 ± 2.3
	333	19 ± 1.7	13 ± 2.3	27 ± 2.1	26 ± 1.9	19 ± 1.7	24 ± 0.9
	1,000	14 ± 0.3	17 ± 2.3	22 ± 1.9	19 ± 0.9	25 ± 4.1	22 ± 0.3
	3,333	17 ± 0.7[c]	13 ± 0.7[c]	23 ± 5.5[c]	22 ± 1.2[c]	21 ± 2.0[c]	20 ± 1.9
	10,000	10 ± 2.9[c]	8 ± 0.3[c]	16 ± 4.2[c]	18 ± 2.2[c]	15 ± 2.9[c]	21 ± 2.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,449 ± 94.2	1,812 ± 30.2	2,027 ± 72.1	428 ± 28.5	1,029 ± 83.0	361 ± 22.0

[a] The detailed protocol and these data are presented in Haworth *et al.* (1983).
[b] Revertants are presented as mean ± standard error from three plates.
[c] Precipitate on plate
[d] The positive controls in the absence of metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 5,5-Diphenylhydantoin[a]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
-S9						
Trial 1						
Dimethylsulfoxide		84	90	69	27	
		73	94	63	29	
		83	112	44	18	
		70	104	65	31	26
Ethyl methanesulfonate		37	42	304	274	
	250	34	47	271	266	270*
Methyl methanesulfonate		35	35	103	99	
	15	46	39	106	77	88*
5,5-Diphenylhydantoin						
	31.25	70	96	61	29	
		59	84	46	26	28
	62.5[c]	69	84	62	30	
		75	84	84	37	34
	125	54	46	58	36	
		57	52	51	30	33
	250	50	32	74	50	
		47	32	30	21	36
	500	46	13	35	25	
		56	31	58	35	30

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 5,5-Diphenylhydantoin (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 2						
Dimethylsulfoxide		99	106	92	31	
		85	98	90	35	
		85	106	121	48	
		74	89	84	38	38
Ethyl methanesulfonate		68	62	631	308	
	250	57	52	540	316	312*
Methyl methanesulfonate			31	19	199	214
	15	29	17	169	195	205*
5,5-Diphenylhydantoin						
	15	76	95	101	44	
		76	100	96	42	43
	30	78	79	80	34	
		78	88	99	42	38
	60	65	60	103	53	
		70	74	112	54	53
	120	58	21	83	48	
		52	35	91	58	53
	240	65	29	162	84	
		58	22	169	96	90*

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 5,5-Diphenylhydantoin (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 3						
Dimethylsulfoxide		78	118	77	33	
		81	124	75	31	
		50	74	80	53	
		52	85	65	41	40
Ethyl methanesulfonate		56	58	648	387	
	250	48	53	555	388	387*
5,5-Diphenylhydantoin						
	17.5	41	58	62	51	
		50	77	82	55	53
	35	49	72	70	47	
		46	73	46	33	40
	70[c]	72	54	91	42	
		64	68	62	33	37
	140	66	31	79	40	
		67	36	56	28	34
	280	73	32	76	35	
		58	28	74	43	39

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 5,5-Diphenylhydantoin (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 4						
Dimethylsulfoxide		95	114	64	22	
		116	88	105	30	
		86	90	58	22	
		106	107	61	19	24
Methyl methanesulfonate		37	18	256	234	
	15	29	17	219	249	241*
5,5-Diphenylhydantoin						
	17.5	102	105	75	25	
		93	77	89	32	28
	35	107	85	98	31	
		101	79	73	24	27
	70	101	78	70	23	
		114	85	125	37	30
	140	77	22	74	32	
		90	27	86	32	32
	280	88	12	69	26	
		85	11	67	26	26

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 5,5-Diphenylhydantoin (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 1						
Dimethylsulfoxide		70	99	72	35	
		86	94	84	32	
		83	104	68	27	
		84	103	88	35	32
Methylcholanthrene		41	28	490	400	
	2.5	50	32	477	319	360*
5,5-Diphenylhydantoin						
	18.75	83	85	80	32	
		84	94	80	32	32
	37.5	76	106	66	29	
		72	112	64	30	29
	75[c]	86	80	88	34	
		72	58	79	37	35
	150	73	48	76	35	
		89	43	60	23	29
	300	63	14	47	25	
		78	15	74	32	28

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 5,5-Diphenylhydantoin (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Dimethylsulfoxide		75	108	31	14	
		81	98	47	19	
		90	103	118	44	
		76	91	50	22	25
Methylcholanthrene		56	36	344	205	
	2.5	66	43	479	241	223*
5,5-Diphenylhydantoin						
	50	82	107	66	27	
		102	123	64	21	24
	100[c]	85	31	70	28	
		74	49	56	25	26
	150	99	34	55	19	
		64	21	36	19	19
	200	96	51	85	30	
		81	66	47	19	24
	250	90	66	96	35	
		93	41	58	21	28
	300	69	32	56	27	
		59	22	68	38	33
	350	88	26	70	26	
		79	21	108	46	36

* Significant positive response ($P \leq 0.05$)
[a] Study performed at Inveresk Research International. The experimental protocol and these data are presented by Myhr *et al.* (1985).
[b] Mean ± standard error from three replicate plates of approximately 10^6 cells each. Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF per 10^6 cells treated); MF = mutant fraction.
[c] Precipitate formed at this and all higher doses of 5,5-diphenylhydantoin.

TABLE E3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 5,5-Diphenylhydantoin[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[b]
-S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,046	502	0.47	10.0	26.0	
Triethylenemelamine	0.0150	50	1,048	1,611	1.53	32.2	26.0	220.31
5,5-Diphenylhydantoin	16	50	1,044	525	0.50	10.5	26.0	4.78
	50	50	1,047	503	0.48	10.1	26.0	0.10
	160	50	1,043	486	0.46	9.7	26.0	–2.91
								P=0.748[c]
+S9								
Trial 1								
Summary: Positive								
Dimethylsulfoxide		50	1,045	395	0.37	7.9	26.0	
Cyclophosphamide	1	50	1,050	1,291	1.22	25.8	26.0	225.28
5,5-Diphenylhydantoin	16	50	1,048	448	0.42	9.0	26.0	13.09
	50	50	1,049	473	0.45	9.5	26.0	19.29
	160	50	1,048	475	0.45	9.5	26.0	19.91
	1,600	50	1,037	488	0.47	9.8	28.0	24.50*
	5,000	50	1,046	485	0.46	9.7	28.0	22.67*
								P=0.001

* Positive (≥20% increase over solvent control)
[a] Study performed at Columbia University. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. The experimental protocol and these data are presented in Galloway *et al.* (1987).
[b] SCEs/chromosome of culture exposed to 5,5-diphenylhydantoin relative to those of culture exposed to solvent
[c] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose

TABLE E4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 5,5-Diphenylhydantoin[a]

-S9 Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells w/Abs	+S9 Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells w/Abs
Trial 1 - Harvest time: 14.0 hours Summary: Negative					**Trial 1** - Harvest time: 14.0 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	2	0.02	2.0		100	3	0.03	3.0
Triethylenemelamine					Cyclophosphamide				
0.1500	100	31	0.31	24.0	15	100	33	0.33	30.0
5,5-Diphenylhydantoin					5,5-Diphenylhydantoin				
500	100	3	0.03	3.0	16	100	5	0.05	5.0
1,600	100	2	0.02	2.0	50	100	8	0.08	7.0
5,000	100	2	0.02	2.0	160	100	8	0.08	8.0
				P=0.560[b]					P=0.051
					Trial 2 - Harvest time: 14.0 hours Summary: Negative				
					Dimethylsulfoxide				
						100	3	0.03	3.0
					Cyclophosphamide				
					15	100	20	0.20	18.0
					5,5-Diphenylhydantoin				
					125	100	4	0.04	4.0
					250	100	7	0.07	6.0
					2,500	100	2	0.02	2.0
					5,000	100	2	0.02	2.0
									P=0.828

[a] Study performed at Columbia University. Abs = aberrations. A detailed presentation of the experimental protocol and these data are presented in Galloway *et al.* (1987).
[b] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose

TABLE E5
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by 5,5-Diphenylhydantoin[a]

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			
				Mating 1	Mating 2	Mating 3	Total[b]
Injection	100	0	0	0/1,956	1/1,941	1/1,856	2/5,753 (0.03%)
	0			1/1,960	2/2,000	2/1,911	5/5,871 (0.09%)
Feeding	5,000	0	1	2/4,026	0/1,757	0/031	2/5,814 (0.03%)
	0			4/4,412	1/1,526	1/076	6/6,014 (0.10%)

[a] Study performed at Brown University. A detailed protocol of the sex-linked recessive lethal assay and these data are presented in Woodruff *et al.* (1985).
[b] Combined total number of lethal mutations/number of X chromosomes tested for three mating trials. Results were not significant at the 5% level (Margolin *et al.*, 1983).

TABLE E6
Induction of Sister Chromatid Exchanges in Mouse Bone Marrow Cells by 5,5-Diphenylhydantoin[a]

Treatment	Dose (mg/kg)	Mean SCEs/Cell[b]
Trial 1 - Standard protocol: 23-hour harvest		
Corn oil		5.29 ± 0.293
Dimethylbenzanthracene[c]	2.5	9.49 ± 1.270*
5,5-Diphenylhydantoin	62.5	4.38 ± 0.414
	125	7.76 ± 0.241*
	250	5.13 ± 1.090
		P=0.475[d]
Trial 2 - Extended protocol: 42-hour harvest		
Corn oil		5.34 ± 1.077
Dimethylbenzanthracene	2.5	13.53 ± 0.713*
5,5-Diphenylhydantoin	25	6.10 ± 0.691
	50	6.85 ± 0.606
	100	7.47 ± 1.226
		P<0.048

* Significantly different from the control group by the one-tailed *t*-test
[a] Study performed at Brookhaven National Laboratory. The protocol and these data are presented in McFee *et al.* (1992).
[b] Mean ± standard error
[c] Positive control
[d] Significance of mean SCEs/cell tested by the one-tailed trend test (Margolin *et al.*, 1986).

