NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 443

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF OXAZEPAM

(CAS NO. 604-75-1)

IN SWISS-WEBSTER 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. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and 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

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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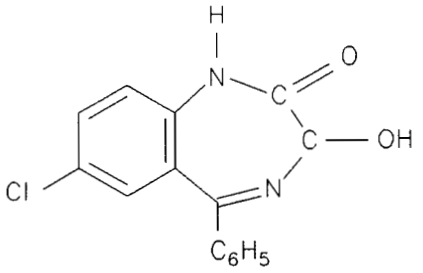
CONTENTS

ABSTRACT



OXAZEPAM

CAS No. 604-75-1

Chemical Formula: $C_{15}H_{11}ClN_2O_2$ Molecular Weight: 286.74

Synonym: 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one
Trade Names: Tazepam, Wy-3498, Serax

Oxazepam is one of a number of benzodiazepines used therapeutically as a sedative-hypnotic and anti-anxiety agent. Toxicology and carcinogenesis studies were performed by administering oxazepam (greater than 99% pure) in feed to male and female Swiss-Webster and B6C3F$_1$ mice for 14 weeks, 57 weeks (Swiss-Webster), or 2 years (B6C3F$_1$). Neurobehavioral assessments were performed during the studies. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells, and peripheral blood samples were analyzed for frequency of micronucleated normochromatic erythrocytes. Supplemental studies were performed to compare the metabolism and toxicokinetics of oxazepam in the two mouse strains, to evaluate the effect on liver cell replication rates, to perform clinical pathology assessments, and to examine the mutation spectrum and frequency of activated H-*ras* oncogenes in liver neoplasms from the 2-year study with B6C3F$_1$ mice.

14-Week Study in Swiss-Webster Mice

Groups of 10 male and 10 female Swiss-Webster mice received oxazepam in feed at concentrations of 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm for 14 weeks. One 625 ppm male and one 10,000 ppm female were killed moribund before the end of the study, and the condition of the female mouse was attributed to oxazepam exposure. Mean body weight gains of exposed groups were similar to those of the controls. Exposed mice displayed chemical-related sedation and lethargy during the first study week, but appeared normal thereafter. In the neurobehavioral studies, reductions in grip strength were evident in both male and female mice at week 2 and persisted in males through week 11. An antianxiety effect was detected in exposed mice in measures of motor activity, startle response, and reactions to thermal stimulus.

At necropsy, absolute and relative liver weights were increased in an exposure-related manner and were approximately two-fold greater in 10,000 ppm mice than in controls. Centrilobular hepatocellular hypertrophy was present only in exposed mice, and the severity increased with dose.

14-Week Study in B6C3F$_1$ Mice

Groups of 10 male and 10 female B6C3F$_1$ mice received oxazepam in feed at concentrations of 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm for 14 weeks. There were no deaths that were clearly related to oxazepam exposure. Mean body weight gains of exposed groups were similar to those of the controls.

Exposed mice displayed chemical-related sedation and lethargy during only the first study week. In neurobehavioral studies, reductions in grip strength were evident in males at week 2 but were no longer observed at week 12. An antianxiety effect was noted in exposed mice in measures of motor activity, startle response, and reactions to a thermal stimulus (females).

At necropsy, absolute and relative liver weights were increased in an exposure-related manner and were approximately two-fold greater in 10,000 ppm mice than in controls. Centrilobular hepatocellular hypertrophy was present only in exposed mice, and the severity increased with dose.

CHRONIC STUDIES

Groups of 60 male and 60 female Swiss-Webster and $B6C3F_1$ mice received oxazepam in feed at concentrations of 0, 2,500, or 5,000 ppm. Additional groups of 60 male and 60 female $B6C3F_1$ mice received 125 ppm in feed to allow for study of a group with projected serum concentrations of oxazepam similar to those achieved in humans taking a therapeutic dose. Ten male and 10 female $B6C3F_1$ mice per group were evaluated at 15 months. Average daily oxazepam consumption varied throughout the studies, and the overall daily average ranged from 10 to 29 mg/kg body weight for the 125 ppm groups, 234 to 512 mg/kg for the 2,500 ppm groups, and 444 to 1,085 mg/kg for the 5,000 ppm groups. Serum oxazepam concentrations determined at 57 weeks in Swiss-Webster mice and at the 15-month interim evaluation of $B6C3F_1$ mice were approximately 1 μg/mL in the 125 ppm groups, 4 to 7 μg/mL in the 2,500 ppm groups, and 7 to 10 μg/mL in the 5,000 ppm groups.

Neurobehavioral assessments during the chronic studies of each strain of mice were confounded by the poor survival and deteriorating condition of mice with hepatic neoplasia. However, within the limitations of the studies, there were no notable changes in the types of behaviors observed compared to those observed in the 14-week studies, nor was there an enhancement in the degree to which they were exhibited.

57-Week Study in Swiss-Webster Mice

Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings

At 57 weeks, survival of exposed mice was significantly lower than that of controls (males: 0 ppm, 45/60; 2,500 ppm, 19/60; 5,000 ppm, 10/60; females: 47/60, 28/59, 17/59), causing the study to be terminated. Mean body weights of exposed males were similar to controls until week 17; afterwards, mean body weights of exposed male groups were lower than those of controls. Final mean body weights of exposed males were 9% lower than that of the controls. The mean body weight of 2,500 ppm females was greater than that of the controls throughout the study. Females receiving 5,000 ppm had a mean body weight greater than that of the controls early in the study; after week 29, the mean body weight of this group was similar to that of the controls. Feed consumption by exposed males and females was slightly lower than that by the controls, and females in all groups, including controls, consumed slightly more feed than males throughout the study. Dietary levels of 2,500 and 5,000 ppm oxazepam resulted in average daily compound consumption levels of 270 and 570 mg/kg for males and 320 and 670 mg/kg for females. Hypoactivity and sedation were observed in exposed mice during the first week of the study. There were no other clinical findings associated with oxazepam exposure.

Pathology Findings

Systemic amyloidosis was the principal cause of death in mice dying before the study was terminated. The lower survival of mice receiving oxazepam was attributed to an increase in the extent and severity of amyloid deposits in many organs, including the heart and kidney. Atrial thrombosis and pulmonary lesions consistent with chronic heart failure occurred at higher incidences and with greater severity in exposed mice.

The incidence of hepatocellular adenomas (males: 1/60, 35/60, 50/60; females: 0/60, 22/59, 47/59) and carcinomas (males: 0/60, 5/60, 19/60; females: 1/60, 1/59, 11/59) were increased in exposed mice. The incidences of eosinophilic foci were also increased in exposed mice (males: 0/60, 22/60, 22/60; females: 0/60, 20/59, 14/59), and there was evidence of

increased centrilobular hepatocyte hypertrophy (males: 12/60, 46/60, 47/60; females: 3/60, 51/59, 53/59).

2-Year Study in B6C3F$_1$ Mice

Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings

Survival of mice receiving 2,500 and 5,000 ppm was significantly lower than that of controls (males: 0 ppm, 45/50; 125 ppm, 44/50; 2,500 ppm, 15/50; 5,000 ppm, 0/50; females: 39/50, 41/50, 2/50, 0/50). Mean body weight gains of exposed male and female mice were similar to controls until about week 15 when weight gains for mice exposed to 2,500 or 5,000 ppm slowed in relation to controls, resulting in weight gains approximately 30% to 40% lower than those of the controls throughout the remainder of the study. Mean body weight gain of male mice exposed to 125 ppm was similar to that of the controls, while that of female mice receiving 125 ppm was 10% to 15% lower than that of the controls after about week 45. Feed consumption by exposed males and females was similar to that by controls. Dietary levels of 125, 2,500, and 5,000 ppm resulted in average daily oxazepam consumption levels of 12, 310, and 690 mg/kg body weight for males and 15, 350, and 780 mg/kg for females. In the 5,000 ppm groups, lethargy and sedation were observed in a few mice during the first week of study.