TABLE E7
Induction of Chromosomal Aberrations in Mouse Bone Marrow Cells by 5,5-Diphenylhydantoin[a]

Treatment	Dose (mg/kg)	% Cells with Abs
Trial 1 - Standard protocol: 17-hour harvest		
Corn oil		1.25
Dimethylbenzanthracene[c]	100	6.50
		P=0.002[d]
5,5-Diphenylhydantoin	125	0.75
	250	0.75
	500	1.75
		P=0.183[e]
Trial 2 - Extended protocol: 36- or 42-hour harvest		
Corn oil		3.25 ± 0.921
Dimethylbenzanthracene	100	25.00 ± 3.836
		P=0.001[d]
5,5-Diphenylhydantoin	125	1.25 ± 0.25
	250	3.50 ± 1.918
	500	1.75 ± 0.701
		P=0.382[e]

[a] Study performed at Brookhaven National Laboratory. The protocol and these data are presented in McFee *et al.* (1992).
[b] Mean ± standard error
[c] Positive control
[d] Significance of percent damaged cells tested by pairwise comparison
[e] Significance tested by the one-tailed trend test (Margolin *et al.*, 1986)

TABLE E8
Frequency of Micronuclei in Bone Marrow Polychromatic Erythrocytes of B6C3F$_1$ Mice Treated with 5,5-Diphenylhydantoin by Intraperitoneal Injection[a]

Dose (mg/kg)	Micronucleated Cells/1,000 Cells[b]	Mice per Dose
Control		
0	2.30 ± 0.98	5
Dimethylbenzanthracene[c]		
12.5	7.30 ± 0.94	5
5,5-Diphenylhydantoin		
17.5	2.80 ± 0.41	5
35.0	3.63 ± 0.85	4
70.0	3.40 ± 1.03	5
	P=0.069[d]	

[a] Study performed at Integrated Laboratory Systems. The protocol and these data are presented in McFee *et al.* (1992).
[b] Mean ± standard error
[c] Positive control
[d] Significance of micronucleated cells/1,000 cells tested by the one-tailed trend test (Margolin *et al.*, 1986)

TABLE E9
Frequency of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Balb/C Mice Treated with 5,5-Diphenylhydantoin by Intravenous Injection[a]

Dose (mg/kg)	Micronucleated Cells/1,000 Cells[b]	Mice per Dose
Control		
0	3.1 ± 0.50	5
Mitomycin C[c]		
0.5	3.9 ± 0.74	5
5.0	9.6 ± 1.38	5
5,5-Diphenylhydantoin		
0.1	2.6 ± 0.59	5
0.5	3.2 ± 0.59	5
1.0	4.1 ± 0.88	5
5.0	3.0 ± 0.82	5
10.0	3.3 ± 0.75	5
20.0	4.0 ± 0.73	5
	P=0.195[d]	

[a] Study performed at Oak Ridge Associated Universities. The protocol and these data are presented in McFee *et al.* (1992).
[b] Mean ± standard error
[c] Positive control
[d] Significance of micronucleated cells/1,000 cells tested by the one-tailed trend test (Margolin *et al.*, 1986)

APPENDIX F
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm	630 ppm 0 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	428 ± 11	422 ± 7	410 ± 6	416 ± 8	411 ± 11	392 ± 7*
Adrenal gland						
Absolute	0.020 ± 0.002	0.016 ± 0.002	0.016 ± 0.002	0.017 ± 0.002	0.017 ± 0.002	0.018 ± 0.001
Relative	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.00
Brain						
Absolute	1.96 ± 0.01	1.95 ± 0.02	1.94 ± 0.02	1.96 ± 0.03	1.91 ± 0.02	1.93 ± 0.02
Relative	4.62 ± 0.13	4.64 ± 0.06	4.73 ± 0.08	4.71 ± 0.11	4.69 ± 0.12	4.93 ± 0.09
Heart						
Absolute	1.11 ± 0.03	1.08 ± 0.03	1.09 ± 0.03	1.14 ± 0.02	1.11 ± 0.06	1.08 ± 0.02
Relative	2.60 ± 0.06	2.57 ± 0.06	2.65 ± 0.04	2.74 ± 0.05	2.70 ± 0.12	2.77 ± 0.06
R. Kidney						
Absolute	1.29 ± 0.02	1.27 ± 0.03	1.27 ± 0.03	1.26 ± 0.03	1.24 ± 0.04	1.24 ± 0.02
Relative	3.01 ± 0.06	3.02 ± 0.06	3.10 ± 0.07	3.02 ± 0.06	3.01 ± 0.04	3.17 ± 0.04
Liver						
Absolute	13.93 ± 0.24	14.09 ± 0.26	15.71 ± 0.22**	13.82 ± 0.39	13.45 ± 0.44	15.15 ± 0.31
Relative	32.70 ± 0.82	33.47 ± 0.70	38.35 ± 0.51**	33.25 ± 0.97	32.71 ± 0.40	38.66 ± 0.62**
Lungs						
Absolute	2.01 ± 0.06	1.97 ± 0.07	1.86 ± 0.07	1.93 ± 0.07	1.81 ± 0.07	1.79 ± 0.06
Relative	4.70 ± 0.13	4.67 ± 0.12	4.56 ± 0.18	4.64 ± 0.15	4.41 ± 0.17	4.58 ± 0.12
Pituitary gland						
Absolute	0.012 ± 0.002	0.012 ± 0.001	0.016 ± 0.003	0.015 ± 0.002	0.012 ± 0.001	0.012 ± 0.001
Relative	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
Prostate gland						
Absolute	0.407 ± 0.059	0.324 ± 0.040	0.302 ± 0.060	0.286 ± 0.039	0.316 ± 0.065	0.237 ± 0.040
Relative	0.95 ± 0.14	0.77 ± 0.10	0.74 ± 0.14	0.69 ± 0.09	0.77 ± 0.15	0.61 ± 0.11
R. Testis						
Absolute	1.55 ± 0.05	1.58 ± 0.03	1.60 ± 0.02	1.52 ± 0.03	1.51 ± 0.04	1.57 ± 0.03
Relative	3.63 ± 0.08	3.76 ± 0.07	3.92 ± 0.06	3.66 ± 0.07	3.67 ± 0.07	4.02 ± 0.08**
Thymus						
Absolute	0.191 ± 0.014	0.174 ± 0.013	0.170 ± 0.013	0.153 ± 0.009	0.146 ± 0.013	0.133 ± 0.009**
Relative	0.45 ± 0.03	0.41 ± 0.03	0.42 ± 0.04	0.37 ± 0.02	0.35 ± 0.03	0.34 ± 0.02
Thyroid gland						
Absolute	0.016 ± 0.002	0.020 ± 0.001	0.019 ± 0.002	0.022 ± 0.002	0.018 ± 0.001	0.017 ± 0.002
Relative	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.01*	0.04 ± 0.00	0.04 ± 0.00

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	210 ppm 800 ppm	63 ppm 240 ppm
Male (continued)		
n	10	10
Necropsy body wt	427 ± 10	440 ± 8
Adrenal gland		
Absolute	0.016 ± 0.002	0.020 ± 0.002
Relative	0.04 ± 0.01	0.05 ± 0.01
Brain		
Absolute	1.92 ± 0.03	1.97 ± 0.02
Relative	4.50 ± 0.07	4.48 ± 0.09
Heart		
Absolute	1.06 ± 0.04	1.10 ± 0.03
Relative	2.49 ± 0.04	2.51 ± 0.06
R. Kidney		
Absolute	1.27 ± 0.05	1.26 ± 0.03
Relative	2.98 ± 0.06	2.87 ± 0.06
Liver		
Absolute	14.27 ± 0.53	13.93 ± 0.41
Relative	33.35 ± 0.71	31.64 ± 0.68
Lungs		
Absolute	1.80 ± 0.10	2.00 ± 0.05
Relative	4.21 ± 0.20	4.55 ± 0.14
Pituitary gland		
Absolute	0.013 ± 0.001	0.015 ± 0.002
Relative	0.03 ± 0.00	0.03 ± 0.01
Prostate gland		
Absolute	0.332 ± 0.048	0.317 ± 0.039
Relative	0.78 ± 0.11	0.72 ± 0.09
R. Testis		
Absolute	1.58 ± 0.04	1.63 ± 0.03
Relative	3.72 ± 0.09	3.70 ± 0.09
Thymus		
Absolute	0.157 ± 0.009	0.183 ± 0.016
Relative	0.37 ± 0.02	0.41 ± 0.03
Thyroid gland		
Absolute	0.019 ± 0.001	0.019 ± 0.002
Relative	0.04 ± 0.00	0.04 ± 0.00

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm	630 ppm 0 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Female						
n	10	10	10	10	9	10
Necropsy body wt	259 ± 3	234 ± 2**	204 ± 3**	251 ± 4	227 ± 3**	202 ± 4**
Adrenal gland						
Absolute	0.023 ± 0.003	0.017 ± 0.002	0.018 ± 0.002	0.019 ± 0.002	0.018 ± 0.002	0.016 ± 0.002
Relative	0.09 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Brain						
Absolute	1.87 ± 0.02	1.76 ± 0.03*	1.75 ± 0.03**	1.78 ± 0.03	1.80 ± 0.02	1.71 ± 0.02**
Relative	7.23 ± 0.10	7.56 ± 0.17	8.58 ± 0.18**	7.10 ± 0.15	7.95 ± 0.11**	8.50 ± 0.14**
Heart						
Absolute	0.753 ± 0.014	0.709 ± 0.013	0.667 ± 0.021**	0.717 ± 0.008	0.715 ± 0.020	0.622 ± 0.013**
Relative	2.91 ± 0.06	3.04 ± 0.06	3.27 ± 0.10**	2.86 ± 0.05	3.15 ± 0.09	3.08 ± 0.06
R. Kidney						
Absolute	0.821 ± 0.022	0.765 ± 0.019	0.677 ± 0.016**	0.771 ± 0.013	0.729 ± 0.020**	0.629 ± 0.011**
Relative	3.17 ± 0.08	3.28 ± 0.08	3.32 ± 0.08	3.07 ± 0.06	3.21 ± 0.08	3.11 ± 0.04
Liver						
Absolute	8.01 ± 0.21	7.25 ± 0.12**	7.14 ± 0.11**	7.95 ± 0.16	7.29 ± 0.21*	6.88 ± 0.19**
Relative	30.92 ± 0.81	31.03 ± 0.47	35.08 ± 0.70**	31.69 ± 0.54	32.08 ± 0.73	34.03 ± 0.69**
Lungs						
Absolute	1.44 ± 0.06	1.31 ± 0.03	1.19 ± 0.03**	1.31 ± 0.07	1.29 ± 0.05	1.10 ± 0.03**
Relative	5.57 ± 0.23	5.60 ± 0.17	5.82 ± 0.15	5.24 ± 0.27	5.68 ± 0.18	5.46 ± 0.14
Ovary						
Absolute	0.064 ± 0.008	0.070 ± 0.011	0.074 ± 0.012	0.071 ± 0.009	0.055 ± 0.005	0.048 ± 0.003
Relative	0.25 ± 0.03	0.30 ± 0.05	0.37 ± 0.07	0.28 ± 0.04	0.24 ± 0.02	0.24 ± 0.01
Pituitary gland						
Absolute	0.016 ± 0.001	0.017 ± 0.002	0.018 ± 0.003	0.016 ± 0.001	0.017 ± 0.002	0.013 ± 0.001[b]
Relative	0.06 ± 0.00	0.07 ± 0.01	0.09 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.06 ± 0.01[b]
Thymus						
Absolute	0.153 ± 0.014	0.140 ± 0.009	0.129 ± 0.006	0.116 ± 0.008*	0.115 ± 0.006*	0.100 ± 0.005**
Relative	0.59 ± 0.05	0.60 ± 0.04	0.63 ± 0.03	0.46 ± 0.03	0.51 ± 0.03	0.49 ± 0.03
Thyroid gland						
Absolute	0.020 ± 0.002	0.018 ± 0.001	0.020 ± 0.002	0.019 ± 0.001	0.016 ± 0.002	0.019 ± 0.003
Relative	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.10 ± 0.01
Uterus						
Absolute	1.105 ± 0.144	0.990 ± 0.089	0.725 ± 0.071	0.970 ± 0.121	1.184 ± 0.190	0.472 ± 0.047**
Relative	4.27 ± 0.56	4.24 ± 0.38	3.56 ± 0.34	3.88 ± 0.48	5.27 ± 0.92	2.34 ± 0.24*

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	210 ppm 800 ppm	63 ppm 240 ppm
Female (continued)		
n	9	9
Necropsy body wt	227 ± 4**	247 ± 4
Adrenal gland		
Absolute	0.015 ± 0.002	0.022 ± 0.003
Relative	0.06 ± 0.01	0.09 ± 0.01
Brain		
Absolute	1.79 ± 0.02	1.82 ± 0.03
Relative	7.92 ± 0.13**	7.38 ± 0.13
Heart		
Absolute	0.685 ± 0.018*	0.739 ± 0.018
Relative	3.02 ± 0.07	3.00 ± 0.08
R. Kidney		
Absolute	0.702 ± 0.017**	0.789 ± 0.019
Relative	3.10 ± 0.04	3.20 ± 0.06
Liver		
Absolute	7.36 ± 0.22*	7.55 ± 0.08
Relative	32.44 ± 0.62	30.66 ± 0.63
Lungs		
Absolute	1.22 ± 0.04**	1.35 ± 0.04
Relative	5.37 ± 0.12	5.48 ± 0.15
Ovary		
Absolute	0.057 ± 0.008	0.066 ± 0.004
Relative	0.25 ± 0.04	0.27 ± 0.02
Pituitary gland		
Absolute	0.014 ± 0.002	0.014 ± 0.001
Relative	0.06 ± 0.01	0.06 ± 0.01
Thymus		
Absolute	0.119 ± 0.010*	0.142 ± 0.008
Relative	0.53 ± 0.04	0.57 ± 0.03
Thyroid gland		
Absolute	0.021 ± 0.001	0.015 ± 0.001
Relative	0.09 ± 0.01	0.06 ± 0.00
Uterus		
Absolute	1.015 ± 0.100	0.979 ± 0.092
Relative	4.47 ± 0.42	3.99 ± 0.41

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 100 ppm	0 ppm 300 ppm	210 ppm 0 ppm	210 ppm 100 ppm	210 ppm 300 ppm
Male						
n	10	10	9	10	10	10
Necropsy body wt	38.9 ± 1.4	35.2 ± 1.4	33.0 ± 0.8*	36.1 ± 1.3	35.7 ± 1.6	32.3 ± 0.8**
Adrenal gland						
Absolute	0.003 ± 0.000	0.003 ± 0.001	0.004 ± 0.000[b]	0.004 ± 0.001	0.004 ± 0.000	0.004 ± 0.001
Relative	0.08 ± 0.01	0.10 ± 0.02	0.11 ± 0.01[b]	0.10 ± 0.02	0.10 ± 0.01	0.12 ± 0.02
Brain						
Absolute	0.437 ± 0.010	0.435 ± 0.004	0.423 ± 0.006	0.423 ± 0.012	0.427 ± 0.010	0.417 ± 0.006
Relative	11.31 ± 0.30	12.53 ± 0.45	12.89 ± 0.33*	11.83 ± 0.50	12.14 ± 0.51	12.94 ± 0.24*
Heart						
Absolute	0.202 ± 0.007	0.176 ± 0.007	0.183 ± 0.007	0.171 ± 0.009*	0.171 ± 0.011*	0.162 ± 0.005**
Relative	5.21 ± 0.10	5.02 ± 0.19	5.57 ± 0.26	4.72 ± 0.15	4.79 ± 0.21	5.03 ± 0.15
R. Kidney						
Absolute	0.388 ± 0.019	0.318 ± 0.015**	0.299 ± 0.011**	0.324 ± 0.014*	0.305 ± 0.014**	0.273 ± 0.010**
Relative	9.99 ± 0.45	9.08 ± 0.40	9.04 ± 0.23	9.00 ± 0.31	8.57 ± 0.22**	8.44 ± 0.20**
Liver						
Absolute	1.94 ± 0.07	1.80 ± 0.12	2.09 ± 0.05	1.71 ± 0.07	1.92 ± 0.16	1.92 ± 0.08
Relative	50.04 ± 1.18	50.98 ± 2.28	63.35 ± 1.16**	47.64 ± 1.47	53.06 ± 2.34	59.33 ± 1.63**
Lungs						
Absolute	0.257 ± 0.013	0.254 ± 0.006	0.245 ± 0.014	0.249 ± 0.012	0.254 ± 0.017	0.229 ± 0.007
Relative	6.59 ± 0.21	7.27 ± 0.21	7.42 ± 0.38	6.89 ± 0.22	7.09 ± 0.22	7.10 ± 0.27
Pituitary gland						
Absolute	0.004 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.004 ± 0.000[c]	0.003 ± 0.000	0.003 ± 0.000
Relative	0.09 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.12 ± 0.01[c]	0.10 ± 0.01	0.09 ± 0.01
Prostate gland						
Absolute	0.045 ± 0.009	0.029 ± 0.003	0.023 ± 0.005*	0.028 ± 0.005	0.041 ± 0.004	0.031 ± 0.003
Relative	1.20 ± 0.26	0.83 ± 0.11	0.69 ± 0.12	0.76 ± 0.11	1.19 ± 0.12	0.98 ± 0.11
R. Testis						
Absolute	0.105 ± 0.005	0.098 ± 0.002	0.100 ± 0.005[b]	0.096 ± 0.005	0.098 ± 0.006[c]	0.101 ± 0.004
Relative	2.72 ± 0.14	2.83 ± 0.13	3.05 ± 0.12[b]	2.68 ± 0.15	2.77 ± 0.18[c]	3.12 ± 0.12
Thymus						
Absolute	0.034 ± 0.004	0.025 ± 0.003	0.027 ± 0.002	0.028 ± 0.004	0.032 ± 0.003	0.029 ± 0.002
Relative	0.89 ± 0.11	0.71 ± 0.08	0.83 ± 0.07	0.76 ± 0.08	0.89 ± 0.06	0.90 ± 0.05
Thyroid gland						
Absolute	0.006 ± 0.000	0.007 ± 0.000	0.006 ± 0.001	0.005 ± 0.000[c]	0.007 ± 0.000	0.006 ± 0.000
Relative	0.16 ± 0.01	0.19 ± 0.01	0.17 ± 0.02	0.16 ± 0.02[c]	0.19 ± 0.02	0.20 ± 0.01

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	70 ppm 100 ppm	21 ppm 30 ppm
Male (continued)		
n	6	10
Necropsy body wt	33.6 ± 1.9	36.5 ± 0.9
Adrenal gland		
Absolute	0.003 ± 0.000	0.003 ± 0.000
Relative	0.08 ± 0.01	0.08 ± 0.01
Brain		
Absolute	0.412 ± 0.011	0.431 ± 0.009
Relative	12.46 ± 0.73	11.85 ± 0.28
Heart		
Absolute	0.176 ± 0.012	0.194 ± 0.007
Relative	5.24 ± 0.19	5.31 ± 0.11
R. Kidney		
Absolute	0.302 ± 0.018**	0.348 ± 0.014
Relative	9.02 ± 0.36	9.51 ± 0.25
Liver		
Absolute	1.80 ± 0.10	1.86 ± 0.05
Relative	53.67 ± 1.56	51.18 ± 0.84
Lungs		
Absolute	0.245 ± 0.014	0.234 ± 0.007
Relative	7.30 ± 0.24	6.41 ± 0.10
Pituitary gland		
Absolute	0.004 ± 0.001	0.003 ± 0.000
Relative	0.12 ± 0.03	0.08 ± 0.01
Prostate gland		
Absolute	0.026 ± 0.004	0.035 ± 0.006
Relative	0.78 ± 0.14	0.94 ± 0.14
R. Testis		
Absolute	0.096 ± 0.005	0.097 ± 0.004
Relative	2.87 ± 0.12	2.65 ± 0.10
Thymus		
Absolute	0.027 ± 0.003	0.021 ± 0.002*
Relative	0.82 ± 0.09	0.58 ± 0.05*
Thyroid gland		
Absolute	0.006 ± 0.001	0.008 ± 0.001
Relative	0.17 ± 0.01	0.22 ± 0.03