Pathology Findings

The early deaths of many of the B6C3F$_1$ mice exposed to oxazepam were attributed to a marked increase in the incidences of hepatoblastoma (males: 0/49, 2/50, 21/50, 13/50; females: 0/50, 1/50, 8/50, 8/50), hepatocellular adenoma (males: 17/49, 18/50, 34/50, 32/50; females: 25/50, 35/50, 35/50, 36/50), and hepatocellular carcinoma (males: 9/49, 5/50, 45/50, 50/50; females: 9/50, 5/50, 49/50, 44/50). Moderate hypertrophy of centrilobular hepatocytes occurred in mice receiving 2,500 and 5,000 ppm (males: 0/49, 2/50, 26/50, 43/50; females: 0/50, 2/50, 11/50, 29/50). An increase in the incidence of follicular cell hyperplasia of the thyroid gland occurred in all exposed groups of mice (males: 4/49, 22/50, 49/50, 47/50; females: 16/50, 34/50, 49/50, 44/50), and thyroid gland follicular cell adenoma was increased in exposed females (0/50, 4/50, 5/50, 6/50). Testicular atrophy occurred in the 2,500 and 5,000 ppm groups (1/50, 0/50, 25/50, 38/50), and the incidence of epididymal lymphocyte infiltration was increased in all exposed groups (2/50, 14/50, 33/50, 21/50).

The frequency of hepatocellular neoplasms with an activated H-*ras* oncogene in the B6C3F$_1$ mice and the mutation spectrum of the H-*ras* gene were determined. The mutation spectrum of the H-*ras* genes in the relatively few neoplasms from exposed mice that did have an activated H-*ras* did not differ from the spectrum of mutations observed in neoplasms from controls, but the proportion of neoplasms with an activated H-*ras* gene decreased with increasing oxazepam dose. While 11 of 19 (58%) neoplasms from control mice had an activated H-*ras* gene, only 1 of 40 neoplasms from mice receiving 2,500 or 5,000 ppm oxazepam exhibited a similar molecular lesion. Thirteen of 37 (35%) neoplasms from mice in the 125 ppm group had an activated H-*ras* oncogene, suggesting that, although the incidence of all liver neoplasms was not statistically increased compared to controls, there was an increase in a similar subset of neoplasms (lacking an activated H-*ras*) that occurred with increased incidence at higher doses.

SUPPLEMENTAL STUDIES

Because exposure to oxazepam caused increased incidences of liver neoplasms, supplemental short-term studies were performed. Oxazepam given in feed to male B6C3F$_1$ mice at 25, 125, 2,500, or 5,000 ppm for up to 13 weeks was found to cause a dose-related increase in nuclear labeling index in studies measuring the incorporation of bromodeoxyuridine into replicating liver cells. This increase was statistically significant at all but the 25 ppm exposure level and was limited to mice evaluated at 15 days. Cell replication rates in most groups evaluated at 30 days and after were similar to control rates. There was minimal evidence suggestive of hepatocyte necrosis either by light microscopy or in clinical chemistry measures. There was, however, evidence of cholestasis, likely due to physical obstruction of bile canaliculi by swollen hepatocytes.

The metabolic fate and toxicokinetics of oxazepam were evaluated in each strain of mice and were compared to published data from human studies.

Both mice and humans form glucuronides of oxazepam and form 3- and 4-hydroxy and methoxy derivatives of the phenyl group. Oxidative metabolism of the phenyl group appears to be more prevalent in mice than is reported for humans. Elimination half-lives of parent compound do not differ between Swiss-Webster and $B6C3F_1$ mice and are similar to values reported for humans.

GENETIC TOXICOLOGY

Oxazepam was not mutagenic in any of several strains of *Salmonella typhimurium*, nor did it induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. These *in vitro* tests were performed with and without S9 metabolic activation. Results from an *in vivo* mouse peripheral blood micronucleus test performed on the $B6C3F_1$ mice used in the 14-week study were also negative.

CONCLUSIONS

Under the conditions of these feed studies, there was *clear evidence of carcinogenic activity** of oxazepam in male and female Swiss-Webster mice based on increased incidences of hepatocellular adenoma and carcinoma. There was *clear evidence of carcinogenic activity* of oxazepam in male and female $B6C3F_1$ mice based on increased incidences of hepatoblastoma and hepatocellular adenoma and carcinoma. Increased incidences of hyperplasia of thyroid gland follicular cells in male and female $B6C3F_1$ mice and of follicular cell adenomas in female $B6C3F_1$ mice were also related to oxazepam exposure.

Administration of oxazepam to Swiss-Webster mice resulted in centrilobular hepatocellular hypertrophy and increased incidences and severity of systemic amyloidosis. Administration of oxazepam to $B6C3F_1$ mice also resulted in centrilobular hepatocellular hypertrophy.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this report appear on page 12.

Summary of the Chronic Carcinogenesis and Genetic Toxicology Studies of Oxazepam

	Male Swiss-Webster Mice	Female Swiss-Webster Mice	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 2,500, or 5,000 ppm (approximately 270 or 570 mg/kg in feed)	0, 2,500, or 5,000 ppm (approximately 320 or 670 mg/kg in feed)	0, 125, 2,500, or 5,000 ppm (approximately 12, 310, or 690 mg/kg in feed)	0, 125, 2,500, or 5,000 ppm (approximately 15, 350, or 780 mg/kg in feed)
Body weights	Exposed groups lower than controls	2,500 ppm group higher than controls	2,500 and 5,000 ppm groups lower than controls	Exposed groups lower than controls
Survival rates[a]	45/60, 19/60, 10/60	47/60, 28/59, 17/59	45/50, 44/50, 15/50, 0/50	39/50, 41/50, 2/50, 0/50
Nonneoplastic effects	Multiple organs: increased incidence and severity of systemic amyloid deposition Liver: centrilobular hypertrophy (12/60, 46/60, 47/60)	Multiple organs: increased incidence and severity of systemic amyloid deposition Liver: centrilobular hypertrophy (3/60, 51/59, 53/59)	Liver: centrilobular hypertrophy (0/49, 2/50, 26/50, 43/50); Thyroid gland: follicular cell hyperplasia (4/49, 22/50, 49/50, 47/50)	Liver: centrilobular hypertrophy (0/50, 2/50, 11/50, 29/50); Thyroid gland: follicular cell hyperplasia (16/50, 34/50, 49/50, 44/50)
Neoplastic effects	Liver: hepatocellular adenoma (1/60, 35/60, 50/60); carcinoma (0/60, 5/60, 19/60)	Liver: hepatocellular adenoma (0/60, 22/59, 47/59); carcinoma (1/60, 1/59, 11/59)	Liver: hepatoblastoma (0/49, 2/50, 21/50, 13/50); hepatocellular adenoma (17/49, 18/50, 34/50, 32/50); carcinoma (9/49, 5/50, 45/50, 50/50)	Liver: hepatoblastoma (0/50, 1/50, 8/50, 8/50); hepatocellular adenoma (25/50, 35/50, 35/50, 36/50); carcinoma (9/50, 5/50, 49/50, 44/50); Thyroid gland: follicular cell adenoma (0/50, 4/50, 5/50, 6/50)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutation:	Negative in strains TA97, TA98, TA100, TA102, and TA1535 with and without S9			
Sister chromatid exchanges Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Chromosomal aberrations Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Micronucleated normochromatic erythrocytes in $B6C3F_1$ mice:	Negative at 14 weeks			

[a] Survival of Swiss-Webster mice based on a 57-week study; survival of $B6C3F_1$ mice based on 2-year study.

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 a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such 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 tumors 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 tumors of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific tumor;
- 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 oxazepam on December 1, 1992, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee 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 December 1, 1992, the draft Technical Report on the toxicology and carcinogenesis studies of oxazepam 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, Research Triangle Park, NC.

Dr. J.R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of oxazepam by discussing the uses and rationale for study, describing the experimental design in Swiss-Webster and $B6C3F_1$ mice, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in both mouse strains. Dr. Bucher reported that due to the marked enhancement of liver neoplasia in both strains, a number of supplemental studies were performed at NIEHS including a study to evaluate rates of replicative DNA synthesis in the liver, metabolic fate and toxicokinetic studies, and analysis of the frequency of occurrence of an activated H-*ras* oncogene in hepatocellular neoplasms in $B6C3F_1$ mice. The proposed conclusions were *clear evidence of carcinogenic activity* of oxazepam in male and female Swiss-Webster mice and in male and female $B6C3F_1$ mice.