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 200 ppm	0 ppm 600 ppm	210 ppm 0 ppm	210 ppm 200 ppm	210 ppm 600 ppm
Female						
n	10	10	10	10	10	10
Necropsy body wt	30.5 ± 1.0	27.1 ± 0.8*	23.2 ± 0.6**	28.5 ± 1.2	28.0 ± 1.0	23.9 ± 0.6**
Adrenal gland						
Absolute	0.003 ± 0.000	0.004 ± 0.000	0.004 ± 0.000	0.004 ± 0.001	0.003 ± 0.000	0.004 ± 0.000
Relative	0.09 ± 0.01	0.14 ± 0.02	0.18 ± 0.02**	0.14 ± 0.03	0.12 ± 0.01	0.16 ± 0.01
Brain						
Absolute	0.431 ± 0.007	0.429 ± 0.006	0.415 ± 0.006	0.428 ± 0.008	0.439 ± 0.004	0.418 ± 0.006
Relative	14.26 ± 0.45	15.96 ± 0.43	17.99 ± 0.44**	15.16 ± 0.51	15.86 ± 0.60	17.56 ± 0.23**
Heart						
Absolute	0.143 ± 0.007	0.133 ± 0.003	0.114 ± 0.005**	0.128 ± 0.005	0.140 ± 0.005	0.119 ± 0.004**
Relative	4.70 ± 0.20	4.93 ± 0.13	4.92 ± 0.22	4.51 ± 0.17	5.06 ± 0.24	5.00 ± 0.17
R. Kidney						
Absolute	0.240 ± 0.006	0.226 ± 0.012	0.171 ± 0.010**	0.208 ± 0.006*	0.192 ± 0.009**	0.179 ± 0.005**
Relative	7.93 ± 0.20	8.35 ± 0.35	7.35 ± 0.35	7.35 ± 0.24	6.85 ± 0.20*	7.51 ± 0.12
Liver						
Absolute	1.54 ± 0.06	1.72 ± 0.05	1.66 ± 0.07	1.43 ± 0.06	1.71 ± 0.06	1.80 ± 0.04*
Relative	50.79 ± 1.57	63.68 ± 1.37**	71.34 ± 1.95**	50.23 ± 1.24	61.28 ± 1.69**	75.62 ± 0.90**
Lungs						
Absolute	0.244 ± 0.015	0.227 ± 0.009	0.188 ± 0.008**	0.214 ± 0.006	0.203 ± 0.009*	0.183 ± 0.007**
Relative	8.00 ± 0.41	8.40 ± 0.30	8.08 ± 0.21	7.55 ± 0.21	7.26 ± 0.31	7.69 ± 0.24
Ovary						
Absolute	0.013 ± 0.001	0.014 ± 0.002[c]	0.012 ± 0.002	0.016 ± 0.002	0.010 ± 0.001	0.013 ± 0.003
Relative	0.44 ± 0.05	0.50 ± 0.06[c]	0.53 ± 0.08	0.56 ± 0.08	0.37 ± 0.04	0.58 ± 0.12
Pituitary gland						
Absolute	0.004 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000
Relative	0.13 ± 0.02	0.13 ± 0.02	0.11 ± 0.02	0.12 ± 0.01	0.10 ± 0.02	0.14 ± 0.01
Thymus						
Absolute	0.032 ± 0.003	0.033 ± 0.004	0.029 ± 0.003	0.032 ± 0.002	0.030 ± 0.005[c]	0.029 ± 0.002
Relative	1.04 ± 0.09	1.23 ± 0.13	1.23 ± 0.11	1.12 ± 0.10	1.09 ± 0.15[c]	1.19 ± 0.07
Thyroid gland						
Absolute	0.007 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.005 ± 0.000	0.007 ± 0.001
Relative	0.22 ± 0.05	0.19 ± 0.02	0.27 ± 0.03	0.22 ± 0.03	0.19 ± 0.02	0.27 ± 0.03
Uterus						
Absolute	0.203 ± 0.013	0.332 ± 0.024**	0.238 ± 0.017	0.233 ± 0.017	0.201 ± 0.019	0.210 ± 0.020
Relative	6.65 ± 0.36	12.30 ± 0.90**	10.41 ± 0.88**	8.43 ± 0.82	7.31 ± 0.83	8.83 ± 0.96

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	70 ppm 200 ppm	21 ppm 60 ppm
Female (continued)		
n	10	10
Necropsy body wt	26.9 ± 0.8*	29.6 ± 1.1
Adrenal gland		
Absolute	0.005 ± 0.001*[c]	0.004 ± 0.000
Relative	0.17 ± 0.02*[c]	0.13 ± 0.02
Brain		
Absolute	0.418 ± 0.01	0.447 ± 0.01
Relative	15.65 ± 0.683	15.25 ± 0.477
Heart		
Absolute	0.131 ± 0.005	0.140 ± 0.005
Relative	4.90 ± 0.24	4.77 ± 0.18
R. Kidney		
Absolute	0.215 ± 0.006	0.239 ± 0.007
Relative	7.98 ± 0.15	8.16 ± 0.33
Liver		
Absolute	1.66 ± 0.07	1.59 ± 0.04
Relative	61.69 ± 1.95**	54.03 ± 1.20
Lungs		
Absolute	0.214 ± 0.008	0.223 ± 0.008
Relative	8.00 ± 0.35	7.55 ± 0.21
Ovary		
Absolute	0.013 ± 0.002[c]	0.012 ± 0.002
Relative	0.50 ± 0.06[c]	0.42 ± 0.08
Pituitary gland		
Absolute	0.004 ± 0.000	0.004 ± 0.001
Relative	0.15 ± 0.02	0.14 ± 0.02
Thymus		
Absolute	0.032 ± 0.004[c]	0.034 ± 0.003
Relative	1.20 ± 0.13[c]	1.13 ± 0.08
Thyroid gland		
Absolute	0.006 ± 0.001	0.006 ± 0.001
Relative	0.21 ± 0.03	0.19 ± 0.02
Uterus		
Absolute	0.255 ± 0.017	0.275 ± 0.026
Relative	9.56 ± 0.73	9.25 ± 0.70

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=8
[c] n=9

APPENDIX G
HEMATOLOGY, CLINICAL CHEMISTRY, AND URINALYSIS RESULTS

HEMATOLOGY, CLINICAL CHEMISTRY, AND URINALYSIS RESULTS

METHODS

At the 9-month interim evaluation, male and female rats were anesthetized and bled from the retroorbital sinus using heparinized capillary tubes. Blood (approximately 0.50 mL) for hematologic determinations was collected into plastic test tubes containing potassium EDTA for anticoagulation (Microtainers, Becton Dickenson, Rutherford, NJ) and into similar tubes devoid of an anticoagulant for biochemical determinations (approximately 1.0 mL). These latter samples were centrifuged after clotting at room temperature for at least 30 minutes, and serum was removed. All hematologic and biochemical analyses were performed the day of sample collection.

Automated hematologic determinations were performed using an Ortho ELT-8 (Ortho Instruments, Westwood, MA) hematology analyzer. The following variables were measured or calculated: erythrocyte, leukocyte, and platelet counts; hemoglobin concentration; hematocrit; and mean cell volume. Leukocyte differentials, morphologic evaluation of blood cells, and counts of nucleated erythrocytes were determined from blood smears stained with Wright-Giemsa. Reticulocytes were stained with new methylene blue, and absolute counts were calculated based on numbers relative to mature erythrocytes.

Clinical chemistry variables were measured using a Gemsac-4 chemistry analyzer (Electro-Nucleonics, Fairfield, NJ). Assays included activities of sorbitol dehydrogenase, alanine aminotransferase, and alkaline phosphatase and concentrations of total protein, albumin, urea nitrogen, creatinine, glucose, total bilirubin, cholesterol, and triglycerides. Reagents for these assays were obtained from the instrument manufacturer, except for the reagent for sorbitol dehydrogenase, which was obtained from Sigma Chemical Company (St. Louis, MO). Urine samples were collected and specific gravity and pH were measured.

TABLE G1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm	630 ppm 0 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	44.7 ± 0.6	46.2 ± 0.7	45.8 ± 0.5	46.1 ± 0.6	45.9 ± 0.8	46.1 ± 0.6
Hemoglobin (g/dL)	14.0 ± 0.3[b]	14.4 ± 0.3	14.1 ± 0.2	14.3 ± 0.2	14.2 ± 0.3	14.1 ± 0.1
Erythrocytes (10^6/μL)	8.53 ± 0.19	8.99 ± 0.14	9.01 ± 0.10*	8.77 ± 0.15	8.86 ± 0.14	9.01 ± 0.12*
Mean cell volume (fL)	52.0 ± 0.4	51.4 ± 0.3	51.0 ± 0.3	52.1 ± 0.4	51.8 ± 0.3	51.2 ± 0.5
Platelets (10^3/μL)	415.4 ± 22.7	530.0 ± 23.8	531.1 ± 7.6**	420.9 ± 20.4	525.0 ± 29.9	530.2 ± 10.2**
Reticulocytes (10^6/μL)	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Leukocytes (10^3/μL)	3.66 ± 0.22	4.09 ± 0.31	4.42 ± 0.61	3.51 ± 0.32	4.27 ± 0.25	4.94 ± 0.54
Segmented neutrophils (10^3/μL)	0.97 ± 0.11	1.35 ± 0.18	1.59 ± 0.48	1.00 ± 0.22	1.15 ± 0.20	1.65 ± 0.32
Lymphocytes (10^3/μL)	2.61 ± 0.18	2.69 ± 0.24	2.73 ± 0.26	2.47 ± 0.27	3.05 ± 0.15	3.20 ± 0.25
Monocytes (10^3/μL)	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Eosinophils (10^3/μL)	0.06 ± 0.02	0.03 ± 0.02	0.08 ± 0.02	0.02 ± 0.01	0.08 ± 0.02	0.08 ± 0.02
Nucleated erythrocytes (10^3/μL)	0.06 ± 0.02	0.04 ± 0.01	0.01 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.04 ± 0.02
Clinical Chemistry						
Urea nitrogen (mg/dL)	18.5 ± 0.6	18.1 ± 0.4	18.3 ± 0.6	18.6 ± 0.5	16.7 ± 0.7	18.8 ± 0.6
Creatinine (mg/dL)	0.60 ± 0.03	0.74 ± 0.11	0.55 ± 0.02	0.49 ± 0.03*	0.52 ± 0.02*	0.52 ± 0.03*
Glucose (mg/dL)	154 ± 6	166 ± 7	158 ± 5	161 ± 4	171 ± 4*	149 ± 5
Total protein (g/dL)	6.5 ± 0.2	6.8 ± 0.2	7.0 ± 0.2	6.5 ± 0.1	6.7 ± 0.2	6.9 ± 0.2
Albumin (g/dL)	4.0 ± 0.1	4.2 ± 0.1	4.3 ± 0.2	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
Total bilirubin (mg/dL)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Cholesterol (mg/dL)	62 ± 2	55 ± 2	61 ± 2	60 ± 1	51 ± 1**	53 ± 3
Triglyceride (mg/dL)	132 ± 9	115 ± 8	77 ± 5**	149 ± 13	83 ± 5**	55 ± 6**
Alkaline phosphatase (IU/L)	101 ± 4	102 ± 5	95 ± 4	109 ± 6	95 ± 4	93 ± 2
Alanine aminotransferase (IU/L)	62 ± 4	54 ± 4	51 ± 5	59 ± 6	39 ± 2**	45 ± 3*
Sorbitol dehydrogenase (IU/L)	20 ± 2	22 ± 3	22 ± 3	16 ± 2	15 ± 1	17 ± 2
Urinalysis						
Specific gravity	1.027 ± 0.001	1.034 ± 0.002	1.032 ± 0.002	1.028 ± 0.002	1.030 ± 0.002	1.033 ± 0.003
pH	6.90 ± 0.15	6.80 ± 0.11	6.40 ± 0.10**	7.30 ± 0.11	6.40 ± 0.10*	6.40 ± 0.10*