Dr. Ward, a principal reviewer, agreed with the proposed conclusions. He said it should be noted that in the $B6C3F_1$ mouse study, the two highest exposure levels exceeded maximum tolerated dose guidelines, but despite the severe depression in body weight gain, liver neoplasms were associated with early mortality and increased feed consumption. Dr. Bucher thought this was a reasonable point for further discussion by the Subcommittee. Dr. Ward said it was important to establish whether the thyroid follicular cell hyperplasia was goiter (diffuse) or focal (not diffuse) in $B6C3F_1$ mice. Dr. Bucher responded that at the two highest exposure levels, hyperplasia was a diffuse goiter type. Dr. Ward asked that the appendixes associated with the supplemental studies be discussed in the Results section.

Dr. Taylor, the second principal reviewer, agreed with the proposed conclusions. He complimented the inclusion of the mechanistic studies and also urged that the appendixes be discussed in the Results section. Dr. Taylor thought the detailed discussion of chlordiazepoxide genotoxicity was not necessary since little of this agent metabolized to oxazepam, while genotoxicity information might be useful on temazepam, which is metabolically converted largely to oxazepam. Dr. Bucher agreed to add genotoxicity information on temazepam if available. (Genotoxicity information was not available in the literature.)

Dr. Ryan, the third principal reviewer, agreed with the proposed conclusions. She said the different patterns of weight gain between male and female Swiss-Webster mice were of some concern, and wondered if these patterns could be explained through the varying incidences of toxicity and neoplasia. Dr. Bucher said there was not a clear-cut cause and effect relationship that would explain the differences. Dr. Ryan asked why no studies were conducted to assess reproductive toxicity since one of the rationales for the study was to examine the use of the drug by pregnant women. Dr. Bucher commented that adequate reproductive and developmental toxicology studies had been conducted as a part of the FDA drug approval process. Dr. Ryan noted that since the 125 ppm exposure level in $B6C3F_1$ mice was included in an attempt to produce a blood level in the therapeutic range for humans, interpretation of the findings for humans should be addressed in the Conclusions. Dr. Bucher said he would add a phrase that there were indications in the study that the amount of oxazepam was sufficient at that level to influence expression of the neoplastic process (page 62).

Dr. Davidson asked that some of the nonneoplastic lesions, notably heart lesions (amyloidosis) in Swiss-Webster mice and testicular lesions in $B6C3F_1$ mice, be summarized in the text along with the appropriate statistical analysis. Dr. Bucher explained that since the amyloidosis was a systemic effect, such a focus on the heart lesions could be misleading. With regard to the testicular lesions, he said it was likely that this was a treatment-related effect but could also be secondary to debilitation of the animal. There was no evidence from the 14-week study that the testis was a target organ. Dr. Davis thought there needed to be a clear presentation in the text of the toxicokinetic studies including area under the curve (AUC) information, noting that the extensive

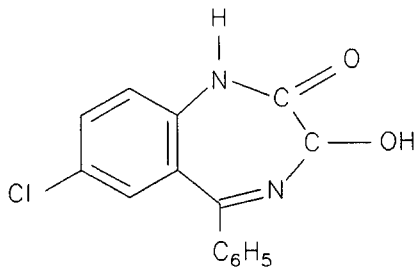
amyloidosis in one strain of mice could affect chemical disposition depending on the organs involved. Dr. Bucher said AUC data were included, and noted that young Swiss-Webster mice were used for the toxicokinetic studies so amyloidosis would not have been present.

In comments from the public, Dr. Michael McClain, Hoffman-LaRoche, stated that the existence of the thyroid follicular cell hypertrophy along with hyperplasia of a diffuse type provided fairly clear evidence that the thyroid gland effects were probably secondary to hormone imbalance. Dr. Klaassen asked whether serum thyroid-stimulating hormone (TSH) levels had been measured.

Dr. Bucher replied that thyroid hormone status was not determined in the studies done to date, but there were plans to measure TSH and other thyroid hormones in further studies. In response to a question by Dr. Klaassen about measurement of P-450 isoforms, Dr. Julian Leakey, NCTR, reported that his laboratory was going to be doing studies in rats and mice treated with oxazepam, looking at induction of specific isoforms of P-450. Dr. Joseph Contrera, Center for Drug Evaluation and Research, FDA, praised the interaction between the FDA and the NTP in the design and conduct of oxazepam studies.

Dr. Ward moved that the Technical Report on oxazepam be accepted with the revisions discussed and with the conclusions as written for male and female Swiss-Webster mice and male and female $B6C3F_1$ mice, *clear evidence of carcinogenic activity.* Dr. Taylor seconded the motion, which was accepted by nine yes votes with one abstention (Dr. van Zwieten).

INTRODUCTION



OXAZEPAM

CAS No. 604-75-1

Chemical Formula: $C_{15}H_{11}ClN_2O_2$ Molecular Weight: 286.74

Synonym: 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one
Trade Names: Tazepam, Wy-3498, Serax

CHEMICAL AND PHYSICAL PROPERTIES

Oxazepam is a bitter tasting, white crystalline powder, insoluble in water, but soluble in alcohol, chloroform, and ether (*Remington's Pharmaceutical Sciences*, 1980). The material is nonhygroscopic and stable in light. It has a melting point range of 205° to 206° C (*Merck*, 1983).

USE AND HUMAN EXPOSURE

Oxazepam and related benzodiazepine drugs are used in the treatment of anxiety. Most clinically useful drugs for this purpose are variants of the 1,4-benzodiazepine structure (Figure 1) consisting of two aromatic rings and a 7-membered heterocycle. One of the aromatic rings is fused to the 7-membered ring and contains a chlorine or other electronegative group as a substituent. All clinically important derivatives contain a 5-aryl or 5-cyclohexenyl group. Most of the drugs vary in substituent groups at the 1-3 positions (*Goodman and Gilman's*, 1990). Oxazepam, known under the trade name Serax®, is produced and sold by Wyeth Laboratories and has been on the market since 1965.

No definite production data are available for oxazepam or for the benzodiazepine drugs; however, the use of benzodiazepine by the general population has been reported as 8% in the United Kingdom, 7% in the United States, and 8% to 10% in Norway (Pedersen and Lavik, 1991). In 1983, 2.6 million prescriptions for oxazepam were written in the United States, and oxazepam ranked 132nd and 125th in overall frequency of prescriptions written for all drugs in 1984 and 1985, respectively (Anonymous, 1986; Baum, 1986). Oxazepam is also a common metabolite of several other benzodiazepines, some of which are more widely prescribed, including diazepam (Valium®). In 1983, 25.5 million U.S. prescriptions were written for diazepam, making it the fourth most prescribed drug.

REGULATORY STATUS

Benzodiazepines are prescription drugs regulated under the Federal Food, Drug, and Cosmetic Act of 1938 and are on Schedule IV of the Drug Enforcement Administration Controlled Substances Code (Tocus *et al.*, 1983). Although production workers and dispensers are exposed to benzodiazepines,

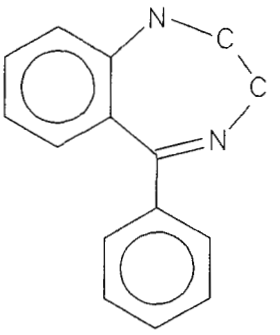


FIGURE 1
1,4-Benzodiazepine Structure

no workplace exposure limits have been recommended for these types of chemicals (ACGIH, 1987). Environmental contamination has not been shown.

PHARMACOLOGY

All benzodiazepines currently in use share a number of effects including sedation, hypnosis, decreased anxiety, muscle relaxation, amnesia, and anticonvulsant activity. They are considered central nervous system (CNS) depressants but are not general depressants and, within therapeutic dose ranges, all effects are related to specific CNS events (*Goodman and Gilman's*, 1990). Each drug differs slightly within this spectrum of actions (e.g., flurazepam has a strong hypnotic effect in humans) (Randall *et al.*, 1969). Other drugs are marketed specifically for use in obstetrics, for epilepsy, or for insomnia (CRM, 1980). These differences may reflect the different intrinsic affinities of the drugs for benzodiazepine receptors. In addition, the various drugs have different pharmacokinetics (Greenblatt and Shader, 1978; Eadie, 1984), and differences in disposition and rates of biotransformation may affect the spectrum of effects. Oxazepam is a relatively short-acting agent typically prescribed for relief of anxiety and given orally, 10 to 15 mg, three or four times per day (PDR, 1991).