TABLE G1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	210 ppm 800 ppm	63 ppm 240 ppm
Male (continued)		
n	10	10
Hematology		
Hematocrit (%)	46.5 ± 0.2*	46.5 ± 0.3*
Hemoglobin (g/dL)	14.5 ± 0.1	14.4 ± 0.1
Erythrocytes ($10^6/\mu L$)	9.12 ± 0.03**	9.09 ± 0.06*
Mean cell volume (fL)	51.0 ± 0.2	51.4 ± 0.3
Platelets ($10^3/\mu L$)	531.7 ± 31.5*	476.1 ± 14.4
Reticulocytes ($10^6/\mu L$)	0.1 ± 0.0	0.1 ± 0.0*
Leukocytes ($10^3/\mu L$)	3.92 ± 0.18	3.74 ± 0.17
Segmented neutrophils ($10^3/\mu L$)	1.10 ± 0.09	1.01 ± 0.11
Lymphocytes ($10^3/\mu L$)	2.79 ± 0.14	2.67 ± 0.17
Monocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00
Eosinophils ($10^3/\mu L$)	0.04 ± 0.02	0.05 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)	0.03 ± 0.01	0.02 ± 0.01
Clinical Chemistry		
Urea nitrogen (mg/dL)	17.5 ± 0.8	19.5 ± 0.6
Creatinine (mg/dL)	0.51 ± 0.03*	0.53 ± 0.02*
Glucose (mg/dL)	167 ± 3	187 ± 7**
Total protein (g/dL)	6.7 ± 0.2	6.7 ± 0.2
Albumin (g/dL)	4.3 ± 0.1	4.2 ± 0.1
Total bilirubin (mg/dL)	0.3 ± 0.0	0.3 ± 0.0
Cholesterol (mg/dL)	59 ± 2	66 ± 2
Triglyceride (mg/dL)	98 ± 6**	122 ± 9**
Alkaline phosphatase (IU/L)	102 ± 4	114 ± 7
Alanine aminotransferase (IU/L)	50 ± 4	71 ± 10
Sorbitol dehydrogenase (IU/L)	17 ± 1	21 ± 2
Urinalysis		
Specific gravity	1.030 ± 0.001	1.028 ± 0.003
pH	6.60 ± 0.07*	6.80 ± 0.21

TABLE G1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 800 ppm	0 ppm 2,400 ppm	630 ppm 0 ppm	630 ppm 800 ppm	630 ppm 2,400 ppm
Female						
Hematology						
n	10	10	10	9	9	10
Hematocrit (%)	46.0 ± 0.5	45.8 ± 0.3	45.4 ± 0.4	45.2 ± 0.5	45.3 ± 0.7	46.4 ± 0.5
Hemoglobin (g/dL)	14.2 ± 0.2	14.2 ± 0.1	13.8 ± 0.2	14.0 ± 0.2	13.7 ± 0.2	14.2 ± 0.1
Erythrocytes (10^6/μL)	7.96 ± 0.09	8.10 ± 0.06	8.07 ± 0.08	7.92 ± 0.08	7.96 ± 0.13	8.28 ± 0.09*
Mean cell volume (fL)	57.7 ± 0.3	56.3 ± 0.3**	56.2 ± 0.3**	57.0 ± 0.4*	56.7 ± 0.3*	56.1 ± 0.5**
Platelets (10^3/μL)	325.7 ± 33.9	444.7 ± 11.1**	434.6 ± 35.2**	405.7 ± 18.7*	466.6 ± 7.6**	468.1 ± 16.9**
Reticulocytes (10^6/μL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Leukocytes (10^3/μL)	2.43 ± 0.21	2.07 ± 0.12	2.76 ± 0.20	2.20 ± 0.19	3.16 ± 0.41	2.54 ± 0.20
Segmented neutrophils (10^3/μL)	0.50 ± 0.07	0.57 ± 0.04	0.82 ± 0.13	0.51 ± 0.07	0.64 ± 0.10	0.50 ± 0.05
Lymphocytes (10^3/μL)	1.90 ± 0.16	1.48 ± 0.12	1.87 ± 0.17	1.67 ± 0.14	2.49 ± 0.35	2.00 ± 0.20
Monocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils (10^3/μL)	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.01
Clinical Chemistry						
n	10	10	10	10	9	10
Urea nitrogen (mg/dL)	19.8 ± 0.7	19.6 ± 0.8	20.3 ± 0.4	20.3 ± 0.6	18.1 ± 0.7	20.2 ± 0.6
Creatinine (mg/dL)	0.71 ± 0.05	0.60 ± 0.03	0.68 ± 0.04	0.78 ± 0.11	0.60 ± 0.02	0.67 ± 0.04
Glucose (mg/dL)	156 ± 6	145 ± 5	137 ± 6	154 ± 6	150 ± 5	147 ± 15
Total protein (g/dL)	7.0 ± 0.2	7.1 ± 0.2	7.3 ± 0.2	7.3 ± 0.3	7.1 ± 0.2	7.0 ± 0.1
Albumin (g/dL)	4.6 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	5.2 ± 0.1**	4.9 ± 0.1	4.6 ± 0.0
Total bilirubin (mg/dL)	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Cholesterol (mg/dL)	89 ± 2	86 ± 1	90 ± 2	97 ± 2	89 ± 3	84 ± 2
Triglyceride (mg/dL)	97 ± 9	56 ± 6**	38 ± 3**	113 ± 15*	46 ± 7**	34 ± 4**
Alkaline phosphatase (IU/L)	115 ± 5	84 ± 3**	71 ± 5**	90 ± 4**	86 ± 4**	82 ± 4**
Alanine aminotransferase (IU/L)	48 ± 2	35 ± 1**	33 ± 2**	43 ± 2	33 ± 1**	42 ± 4
Sorbitol dehydrogenase (IU/L)	14 ± 2	11 ± 1	12 ± 1	15 ± 2	9 ± 1	12 ± 1[b]
Urinalysis						
n	10	10	10	10	9	10
Specific gravity	1.030 ± 0.005	1.024 ± 0.004	1.027 ± 0.004	1.036 ± 0.005	1.024 ± 0.003	1.036 ± 0.006
pH	6.90 ± 0.12	6.65 ± 0.13	6.75 ± 0.11	6.75 ± 0.13	6.61 ± 0.14	6.80 ± 0.08

TABLE G1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of 5,5-Diphenylhydantoin (continued)

F_0 Concentration F_1 Concentration	210 ppm 800 ppm	63 ppm 240 ppm
Female (continued)		
Hematology		
n	9	8
Hematocrit (%)	46.3 ± 0.3	46.5 ± 0.7
Hemoglobin (g/dL)	14.4 ± 0.1	14.4 ± 0.1
Erythrocytes (10^6/μL)	8.30 ± 0.06*	8.19 ± 0.09*
Mean cell volume (fL)	55.7 ± 0.3**	56.9 ± 0.4**
Platelets (10^3/μL)	424.7 ± 28.1**	444.1 ± 10.1**
Reticulocytes (10^6/μL)	0.1 ± 0.0	0.1 ± 0.0
Leukocytes (10^3/μL)	2.68 ± 0.31	2.43 ± 0.19
Segmented neutrophils (10^3/μL)	0.76 ± 0.12	0.55 ± 0.10
Lymphocytes (10^3/μL)	1.90 ± 0.25	1.85 ± 0.16
Monocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00
Eosinophils (10^3/μL)	0.01 ± 0.01	0.02 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.04 ± 0.02	0.06 ± 0.02
Clinical Chemistry		
n	9	10
Urea nitrogen (mg/dL)	17.2 ± 0.9	14.0 ± 1.9**
Creatinine (mg/dL)	0.81 ± 0.17	0.62 ± 0.15
Glucose (mg/dL)	146 ± 3	134 ± 17
Total protein (g/dL)	7.4 ± 0.2	5.5 ± 0.9
Albumin (g/dL)	5.0 ± 0.1	3.7 ± 0.6
Total bilirubin (mg/dL)	0.4 ± 0.1	0.4 ± 0.2
Cholesterol (mg/dL)	87 ± 4	77 ± 10
Triglyceride (mg/dL)	61 ± 12**	44 ± 11**
Alkaline phosphatase (IU/L)	75 ± 4**	82 ± 5**
Alanine aminotransferase (IU/L)	38 ± 2	37 ± 5
Sorbitol dehydrogenase (IU/L)	13 ± 2	14 ± 2
Urinalysis		
n	9	9
Specific gravity	1.030 ± 0.005	1.041 ± 0.023
pH	6.83 ± 0.14	6.67 ± 0.12

* Significantly different (P≤0.05) from the control 0:0 group by Dunn's test
** P≤0.01
[a] Mean ± standard error
[b] n=9

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF 5,5-DIPHENYLHYDANTOIN

5,5-Diphenylhydantoin was obtained from Parke-Davis and Company (Detroit, MI) in one lot (H-732008), which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Stability studies performed by the manufacturer confirmed the stability of 5,5-diphenylhydantoin for at least 12 months at room temperature. The reports on analyses performed in support of the 5,5-diphenylhydantoin studies are on file at the National Institute of Environmental Health Sciences.