The therapeutic effects of the benzodiazepines are thought to be due to a receptor-mediated response that increases the efficiency of submaximal GABAergic transmission mediated by a variety of long-fiber neurons and interneurons in the CNS (Richards *et al.*, 1986). A GABAergic receptor protein complex has been isolated from brain tissue. This complex is associated with a chloride ion channel and has associated proteins that are separate binding sites for barbiturates and the benzodiazepines (Barnard *et al.*, 1984). The benzodiazepine binding site is on the alpha subunit (Levitan *et al.*, 1988). The complex is subject to a complicated pattern of allosteric interactions which ultimately affect chloride conductance in the neuron. The clinically useful benzodiazepines all act to increase the permeability of the GABA receptor complex to chloride (Richards *et al.*, 1986). GABAergic neurons are in highest concentration in the substantia nigra, globus pallidus, and hypothalamus in the human brain (Cooper *et al.*, 1978). However, the density of CNS-type benzodiazepine receptors is highest in the cortical regions of the cerebrum and cerebellum, suggesting other functions for the CNS-type receptors (Saano, 1988). The anxiety-reducing effect of benzodiazepines in the rat brain has been associated with GABAergic circuits in the mammillary body (Kataoka *et al.*, 1982). At least one other benzodiazepine receptor type has been identified in the brain, specifically in glial tissues in the pineal gland and olfactory bulb, and is also found in heart, liver, lung, testis, and other tissues. The role of this receptor is not clear, but it appears to be a mitochondrial protein that may use porphyrins as endogenous ligands (Snyder *et al.*, 1987; Verma and Snyder, 1989; Calvo *et al.*, 1991), and may be involved in the regulation of steroid biosynthesis (Krueger and Papadopoulos, 1992).

ABSORPTION, DISPOSITION, METABOLISM, AND EXCRETION

Experimental Animals

In the mouse, 27.3% of an oral gavage dose of 22 mg/kg was recovered in urine and 57.8% in feces during 5 days following administration of ^{14}C oxazepam (labeled at the 2 carbon). The majority of urinary radioactivity was found to represent oxazepam glucuronide and 4′-hydroxyoxazepam glucuronide. Lesser amounts of 6-chloro-4-phenyl-2-(1H)-quinazoline carboxylic acid and 4′-hydroxy-3′-methoxyoxazepam were also identified, the latter as a sulfate. Fecal metabolites were not identified (Sisenwine *et al.*, 1987).

In the rat, following a single oral dose of 2 mg/kg with similarly labeled material as was given to mice, the radiolabel was found in most tissues within 30 minutes. Liver radiolabel peaked early and cleared within 24 hours (Walkenstein *et al.*, 1964). During the first 48 hours, 65% of the label appeared in the feces as unidentified metabolites. Seven labeled metabolites were found in the urine.

Sisenwine *et al.* (1972) identified a number of oxazepam metabolites collected in the urine over a 5-day dosing period in which 40 mg/kg per day was given orally to rats. The major peak appeared to be the 4′-hydroxyoxazepam glucuronide. Other metabolites included oxazepam substituted with a hydroxyl and a methoxy group on the phenyl ring, a metabolite in which the diazepine ring was condensed to a six-membered quinazolinone, and unchanged drug.

Labeled drug was administered in 0.5% Tween 80 to rats, and urine, bile, and feces were collected over 48 hours; 66% of the dose was recovered from the bile primarily within 12 hours (Sisenwine and Tio, 1986). Additional metabolites identified in the urine were 3′-hydroxyoxazepam and an unidentified compound thought to be a dihydrodiol. Major biliary and fecal metabolites included 4′-hydroxyoxazepam, the tentative dihydrodiol, and unchanged drug.

Humans

Oxazepam is readily absorbed following oral administration, and peak blood levels in humans are achieved in 0.75 to 8 hours when given in tablet form, with an average of 2.7 hours (Shader and Greenblatt, 1981). The half-life of oxazepam in the blood of humans is 6.8 ± 1.3 hours. It has a volume of distribution of 0.6 ± 0.2 L/kg and a clearance of 1.05 ± 0.36 mL/min/kg. Approximately 98% of the drug is bound to plasma proteins (*Goodman and Gilman's*, 1990). About 95% is converted to the C3 glucuronide conjugate by UDPglucuronyl transferase 2 (Rajaonarison *et al.*, 1991) and excreted in the urine; minor amounts of six other metabolites have been identified (Sisenwine *et al.*, 1972). Only the parent compound is thought to have antianxiety activity.

TOXICITY

Experimental Animals

Oral LD_{50} values have been reported to range from about 1,500 mg/kg to greater than 5,000 mg/kg in various strains of mice (Marcucci *et al.*, 1968; Randall *et al.*, 1970; Scrollini *et al.*, 1975; Petrescu *et al.*, 1981) and were greater than 5,000 mg/kg in Wistar and Charles River CD rats (Owen *et al.*, 1970; Scrollini *et al.*, 1975).

Owen *et al.* (1970) administered oxazepam in feed to Charles River CD rats at concentrations of 0.06%, 0.125%, 0.25%, and 0.5%. After 6 weeks, 2/20 high-dose rats had died, and weight gain was decreased in the 0.25% males. Liver, adrenal gland, and kidney weights were greater in dosed rats than in controls. The only histopathologic finding was an increase in liver parenchymal fat.

Groups of 30 male and 30 female rats were fed diets containing 0, 0.015%, 0.03%, 0.06%, or 0.12% oxazepam for 55 weeks. Deaths were not clearly chemical related, and other than increased liver weights, no effects on body weight gain or hematology parameters or significant chemical-related gross or histopathologic lesions were observed (Owen *et al.*, 1970).

The increased liver weights observed in these and other studies with benzodiazepines suggest stimulation of proliferation of smooth endoplasmic reticulum (Orlandi *et al.*, 1975). However, the benzodiazepines do not appear to stimulate their own metabolism and have been found to inhibit metabolism of other drugs such as morphine or aminopyrine in Wistar rats (Vega *et al.*, 1984) and to stimulate metabolism of certain chemicals (i.e., benzene and aniline) (Jablonska *et al.*, 1975). This coincides with their reputation of not producing significant tolerance during long-term therapy (*Goodman and Gilman's*, 1990). Physical dependence has been

demonstrated in rats with several of the drugs including diazepam (Martin *et al.*, 1982).

Humans

The benzodiazepines are a poor choice for suicide purposes and, despite many attempts, deaths by overdose are rare (Finkle *et al.*, 1979). Overdoses of oxazepam commonly result in drowsiness, blurred vision, and ataxia. As in rats, stimulation of proliferation of smooth endoplasmic reticulum has been shown in liver biopsies from humans taking diazepam (Orlandi *et al.*, 1975). Physical dependence is produced in humans given benzodiazepines.

CARCINOGENICITY

Experimental Animals

A number of long-term rodent studies have been performed with the benzodiazepines. Fox and Lahcen (1974) observed liver neoplasms in oxazepam-treated Swiss-Webster mice during the course of reproductive toxicity studies. Mice were housed as breeding pairs from 3 to 12 months of age and were fed an oxazepam-supplemented diet at doses of 0.05% and 0.15%. They were killed at 14 months of age. The incidences of liver neoplasms increased in males (0/13, 3/12, 8/13) and females (0/10, 0/10, 5/8) with dose. The neoplasms were generally multiple and gave the livers a massively nodular appearance. Histopathologically, the neoplasms were diagnosed as hepatocellular adenomas, which showed peliosis and extramedullary hematopoiesis.

De la Iglesia *et al.* (1981) fed diazepam or prazepam in the diet at concentrations sufficient to result in doses up to 75 mg/kg per day to male and female CF_1 mice and Wistar rats for 80 and 104 weeks, respectively. The incidence of malignant liver neoplasms was increased in male mice receiving diazepam. Temazepam, which is metabolized to oxazepam in the mouse, was administered in the diet to CRCD rats for 2 years and to CRCD-1 mice for 18 months at doses of 10 to 160 mg/kg per day. Female mice had a slightly increased incidence of liver adenomas (Robinson *et al.*, 1984).

PROMOTION STUDIES

The benzodiazepines have been tested in various promotion assays because of reports, primarily from one laboratory, that diazepam treatment accelerated the growth of intrarenally implanted neoplasm cells (Walker 256) (Horrobin *et al.*, 1979) and that it was positive in an *in vitro* metabolic cooperation assay for neoplasm promoters (Trosko and Horrobin, 1980). These reports appeared following publication of an epidemiological study that suggested an association between increased incidences of breast cancer and benzodiazepine use in women (Stoll, 1976). This association was later discounted (Kleinerman *et al.*, 1984), but further animal experimentation has provided mixed results.

Remandet *et al.* (1984) fed F344 rats *N*-2-fluorenylacetamide for 8 weeks and followed this for 12 weeks with diets containing one of six benzodiazepines. They reported no increased incidences of liver neoplasms or enzyme-altered foci. Preat *et al.* (1987) reported positive promotional activity with oxazepam in Wistar rats in two different assays for hepatocarcinogenesis. In one, animals were initiated with diethylnitrosamine (DEN) and were treated with 2-acetylaminofluorene and carbon tetrachloride during the next 2 weeks; they then received oxazepam in the diet for 30 weeks. In the other protocol, initiation with DEN was preceded by partial hepatectomy, and promotion was effected by dietary administration for 1 year. Diwan *et al.* (1986) found diazepam and oxazepam to be promoters of DEN-initiated liver neoplasms in mice. In this study, groups of $B6C3F_1$ mice received injections of DEN at 5 weeks of age; at 7 weeks they were fed diets containing diazepam or oxazepam at 0.05% or 0.15%, or given phenobarbital in water at 500 ppm. Mice were killed periodically through 60 weeks of age. The incidence of neoplasms was increased in mice receiving diazepam and in those receiving 0.15% oxazepam. A few adenomas were also observed in uninitiated mice receiving 0.15% diazepam (3/15) or 0.05% oxazepam (2/16), and none were observed in mice receiving only phenobarbital. Diazepam and oxazepam were also found to induce hepatic P-450 content and increase aminopyrine *N*-demethylase activity. Diwan *et al.* (1986) have proposed that promotion of hepatocellular carcinogenesis is associated with induction of *N*-demethylase activity and appears to be quite species and strain specific. Diazepam did not induce cytochrome P-450 in the liver of Sprague-Dawley rats (Vorne and Idanpaan-Heikkila, 1975), and this was considered consistent with the negative promotional findings of Remandet *et al.* (1984) in their study with F344 rats.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Although the benzodiazepines have been used in treating toxemia and preeclampsia as well as the psychiatric complications of pregnancy (Shannon *et al.*, 1972; Kanto, 1982), there are many reports of fetotoxic and teratogenic effects of these and other minor tranquilizers when given to pregnant animals. Tucker (1985) provided a critical review of studies of developmental toxicity of benzodiazepines in the rat. Saito *et al.* (1984) found increases in fetal toxicity (resorptions, dead fetuses, and malformations) in pregnant rats given doses of diazepam or chlordiazepoxide of 100 mg/kg *per os* during days 7 to 14. Miller and Becker (1975) first found diazepam produced cleft palate following oral administration of 87.5 or 125 mg/kg to Swiss-Webster mice on days 11 to 13. This has since received considerable study and is now attributed to potentiation of the GABAergic inhibition of the palate shelf reorientation (Wee and Zimmerman, 1983). In general, exposures to high doses *in utero* produce decreased litter sizes, decreased pup weights, and increases in malformations. Exposures to lower doses (5 to 20 mg/kg per day) during critical periods (after day 14 in rats) produce no immediately obvious effects at birth but result in various behavioral deficits during later life and a variety of poorly understood changes in the concentration of neurotransmitters in various brain areas (Livezey *et al.*, 1986a; Ryan and Pappas, 1986; Shibuya *et al.*, 1986). Central to these studies have been attempts to correlate changes in benzodiazepine receptor concentration with altered behavior. Livezey *et al.* (1986b) have argued that *in utero* exposure to benzodiazepines during the period of receptor development (after gestational day 14 in rats) results in a decreased benzodiazepine receptor concentration and results in a rat that suffers chronic anxiety demonstrated by hyperarousal, inability of the animals to habituate to a novel environment, and a large reduction in the amount of deep slow-wave sleep.

Humans

Exposure of the human fetus to diazepam results in a set of symptoms collectively known as the "floppy infant syndrome," which includes hypothermia, hyperbilirubinemia, hypotonia, asphyxia, respiratory complications, and poor sucking response. This is likely due to the ready transfer of the drugs across the placenta. Pharmacologic effects are exaggerated in the unborn because higher levels accumulate due to the slower elimination from the fetus. There have been reports of increases in severe congenital anomalies in infants whose mothers took chlordiazepoxide and other benzodiazepines (including oxazepam) during pregnancy (Milkovich and van den Berg, 1974); there have also been reports to the contrary (Hartz *et al.*, 1975).

GENETIC TOXICITY

Oxazepam has not been tested extensively for mutagenicity, but the data reported for oxazepam and its structural analogues indicate that this class of chemicals is probably not genotoxic. Positive responses in a *Salmonella* gene mutation assay were reported only by Batzinger *et al.* (1978). They described an increase in revertants for strains TA100 and TA98 when exposure was carried out in the presence of rat liver S9 activation enzymes. Insufficient data were reported to allow an evaluation of the results. In a brief abstract that presented little experimental detail, Matula and Downie (1983) reported negative results in strains TA100 and TA98, with and without S9. Balbi *et al.* (1980) detected no mutagenic activity with oxazepam in four strains of *Salmonella*, with or without S9, but their report did not include complete data tables for those tests that gave negative results.

No evidence of chromosome nondisjunction was observed in *Aspergillus nidulans* treated with an unspecified concentration of oxazepam in the absence of S9 (Bignami *et al.*, 1974). Unscheduled DNA synthesis was not detected in rat liver cells *in vitro* (Swierenga *et al.*, 1983), and no induction of chromosomal aberrations was observed in bone marrow cells of mice administered oxazepam in doses of 0.85 mg/kg body weight by intraperitoneal injection, five times weekly for 8 weeks (Degraeve *et al.*, 1985).

A variety of genotoxicity tests have been performed with two of the widely used structural analogues of oxazepam, diazepam, and chlordiazepoxide. Diazepam was nonmutagenic in *Salmonella* (Batzinger *et al.*, 1978; Waskell, 1978; Preiss *et al.*, 1982; Zeiger *et al.*, 1992). There was no evidence of diazepam-induced chromosome loss or nondisjunction in yeast (Bignami *et al.*, 1974; Matula and Downie, 1983; Crebelli *et al.*, 1989; Parry *et al.*, 1989; Whittaker *et al.*, 1990; Crebelli *et al.*, 1991). The effects reported for diazepam in cultured mammalian cells varied. Two laboratories (Ishidate *et al.*, 1978;

Matsuoka *et al.*, 1979) found no induction of chromosomal aberrations in cultured Chinese hamster ovary cells with or without S9. However, a positive study for induction of chromosomal aberrations in cultured Chinese hamster ovary cells without S9 was reported (Lafi and Parry, 1988), and disruption of mitosis with concomitant chromosome loss was observed in cultured Chinese hamster ovary cells following treatment with diazepam, without S9 (Hsu *et al.*, 1983; Parry *et al.*, 1986; Lafi *et al.*, 1987). Results of tests for induction of chromosomal aberrations and sister chromatid exchanges in human lymphocytes (Staiger, 1970; Zhurkov, 1975) or fibroblasts (Staiger, 1969; Kawachi *et al.*, 1980; Sasaki *et al.*, 1980) treated *in vitro* with diazepam were uniformly negative. Unscheduled DNA synthesis was not detected in rat liver cells treated *in vitro* with diazepam (Swierenga *et al.*, 1983; Williams *et al.*, 1989).