The chemical was identified as 5,5-diphenylhydantoin by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopies. All spectra were consistent with those expected for the structure and with the literature spectra (*Sadtler Standard Spectra*) of 5,5-diphenylhydantoin (Figures H1 and H2).

The purity of 5,5-diphenylhydantoin was determined by elemental analyses, Karl Fischer water analysis, titration of the imide group, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Titration of the imide group was performed with tetrabutylammonium hydroxide. TLC was performed on Silica Gel 60 F-254 plates with two solvent systems: A) toluene: methanol (90:10) and B) chloroform:acetone (73:27). Benzimidazole was used as an internal standard. Visualization was accomplished with ultraviolet (254 nm) light and a spray of silver nitrate/ diphenylcarbazone. HPLC was performed with a μBondapak C_{18} column with a solvent system of water:methanol (45:55) at a flow rate of 1 mL/minute. Ultraviolet detection was at 254 nm.

Elemental analysis for carbon was slightly high; elemental analyses for oxygen, hydrogen, and nitrogen were in agreement with the theoretical values for 5,5-diphenylhydantoin. Karl Fischer analysis indicated 0.28 ± 0.15% water. Titration of the imide group indicated a purity of 99.97 ± 0.74%. TLC analysis indicated one trace impurity by system A and one slight trace impurity by system B. HPLC indicated two impurities with areas of 0.09% and 5.5% of the major peak area. Analysis of a United States Pharmacopeia (USP) standard of 5,5-diphenylhydantoin by the same system indicated no impurities. Comparison of the standard with lot H-732008 using the HPLC system described above but with a solvent ratio of 25:75 A:B and a flow rate of 2 mL/minute indicated the same major areas (± 2%), with the 5.5% impurity eluting in lot H-732008; this probably indicates that the impurity had a higher absorbance and was present at a much lower concentration than 5.5%. Analysis of the chemical by HPLC using a solvent ratio of 60:40 indicated no additional impurities. The overall purity of the chemical was estimated to be 98% or greater.

Stability studies performed using HPLC with the system described for the analysis of the USP sample indicated that 5,5-diphenylhydantoin was stable for 2 weeks at temperatures up to 60° C. Periodic reanalysis of 5,5-diphenylhydantoin by the study laboratory using infrared spectroscopy and HPLC with a Spherisorb S5 ODS column and a solvent system of acetonitrile:water:glacial acetic acid (35.7:63.8:0.5) at a flow rate of 1 mL/minute and ultraviolet detection at 254 nm indicated no significant deterioration during the studies.

PREPARATION AND CHARACTERIZATION OF DOSE FORMULATIONS

The dose formulations were prepared every 2 weeks by mixing 5,5-diphenylhydantoin with feed in a Patterson-Kelly twin-shell blender (Table H1). The formulations were stored in plastic-lined tin cans for no longer than 2 weeks.

Dose formulations of 5,5-diphenylhydantoin were analyzed by the study laboratory using HPLC with an Excalibar Spherisorb ODS column with a solvent system of acetonitrile:water:acetic acid (with ratios from 30:69.5:0.5 to 37.5:63.8:0.5) at a flow rate of 1.0 to 1.5 mL/minute. Ultraviolet detection was at 254 nm. Dose formulations were analyzed once during the 13-week and maximum neonatal dose determination studies; all dose formulations were within 10% of the target concentrations (Tables H2 and H3). During the 2-year studies, dose formulations were analyzed approximately every 2 months. For rats, 38 of the 39 dose formulations were within 10% of the target concentrations; 62 of 67 dose formulations for mice were within specifications. Results of the dose formulation analyses for the 2-year studies are presented in Table H4.

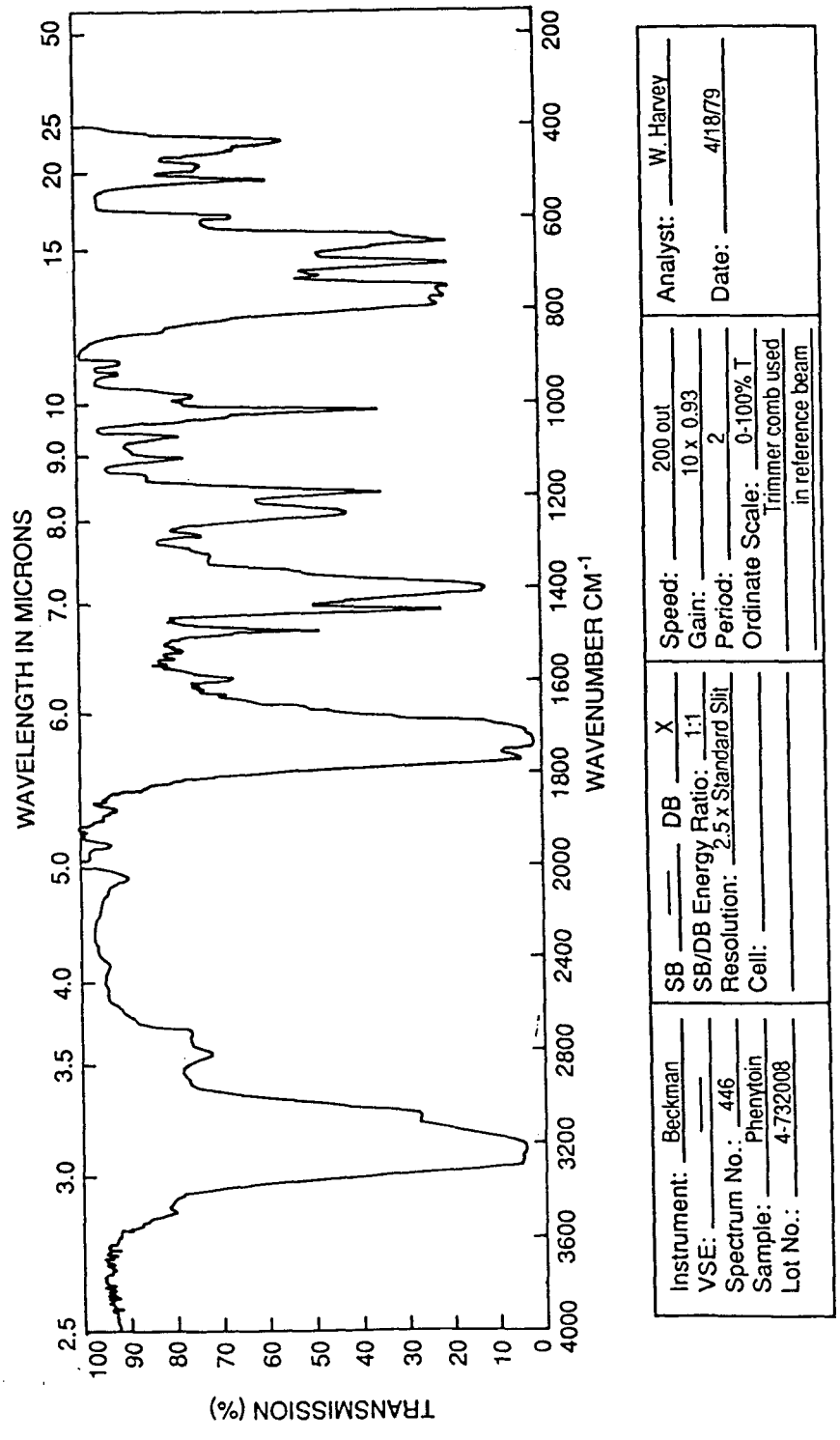

FIGURE H1
Infrared Absorption Spectrum of 5,5-Diphenylhydantoin

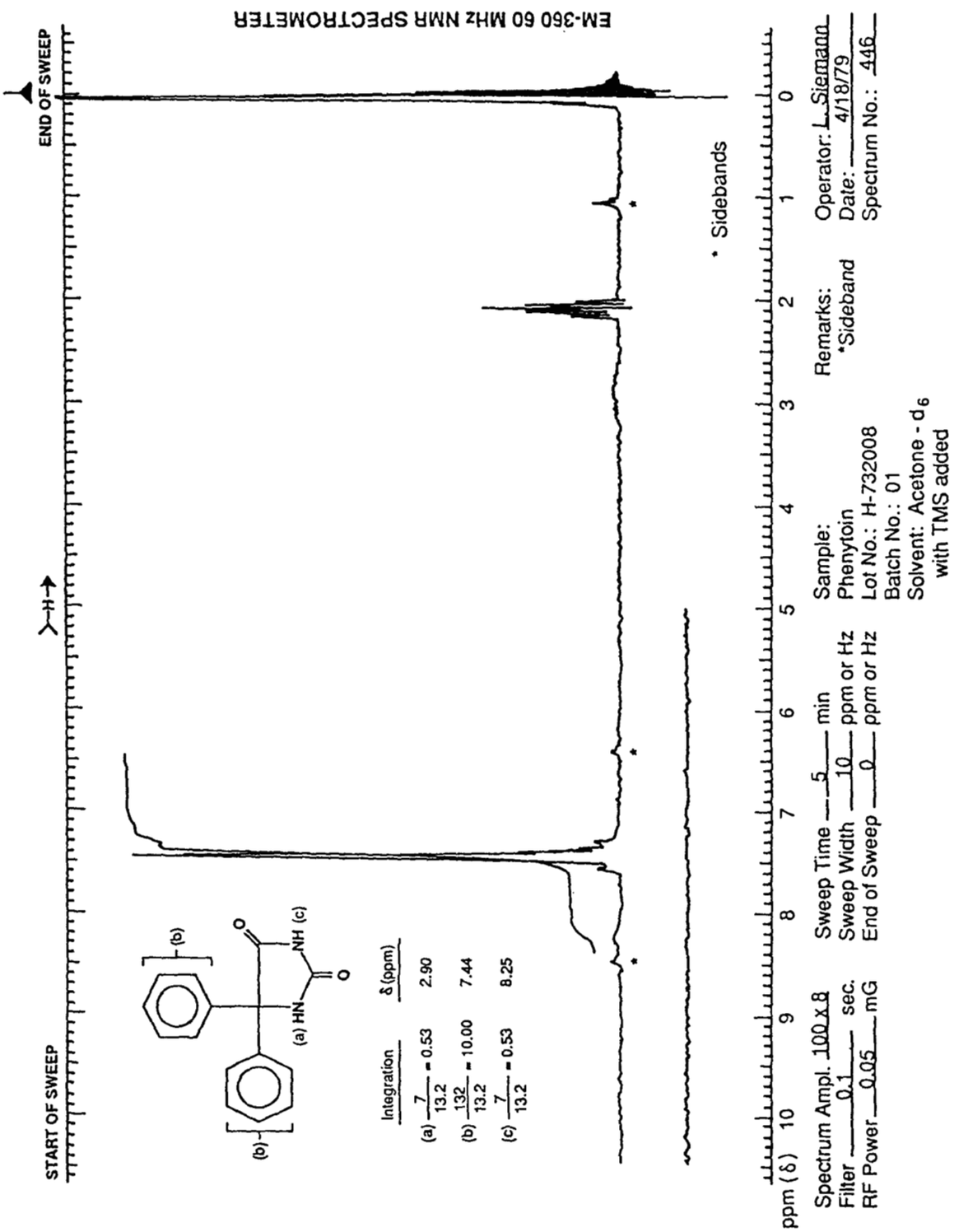

FIGURE H2
Nuclear Magnetic Resonance Spectrum of 5,5-Diphenylhydantoin

TABLE H1
Preparation and Storage of Dose Formulations in the 13-Week, Maximum Perinatal Dose, and 2-Year Feed Studies of 5,5-Diphenylhydantoin