In vivo tests with diazepam showed little indication of genotoxic activity. No evidence of mitotic disruption or induction of chromosomal aberrations was observed in mouse bone marrow cells following administration of 100 to 150 mg/kg diazepam (Miller and Adler, 1989; Xu and Adler, 1990). Diazepam did not induce chromosomal aberrations in bone marrow cells of hamsters (Schmid and Staiger, 1969) or rats (Ishimura *et al.*, 1975; Kawachi *et al.*, 1980). In addition, no increases in chromosomal aberrations (Stenchever *et al.*, 1970a; White *et al.*, 1974) or sister chromatid exchanges (Torigoe, 1979; Husum *et al.*, 1985) were observed in peripheral lymphocytes obtained from patients treated with diazepam either chronically, as a management for anxiety or muscle spasm, or acutely, as part of a surgical routine.

Fewer genotoxicity test results are available for chlordiazepoxide, but indications are that it, too, is not genetically active. Chlordiazepoxide did not induce nondisjunction in *A. nidulans* (Bignami *et al.*, 1974), or chromosomal aberrations in cultured Chinese hamster ovary cells (Sasaki *et al.*, 1980), human fibroblasts (Staiger, 1969), or leukocytes (Bregman, 1970; Stenchever *et al.*, 1970b). *In vitro* micronucleus tests with hamster and human cells were negative (Sasaki *et al.*, 1980). Results of *in vivo* investigations indicate that chlordiazepoxide does not induce chromosomal aberrations in mouse (Peterson *et al.*, 1978; Degraeve *et al.*, 1985) or hamster (Schmid and Staiger, 1969) bone marrow cells. Finally, no induction of chromosomal aberrations was observed in lymphocytes obtained from patients administered chlordiazepoxide (up to 200 mg/day) (Stenchever *et al.*, 1970b).

STUDY RATIONALE

Oxazepam and four other benzodiazepines (chlordiazepoxide HCl, chlorazepate, diazepam, and flurazepam) were nominated for study by the Food and Drug Administration (FDA) and by NIEHS based on their high use volume, use by pregnant women, and the lack of adequate rodent carcinogenicity studies. An agreement was reached with Hoffman-LaRoche, Inc., the manufacturer of chlordiazepoxide HCl, diazepam, and flurazepam, for studies to be carried out on these drugs under their auspices in cooperation with the NTP. These studies are currently underway. No studies were performed on chlorazepate because of the very similar metabolite profile between this drug and diazepam. Oxazepam was evaluated in 14-week and chronic studies by the NTP, and this Technical Report contains the results of studies performed with the Swiss-Webster and B6C3F$_1$ strains of mice. Studies with rats were not initiated at the same time as the mouse studies because adequate carcinogenicity studies of oxazepam with the Sprague-Dawley rat strain had been submitted to FDA by the manufacturer, Wyeth Laboratories. Subsequently, because of the marked neoplastic responses found in the two mouse strains reviewed in this report, the NTP initiated further 2-year studies of oxazepam with the Fischer 344/N rat.

Swiss-Webster mice were used in addition to the B6C3F$_1$ strain because of the evidence of oxazepam-induced hepatocellular neoplasia in this strain reported by Fox and Lahcen (1974). The current studies include neurobehavioral assessments, measures of serum oxazepam concentrations, and histopathologic evaluation of tissues. Because evidence of hepatocellular neoplasia was found in the 2-year studies, an additional set of studies was performed to evaluate the comparative metabolism of oxazepam in the Swiss-Webster and B6C3F$_1$ mouse, the mitogenic properties of oxazepam on the liver, and the incidence of liver neoplasms with activated H-*ras* oncogenes and their mutational spectrum. Studies were also conducted to establish the pharmacokinetics of oxazepam administered to animals in feed.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF OXAZEPAM

Oxazepam was obtained from Roussel Corporation (Englewood Cliffs, NJ) in one lot (86017.01), which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). The reports on analyses performed in support of the oxazepam studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a white, powdered solid, was identified as oxazepam by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of oxazepam was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography, and high-performance liquid chromatography.

Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were in agreement with the theoretical values for oxazepam. Karl Fischer analysis indicated less than 0.03% water. Functional group titration indicated a purity of 101%. Thin-layer chromatography was performed using two systems: one indicated a major spot and one trace impurity, and the other indicated a major spot. High-performance liquid chromatography resolved a major peak with no impurity peaks with areas 0.1% or greater relative to the major peak. Major peak comparison between this lot and a United States Pharmacopeia XXI standard indicated a relative purity of 103%. The overall purity was determined to be greater than 99%.

Stability studies performed by the analytical chemistry laboratory using high-performance liquid chromatography indicated that oxazepam was stable for 2 weeks when stored protected from light at temperatures up to 60° C. The stability of the bulk chemical was monitored periodically at the study laboratory using infrared spectroscopy and high-performance liquid chromatography. No degradation of the bulk chemical was observed throughout the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared monthly for the 14-week studies and every 2 weeks for the chronic studies by mixing oxazepam and feed in a Patterson-Kelley twin-shell blender (Table H1). The mixture was stored in sealed, labeled, plastic buckets for up to 3 weeks at 5° C.

Homogeneity and dose formulation stability analyses of the 500 ppm concentration were performed at the analytical chemistry laboratory using high-performance liquid chromatography. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 3 weeks when stored protected from light at 5° C.

Periodic analyses of the dose formulations were conducted at the study laboratory and at the analytical chemistry laboratory using high-performance liquid chromatography. During the 14-week studies, all dose formulations for Swiss-Webster and B6C3F$_1$ mice were within 10% of the target concentrations (Table H2). During the chronic studies, dose formulations were analyzed approximately every 8 weeks; all dose formulations for Swiss-Webster and B6C3F$_1$ mice were within 10% of the target concentrations except two 125 ppm formulations for B6C3F$_1$ mice. These dose formulations were remixed. Results of the dose formulation analyses for the chronic studies are presented in Table H3. Results of periodic referee analyses performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratories (Table H4).

14-WEEK STUDIES

The 14-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to oxazepam and to determine the appropriate doses to be used in the chronic studies.

Male and female Swiss-Webster mice were obtained from Charles River Breeding Laboratories

(Portage, MI) and male and female B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). At receipt, the animals were 34 to 40 days old. The mice were quarantined for 13 or 14 days before dosing began. Before the beginning of the studies, five males and five females of each strain were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five males and five females of each strain using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female Swiss-Webster and 10 male and 10 female B6C3F$_1$ mice were assigned to the core study and received 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm oxazepam in feed for 14 weeks. A second group of 10 male and 10 female Swiss-Webster and 10 male and 10 female B6C3F$_1$ mice were assigned to the special study and were maintained on dosed feed until scheduled terminations during weeks 2 and 12. Animals were housed individually; water and feed were available *ad libitum*. Clinical findings were recorded once weekly. The animals were weighed at the beginning of the studies, weekly, and at the end of the studies. Further details of study design and animal maintenance are summarized in Table 1.

The core study mice were subjected to a series of neurobehavioral tests prior to the beginning of the 14-week studies and during weeks 2 and 12 of the studies. The neurobehavioral tests included undifferentiated motor activity, forelimb and hindlimb grip strengths, thermal sensitivity, and acoustic startle responsiveness (Appendix G).

Ten mice per exposure group in the special study were anesthetized with CO_2 and blood samples were collected by cardiac puncture during weeks 2 and 12 for serum oxazepam determinations. At the end of the 14-week study, a necropsy was performed on all remaining animals. The heart, right kidney, liver, lung, right testis, and thymus of mice were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all control and 10,000 ppm animals, and on all core study animals dying before the end of the study. Table 1 lists the tissues and organs routinely examined.

CHRONIC STUDIES

Study Design

Groups of 60 male and 60 female Swiss-Webster mice received 0, 2,500, or 5,000 ppm oxazepam in feed for 57 weeks; groups of 60 male and 60 female B6C3F$_1$ mice received 0, 125, 2,500, or 5,000 ppm oxazepam in feed for 104 to 105 weeks. Ten male and 10 female B6C3F$_1$ mice per exposure group were evaluated after 15 months of chemical exposure.

Source and Specification of Animals

Male and female Swiss-Webster mice were obtained from Charles River Breeding Laboratories and male and female B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc., for use in the chronic studies. The mice were quarantined for 13 to 15 days before the beginning of the studies. Five male and five female Swiss-Webster and B6C3F$_1$ mice were selected for parasite evaluation and gross observation of disease. Serology samples were collected for viral screening. Swiss-Webster mice were approximately 46 days old and B6C3F$_1$ mice were approximately 44 days old at the beginning of the chronic studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

All animals were housed individually. Feed and water were available *ad libitum*. Feed consumption was recorded every 4 weeks for a 7-day period (Appendix I). Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks. Animals were weighed weekly for the first 13 weeks and every 4 weeks thereafter. Ten male and 10 female Swiss-Webster mice from each exposure group were anesthetized with a mixture of CO_2 and oxygen at the end of the study, and blood was drawn by cardiac puncture to determine serum oxazepam concentrations. Ten male and 10 female B6C3F$_1$ mice from each group were selected for interim evaluations after

15 months. All $B6C3F_1$ mice selected for the 15-month interim evaluation and 10 male and 10 female $B6C3F_1$ mice in the 0, 125, and 2,500 ppm groups (excluding animals selected for neurobehavioral evaluation) were anesthetized with a mixture of CO_2 and oxygen and blood was collected by cardiac puncture for determination of serum oxazepam concentrations.

A necropsy was performed on all animals. The kidneys and livers of $B6C3F_1$ mice were weighed. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. A complete histopathologic examination was performed on all animals and on tissues with grossly visible lesions. Tissues examined are listed in Table 1.

Groups of 10 male and 10 female Swiss-Webster mice from each group were evaluated by noninvasive procedures for neurobehavioral toxicity during the prestudy period and after 6 and 12 months of exposure. Similarly, groups of 10 male and 10 female $B6C3F_1$ mice were evaluated for neurobehavioral toxicity during the prestudy period and after 6, 12, 18, and 24 months of exposure. The same animals were tested at each time point, but animals dying early were replaced by mice randomly selected from the survivors. The tests included motor activity, startle responsiveness, forelimb and hindlimb grip strength, and thermal sensitivity. Further details of these studies are outlined in Appendix G.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscopic slides, paraffin blocks, and residual wet tissues were sent to the NTP Archive for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated by the quality assessment laboratory. The quality assessment pathologist microscopically reviewed selected neoplasms and nonneoplastic lesions.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues for which a disagreement in diagnosis between the laboratory and quality assessment pathologist existed. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologist, or lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of exposure levels or previously rendered diagnoses. For the chronic studies, tissues examined included heart, right kidney, liver, lung, pancreas (males), skeletal muscle (males), and spleen. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals accidentally killed or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A4, B1, B4, C1, C5, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance,

the incidences of most neoplasms (Tables A3, B3, C3, and D3) and of all nonneoplastic lesions are given as the number of affected animals and 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

With the exception of malignant liver neoplasms, the 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 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 the fit of the model was not significantly enhanced. The neoplasm incidences of 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, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms and used in the evaluation of hepatocellular carcinomas and hepatoblastomas in this Technical Report, 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.

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

Analysis of Nonneoplastic Lesion Incidences

Amyloid deposition in the heart was a major cause of death in Swiss-Webster mice. Because the remaining 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 nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Serum oxazepam concentration data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison method of Shirley (1977). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's test). Neurobehavioral data were analyzed using Dunnett's test. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, 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

The 14-week and chronic studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the chronic studies were submitted to the NTP Archives, these studies 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 preliminary review draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of oxazepam was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, and increases in micronucleated B6C3F$_1$ mouse peripheral blood erythrocytes. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of oxazepam 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 and responses of the chemical 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 complimentarity 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 Oxazepam

	14-Week Studies	Chronic Studies
Study Laboratory	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species	Swiss-Webster and B6C3F$_1$ mice	Swiss-Webster and B6C3F$_1$ mice
Animal Source	Swiss-Webster mice: Charles River Breeding Laboratories (Portage, MI) B6C3F$_1$ mice: Simonsen Laboratories, Inc. (Gilroy, CA)	Swiss-Webster mice: Charles River Breeding Laboratories (Portage, MI) B6C3F$_1$ mice: Simonsen Laboratories, Inc. (Gilroy, CA)
Size of Study Groups	Core study: 10 males and 10 females Special study: 10 males and 10 females	60 males and 60 females
Doses	0, 625, 1,250, 2,500, 5,000, or 10,000 ppm in feed	Swiss-Webster mice: 0, 2,500, or 5,000 ppm in feed B6C3F$_1$ mice: 0, 125, 2,500, or 5,000 ppm in feed
Time Held Before Studies	13-14 days	13-15 days
Average Age When Studies Began	Swiss-Webster mice: 48 days B6C3F$_1$ mice: 53 days	Swiss-Webster mice: 46 days B6C3F$_1$ mice: 44 days
Date of First Dose	Swiss-Webster mice: 8 June 1988 (males) or 9 June 1988 (females) B6C3F$_1$ mice: 18 May 1988 (males) or 19 May 1988 (females)	Swiss-Webster mice: 13 July 1989 (males) or 14 July 1989 (females) B6C3F$_1$ mice: 22 June 1989 (males) or 23 June 1989 (females)
Duration of Dosing	14 weeks	Swiss-Webster mice: 57 weeks B6C3F$_1$ mice: 104-105 weeks
Date of Last Dose	Swiss-Webster mice: 8 September 1988 (males) or 9 September 1988 (females) B6C3F$_1$ mice: 18 August 1988 (males) or 19 August 1988 (females)	Swiss-Webster mice: 13 August 1990 (males) or 14 August 1990 (females) B6C3F$_1$ mice: 21 June 1991 (males) or 28 June 1991 (females)
Necropsy Dates	Swiss-Webster mice: 8-9 September 1988 B6C3F$_1$ mice: 18 August 1988 (males) or 19 August 1988 (females)	Swiss-Webster mice: 13 August 1990 (males) or 14 August 1990 (females) B6C3F$_1$ mice: 20-21 June 1991 (males); 27-28 June 1991 (females)

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Oxazepam (continued)

14-Week Studies	Chronic Studies
Average Age at Necropsy	
Swiss-Webster mice: 140 days $B6C3F_1$ mice: 145 days	Swiss-Webster mice: 63 weeks $B6C3F_1$ mice: 110-111 weeks
Method of Sacrifice	
CO_2 asphyxiation	Same as 14-week studies
Method of Animal Distribution	
Animals were randomized by weight with a computer randomization program.	Same as 14-week studies
Animals per Cage	
1	Same as 14-week studies
Method of Animal Identification	
Tail tattoo and ear tag	Tail tattoo
Diet	
Zeigler NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly or as necessary	Same as 14-week studies. Feed consumption recorded every 4 weeks for a 7-day period.
Water	
Tap water (City of Columbus) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 14-week studies
Cages	
Polycarbonate (Lab Products, Inc., Maywood, NJ), changed weekly. There were three racks of cages. Vertical columns containing five cages of like exposure group were randomly assigned to positions on the racks. Every 2 weeks, cages were rotated vertically within each column and racks were rotated clockwise.	Cages and rotation same as 14-week studies. There were three (Swiss-Webster) or four ($B6C3F_1$) racks of cages. Vertical columns containing six or seven cages of like exposure group or five cages of sentinel mice were randomly assigned to positions on the racks. Initial cage placements are on file at NIEHS.
Bedding	
Sani-Chip® heat-treated hardwood chips (P.J. Murphy Forest Products Corp., Rochelle Park, NJ), changed weekly	Same as 14-week studies but supplied by P.J. Murphy Forest Products Corp., Montville, NJ
Cage Filters	
Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed once every 2 weeks	Same as 14-week studies
Racks	
Stainless steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks	Same as 14-week studies