Preparation
A premix of feed and 5,5-diphenylhydantoin was prepared, then remaining feed was blended into the premix in a Patterson-Kelly twin-shell blender. Doses were prepared every two weeks.

Chemical Lot Number
H-732008

Maximum Storage Time
2 weeks

Storage Conditions
In plastic-lined tin cans at room temperature

Study Laboratory
Battelle Columbus Laboratories, Columbus, OH

Referee Laboratory
Midwest Research Institute, Kansas City, MO

TABLE H2
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week Feed Studies of 5,5-Diphenylhydantoin

Date Prepared	Date Analyzed	Target Concentration[a] (ppm)	Determined Concentration[b] (ppm)	Difference from Target (%)
8 January 1980	22 January 1980	75[c]	76.0	+1
		75[d]	76.5	+2
		75[e]	74.0	–1
		150	163	+9
		300	318	+6
		600	550	–8
		1,200	1,170	–3
		2,400	2,285	–5
		4,800[c]	4,468	–7
		4,800[d]	4,578	–5
		4,800[e]	4,405	–8

[a] Target concentrations for rats: 300, 600, 1,200, 2,400, and 4,800 ppm. Target concentrations for mice: 75, 150, 300, 600, and 1,200 ppm.
[b] Results of duplicate analyses
[c] Sample selection from top left of twin-shell blender
[d] Sample selection from top right of twin-shell blender
[e] Sample selection from bottom of twin-shell blender

TABLE H3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the Maximum Perinatal Dose Determination Feed Studies of 5,5-Diphenylhydantoin

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
19 January 1981	19 January 1981	80	83	+4
		240	222	–8
		800	815	+2
		2,400	2,395	0
Mice				
19 January 1981	19 January 1981	20	22	+8
		60	61	+2
		200	217	+8
		600	588	–2

[a] Results of single analysis

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 5,5-Diphenylhydantoin[a]

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[b] (ppm)	Difference from Target (%)
11 November 1982	20 November 1982	100	98.8	–1
24 November 1982	8 December 1982	30	30.7	+2
		60	65.1	+9
		100	97.4	–3
		200	173	–14[c]
		240	200	–17[c]
		300	257	–14[c]
26 November 1982	9 December 1982	600	567	–5
		800	745	–7
		2,400	2,305	–4
27 January 1983	31 January 1983	240	237	–1
		600	593	–1
		800	797	0
		2,400	2,438	+2
28 January 1983	31 January 1983	30	29.8	–1
		60	61.8	+3
		100	94.8	–5
		200	192	–4
		300	282	–6
24 March 1983	7 April 1983	30	28.7	–4
		60	60.5	+1
		100	101	+1
		200	209	+4
		300	288	–4
25 March 1983	8 April 1983	240	223	–7
		600	579	–4
		800	786	–2
		800	751	–6
		2,400	2,373	–1
19 May 1983	23 May 1983	30	34.4	+15[c]
		60	63.3	+5
		100	94.5	–6
		200	201	0
		240	238	–1
		300	306	+2
		600	572	–5
		800	816	+2
		800	802	0
		2,400	2,346	–2

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 5,5-Diphenylhydantoin (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
15 July 1983	15 July 1983	240	225	–6
		300	272	–9
		600	560	–7
		800	769	–4
		800	824	+3
		2,400	2,339	–3
22 July 1983	25 July 1983	30	29.5	–2
		60	57.3	–5
		100	105	+5
		200	196	–2
8 September 1983	12 September 1983	240	217	–10
		300	290	–3
		600	639	+7
		800	785	–2
		2,400	2,241	–7
	19 September 1983	30	28.9	–4
		60	56.0	–7
		100	60.6	–39[c]
		200	114	–43[c]
17 November 1983	21 November 1983	240	235	–2
		300	308	+3
		600	589	–2
		800	785	–2
		2,400[d]	2,393	0
		2,400[e]	2,344	–2
		2,400[f]	2,308	–4
	22 November 1983	30[d]	31.2	+4
		30[e]	27.7	–8
		30[f]	30.8	+3
		60	55.4	–8
		100	92.3	–8
		200	192	–4
18 January 1984	20 January 1984	30	31.6	+5
		60	56.1	–6
		100	96.8	–3
		200	188	–6
		240	233	–3
		300	282	–6
		600	578	–4
		800	762	–5
		2,400	2,290	–5

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 5,5-Diphenylhydantoin (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
8 March 1984	9 March 1984	240	229	–5
		300	276	–8
		600	571	–5
		800	787	–2
		2,400	2,308	–4
	12 March 1984	30	31.2	+4
		60	58.0	–3
		100	96.8	–3
		200	186	–7
9 May 1984	11 May 1984	240	228	–5
		300	292[g]	–3
		600	587	–2
		800	759[g]	–5
		2,400	2,288[h]	–5
	18 May 1984	30	33.0[h]	+10
		60	62.1	+3
		100	96.9	–3
		200	198	–1
21 June 1984	26 June 1984	240	259	+8
		300	313	+4
		600	590	–2
		800	788	–2
		2,400	2,179	–9
	27 June 1984	30	30.6	+2
		60	64.6	+8
		100	95.8	–4
		200	190	–5
9 August 1984	15 August 1984	240	242	+1
		800	811	+1
		2,400	2,402	0

[a] Target concentrations for F_1 rats: 0, 240, 800, and 2,400 ppm. Target concentrations for F_1 mice: 0, 30, 100, and 300 ppm (males) or 0, 60, 200, and 600 ppm (females)
[b] Results of duplicate analyses
[c] Used for dosing; because 100 and 200 ppm dose formulations analyzed 19 September 1983 were out of range, formulations at these dose levels were analyzed again the following week and were within 10% of the target concentration.
[d] Sample selection from top left of twin-shell blender
[e] Sample selection from top right of twin-shell blender
[f] Sample selection from bottom of twin-shell blender
[g] Results of quadruplicate analyses
[h] Results of triplicate analyses

APPENDIX I
FEED CONSUMPTION

TABLE I1
Feed Consumption by Male Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

Month	0:0 ppm	630:0 ppm	63:240 ppm	0:800 ppm	210:800 ppm	630:800 ppm	0:2,400 ppm	630:2,400 ppm
1	19.5	17.7	22.0	20.8	19.9	20.2	18.3	17.3
2	17.7	17.4	15.9	15.6	16.6	18.4	16.0	15.7
3	12.7	13.1	12.9	12.3	13.7	12.0	12.1	11.2
4	16.5	16.2	17.7	15.6	17.0	16.1	16.2	16.3
5	20.7	19.9	18.5	20.2	19.9	19.0	18.5	19.4
6	19.6	18.9	19.3	17.5	18.6	17.5	16.9	17.3
7	18.5	19.1	18.7	18.8	19.5	14.8	17.2	16.8
8	19.1	18.2	18.3	18.0	18.7	17.3	17.4	17.5
9	19.1	17.9	19.3	18.7	18.3	18.2	17.2	16.8
10	17.5	17.1	17.4	17.9	18.0	17.6	16.9	17.0
11	16.5	16.4	16.1	17.8	17.3	17.6	17.4	17.2
12	20.6	19.6	18.9	19.0	21.0	19.8	19.3	18.2
13	19.0	18.6	18.0	18.9	18.7	19.7	18.2	17.9
14	16.9	18.5	17.5	17.3	18.3	17.9	17.3	17.1
15	16.0	15.9	17.4	15.9	16.8	16.6	15.8	16.2
16	19.0	17.4	17.3	17.4	17.8	17.4	17.0	16.8
17	17.0	17.4	17.5	16.5	17.3	17.6	16.8	16.5
18	17.3	17.1	17.4	16.3	17.8	18.2	17.1	17.4
19	17.4	17.4	18.5	17.0	16.6	17.3	17.5	17.2
20	16.7	16.6	17.2	13.4	13.6	14.6	13.4	13.7
21	16.0	17.0	17.4	17.1	16.7	17.7	16.0	16.4
22	15.8	15.7	17.8	18.1	19.7	16.8	15.8	16.4
23	20.3	20.7	20.2	20.2	22.4	22.3	19.4	24.8
24	19.0	17.3	19.2	21.7	20.3	21.5	21.3	20.0

[a] Feed consumption is given as grams of feed consumed per animal per day.

TABLE I2
Feed Consumption by Female Rats in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

Month	0:0 ppm	630:0 ppm	63:240 ppm	0:800 ppm	210:800 ppm	630:800 ppm	0:2,400 ppm	630:2,400 ppm
1	12.6	12.7	10.8	11.3	12.1	11.1	9.3	9.7
2	11.8	11.1	11.9	10.8	10.5	10.8	10.0	10.3
3	12.0	11.0	10.9	11.0	10.6	10.4	9.7	10.0
4	13.6	12.4	12.6	11.1	10.7	11.2	10.1	9.6
5	12.3	11.4	11.7	11.3	10.9	11.0	10.8	9.8
6	12.3	11.6	13.1	11.3	10.5	10.9	9.3	9.4
7	13.7	12.7	12.7	11.8	11.8	11.7	10.3	10.0
8	12.6	12.4	12.0	11.3	11.5	11.4	9.4	9.4
9	12.4	11.9	12.6	10.8	10.6	10.9	9.8	9.9
10	11.1	11.5	11.8	10.8	10.8	10.6	9.0	9.5
11	11.7	10.9	12.0	12.2	11.8	11.5	9.7	10.3
12	13.1	12.6	12.9	12.0	12.4	12.2	9.5	10.0
13	14.2	14.1	13.5	11.7	12.5	12.7	10.6	10.5
14	12.3	11.9	11.5	11.9	11.3	11.5	9.6	9.7
15	12.0	11.4	11.5	12.0	11.8	11.8	9.8	10.0
16	14.2	14.2	13.8	12.9	12.7	13.6	10.8	10.5
17	13.8	13.2	13.1	11.4	11.7	11.6	9.8	10.3
18	14.1	12.3	13.3	12.7	12.4	12.3	10.3	10.9
19	13.8	13.0	13.5	12.4	12.8	12.9	10.8	10.7
20	14.2	13.0	14.2	14.7	13.2	12.5	11.1	11.6
21	13.3	12.5	11.8	12.5	13.2	13.0	12.6	13.2
22	14.5	13.0	13.9	13.5	12.6	13.5	13.1	13.2
23	13.8	13.6	13.0	12.8	13.4	13.7	14.0	11.9
24	14.1	12.2	13.3	14.3	15.2	14.8	13.6	12.2

[a] Feed consumption is given as grams of feed consumed per animal per day.

TABLE I3
Feed Consumption by Male Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

Month	0:0 ppm	210:0 ppm	21:30 ppm	0:100 ppm	70:100 ppm	210:100 ppm	0:300 ppm	210:300 ppm
1	5.4	5.8	6.0	5.5	6.0	5.9	5.4	6.4
2	9.9	8.7	10.2	11.2	9.7	9.9	9.8	9.5
3	7.9	7.4	7.8	7.3	7.9	8.3	8.1	7.0
4	9.9	9.3	9.5	8.5	8.9	9.1	8.7	8.9
5	8.7	8.8	9.1	8.8	8.5	9.3	8.2	7.5
6	8.9	9.4	8.9	8.4	8.1	8.5	7.5	8.3
7	8.4	8.7	9.1	8.5	9.5	8.4	8.0	9.3
8	8.8	8.8	9.3	8.3	8.1	9.0	7.6	7.7
9	7.9	7.9	7.9	7.9	7.6	8.1	7.7	7.4
10	7.6	7.6	9.3	8.2	8.4	8.7	8.3	8.6
11	8.9	9.2	7.8	8.8	8.3	8.3	8.0	7.7
12	7.9	7.7	7.2	8.2	8.0	7.7	9.0	7.1
13	7.8	7.9	8.7	8.5	9.2	8.9	8.0	8.2
14	8.4	9.1	8.4	8.3	8.1	8.8	8.8	8.1
15	9.2	9.2	7.4	8.7	8.1	8.2	8.4	8.7
16	9.8	9.9	9.4	10.0	9.7	9.9	10.2	10.5
17	10.5	9.9	10.1	10.5	9.3	10.0	10.4	8.7
18	10.5	10.1	9.6	10.8	9.5	10.3	9.6	10.0
19	4.4	4.2	4.4	4.0	3.7	3.9	3.7	3.7
20	4.2	3.9	4.6	4.2	4.0	4.2	3.8	4.4
21	4.4	4.2	5.0	4.5	4.7	4.5	3.9	4.7
22	4.2	4.5	5.0	4.6	5.2	4.7	4.5	4.7
23	4.5	4.2	4.7	4.5	4.3	5.0	4.5	4.4
24	4.3	4.5	4.8	4.4	5.1	4.6	4.6	4.6

[a] Feed consumption is given as grams of feed consumed per animal per day.

TABLE I4
Feed Consumption by Female Mice in the 2-Year Feed Study of 5,5-Diphenylhydantoin[a]

Month	0:0 ppm	210:0 ppm	21:60 ppm	0:200 ppm	70:200 ppm	210:200 ppm	0:600 ppm	210:600 ppm
1	5.2	5.8	5.3	5.8	5.7	6.3	5.7	6.2
2	10.5	10.1	10.0	10.4	9.7	9.7	10.0	9.6
3	7.8	7.9	7.2	8.4	8.4	8.3	8.0	8.2
4	9.5	8.9	9.2	8.9	8.8	8.4	7.4	7.4
5	9.2	8.2	9.2	9.7	8.5	8.9	8.7	7.8
6	9.8	9.8	9.9	9.3	8.6	8.6	9.2	7.2
7	10.1	9.2	9.4	9.8	9.7	8.4	8.4	9.3
8	9.9	10.1	7.7	9.1	8.9	8.4	8.3	7.8
9	9.6	7.7	8.7	8.9	8.4	8.0	6.9	7.0
10	7.1	7.9	8.2	8.0	7.5	7.6	7.8	7.8
11	7.8	7.8	8.1	8.4	8.6	7.8	8.6	7.8
12	7.9	7.6	8.6	8.4	8.4	8.4	8.2	7.1
13	8.1	7.6	8.4	8.2	8.2	7.8	7.7	7.7
14	9.0	8.0	7.9	8.0	7.7	8.3	7.6	6.8
15	7.4	7.5	8.2	7.1	7.7	8.5	7.2	7.2
16	11.0	9.7	10.9	12.8	11.4	11.2	10.4	8.9
17	10.2	8.7	10.0	10.3	9.7	9.5	6.9	7.0
18	8.9	8.2	9.7	9.4	9.8	10.1	7.4	7.3
19	3.6	3.3	3.5	3.8	3.5	3.9	3.2	3.0
20	3.9	3.6	4.2	4.2	4.0	3.7	3.4	2.9
21	3.7	3.9	4.3	4.7	3.9	3.9	4.0	3.6
22	3.8	4.0	4.2	4.2	4.0	4.0	3.7	2.8
23	4.2	3.9	3.9	4.2	3.9	4.1	3.9	3.1
24	3.7	3.9	3.7	4.4	3.7	3.9	3.5	2.8

[a] Feed consumption is given as grams of feed consumed per animal per day.

APPENDIX J
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Rats

During the two-year studies, 30 F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Ten animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Samples for viral screening at 24 months were collected from five control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was diluted 1:5 with buffered saline and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the viral antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Complement Fixation	
RCV (rat coronavirus)	6 months
ELISA	
Mycoplasma arthritidis	24 months
Mycoplasma pulmonis	24 months
PVM (pneumonia virus of mice)	24 months
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	12, 18, and 24 months
Sendai	24 months
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	6, 12, 18, and 24 months
KRV (Kilham rat virus)	6, 12, 18, and 24 months
PVM	6, 12, and 18 months
Sendai	6, 12, and 18 months

Mice

During the 2-year studies, 30 $B6C3F_1$ mice of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Ten animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Samples for viral screening at 24 months were collected from five control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was diluted 1:5 with buffered saline and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the viral antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Complement Fixation	
Mouse adenoma virus	6, 12, 18, and 24 months
LCM (lymphocytic choriomeningitis virus)	6, 12, 18, and 24 months
ELISA	
GDVII (mouse encephalomyelitis virus)	24 months
MHV (mouse hepatitis virus)	6, 12, 18, and 24 months
Hemagglutination Inhibition	
Ectromelia virus	6, 12, 18, and 24 months
GDVII	6, 12, and 18 months
MVM (minute virus of mice)	6, 12, 18, and 24 months
Polyoma virus	6, 12, 18, and 24 months
PVM	6, 12, 18, and 24 months
Reovirus 3	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months
Immunofluorescence Assay	
MHV	24 months

Test results are presented in Table J1.

TABLE J1
Murine Virus Antibody Determinations for Rats and Mice in the 2-Year Feed Studies of 5,5-Diphenylhydantoin

	Interval (months)	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
Rats			
	6	19/20	Sendai
	12	16/20	Sendai
	18	17/19	Sendai
	24	1/10	*M. arthritidis*
		10/10	Sendai
Mice			
	6	5/20	MHV
	12	7/20	MHV
	18	16/16	MHV
		1/16	PVM
	24	16/20	GDVII
		12/20	MHV

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201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-Ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-Methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichlorethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosponium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Pariffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3

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340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3′-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
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406	γ-Butyrolactone
407	C.I. Pigment Red 3
408	Mercuric Chloride
409	Quercetin
410	Naphthalene
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413	Ethylene Glycol
414	Pentachloroanisole
415	Polysorbate 80
416	*o*-Nitroanisole
417	*p*-Nitrophenol
418	*p*-Nitroaniline
419	HC Hellow 4
421	Talc
422	Coumarin
423	Dihydrocoumarin
427	Turmeric Oleoresin
431	Benzyl Acetate
434	1,3-Butadiene
443	Oxazepam

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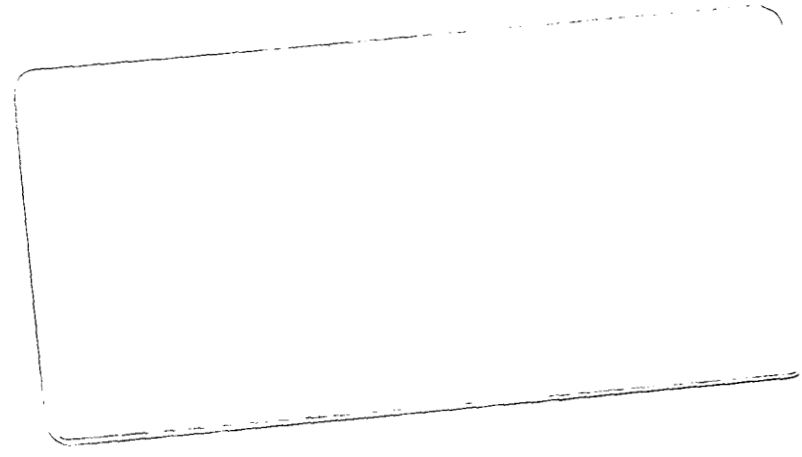

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