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Oxazepam (continued)

	14-Week Studies	Chronic Studies
Animal Room Environment	Average temperature: 21°-24° C Relative humidity: 35%-65% Fluorescent light: 12 hours/day Room air changes: 10 changes/hour	Average temperature: 20°-26° C Relative humidity: 25%-70% (Swiss-Webster); 30%-70% ($B6C3F_1$) Fluorescent light: 12 hours/day Room air changes: 10 changes/hour
Type and Frequency of Observation	Animals were observed and clinical observations were recorded weekly; animals were weighed initially, weekly, and at the end of the studies.	Animals were observed twice daily and clinical observations were recorded every 4 weeks; animals were weighed weekly during first 13 weeks and at 4-week intervals thereafter.
Necropsy	Necropsy was performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsy was performed on all animals. Organs weighed were kidney and liver ($B6C3F_1$ mice).
Clinical Pathology	Blood was collected by cardiac puncture during weeks 2 and 12 for serum oxazepam determinations.	Blood was collected by cardiac puncture at the 15-month interim evaluation ($B6C3F_1$ mice only) and at the end of the studies (Swiss-Webster and $B6C3F_1$). Mice were allowed free access to dosed feed until immediately before blood collection. All blood samples were drawn between 9 a.m. and 11 a.m.
Histopathology	Complete histopathology was performed on all control animals, all mice receiving 10,000 ppm, and all mice dying before the end of the study. In addition to gross lesions, the tissues examined included: adrenal gland, brain, clitoral gland, epididymis, esophagus, femur and marrow, gallbladder, heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach (forestomach and glandular), testis, thigh muscle, thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the liver of all male mice, of all female Swiss-Webster mice, and of all female $B6C3F_1$ mice except those receiving 625 ppm and the adrenal gland of all female Swiss-Webster mice were examined.	Complete histopathology was performed on all mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland, epididymis, esophagus, femur and marrow, gallbladder, heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Neurobehavioral Studies	Core study animals were administered neurobehavioral tests prior to the study and during weeks 2 and 12. These tests included: undifferentiated motor activity, forelimb and hindlimb grip strength, thermal sensitivity, and startle responsiveness.	Ten male and 10 female mice per strain per exposure group were administered neurobehavioral tests prior to the study and after 6, 12, 18 ($B6C3F_1$), and 24 ($B6C3F_1$) months of exposure. These tests included: motor activity, startle responsiveness, forelimb and hindlimb grip strength, and thermal sensitivity.

RESULTS

SWISS-WEBSTER MICE

14-WEEK STUDY

One male mouse in the 625 ppm group and one female in the 10,000 ppm group were killed moribund during the study. One female receiving 1,250 ppm was killed accidentally. The 625 ppm male mouse that died early was found to have lymphoma, and this death was not considered related to oxazepam exposure. No remarkable lesions were found in the 10,000 ppm female mouse, and this early death was considered related to oxazepam exposure. All other mice survived until the end of the study (Table 2). The final mean body weights of all exposed female groups were greater than that of the control group, and those of the 625 and 2,500 ppm groups were significantly greater. Mean body

TABLE 2
Survival, Mean Body Weights, and Feed Consumption of Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption[c] Week 2	Week 14
Male							
0	10/10	26.1 ± 0.3	35.3 ± 0.9	9.3 ± 0.8		4.3	4.8
625	9/10[d]	25.9 ± 0.3	38.0 ± 0.8	12.0 ± 0.9	108	5.0	4.0
1,250	10/10	26.1 ± 0.4	34.4 ± 0.7	8.4 ± 0.6	97	4.6	4.0
2,500	10/10	26.5 ± 0.3	38.1 ± 1.0	11.7 ± 0.8	108	4.6	4.3
5,000	10/10	25.4 ± 0.4	35.1 ± 1.0	9.7 ± 0.9	99	4.3	4.2
10,000	10/10	26.0 ± 0.4	35.1 ± 0.7	9.1 ± 0.6	99	3.8	4.2
Female							
0	10/10	21.5 ± 0.4	29.9 ± 0.7	8.4 ± 0.8		4.4	4.8
625	10/10	21.9 ± 0.2	32.3 ± 0.7*	10.4 ± 0.5	108	4.8	4.7
1,250	9/10[e]	22.3 ± 0.3	31.9 ± 0.5	9.5 ± 0.5	107	4.8	4.3
2,500	10/10	22.1 ± 0.3	32.5 ± 0.8*	10.4 ± 0.8	109	4.5	5.0
5,000	10/10	21.4 ± 0.3	31.6 ± 0.5	10.2 ± 0.3	106	4.0	4.7
10,000	9/10[f]	21.7 ± 0.4	31.2 ± 0.4	9.5 ± 0.4	104	3.7	4.5

* Significantly different (P≤0.05) 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. Subsequent calculations are based on animals surviving to the end of the study.
[c] Feed consumption is expressed as grams per animal per day; data for week 2 are based on 20 animals; data for week 14 are based on 10 animals.
[d] Week of death: 10
[e] Week of death: 6 (accidental death)
[f] Week of death: 13

weight gains of exposed males and females were similar to those of the controls (Table 2). Feed consumption by 10,000 ppm groups was somewhat lower than that by the control groups throughout the study (Table 2). Dietary levels of 625, 1,250, 2,500, 5,000, and 10,000 ppm resulted in average daily consumption levels of 80, 170, 330, 680, and 1,400 mg/kg body weight in males and 100, 220, 440, 830, and 1,620 mg/kg in females. Chemical-related clinical findings in all male and female exposure groups included hypoactivity, drowsiness, lethargy, and decreased exploratory and spontaneous locomotor activity. These findings were observed primarily during the first week of the study, and the short duration of these findings was attributed to the development of tolerance. Serum oxazepam concentrations increased with exposure level in males and females, but the increases were not proportionate to dose. Except in the 5,000 and 10,000 ppm groups at week 2, serum oxazepam concentrations in each exposure group were similar between males and females (Table 3).

Short-term administration (1 week) of oxazepam produced deficits in grip strength at high exposure levels in both males and females (Tables G1 and G2). These deficits were temporary in female mice but persisted in male mice with continued administration (11 weeks). Decreased paw lick latencies in response to thermal stimulation were observed in exposed males and females at 12 weeks (Table G3). Increases in motor activity were observed at all exposure levels at study week 2 (Table G4). This disinhibitory effect may be indicative of an anxiety-reducing effect of oxazepam. Increased motor activity abated in males, but was still evident in females at week 12. Oxazepam produced a general reduction in startle response in males at weeks 2 and 12 and in females at 12 weeks (Table G5). Sensitivity to an auditory prepulse as part of the startle response assessment was not affected by oxazepam administration (Table G6). Oxazepam effects on the sensory system in general may be involved in changes observed in startle behavior as well as altered thermal sensibility measurements.

Except for relative liver weights of mice receiving 625 ppm, the absolute and relative liver weights of all exposed males and females were significantly greater than those of the controls (Tables 4 and F1). These increases were marked and were clearly dose related. In males, absolute and relative heart weights of the 1,250 and 10,000 ppm groups and the relative heart weight of the 2,500 ppm group were significantly lower than those of the controls. This finding was not clearly dose related, and no cause for this change could be determined. The absolute kidney weights of females exposed to 1,250, 2,500, 5,000, or 10,000 ppm were significantly greater than that of the controls.

TABLE 3
Serum Oxazepam Concentrations in Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	0	625	1,250	2,500	5,000	10,000
Male						
2 weeks	0.00 ± 0.00	5.91 ± 0.66	9.96 ± 0.72	13.6 ± 1.23	18.6 ± 1.3	29.3 ± 3.18
12 weeks	0.00 ± 0.00	6.21 ± 0.53	9.30 ± 1.14	12.1 ± 0.94	20.3 ± 2.15	22.0 ± 1.06
Female						
2 weeks	0.00 ± 0.00	6.05 ± 0.62	9.08 ± 0.77	15.2 ± 1.61	29.3 ± 4.04	36.9 ± 4.49
12 weeks	0.00 ± 0.00	6.52 ± 0.93	9.22 ± 0.43	12.1 ± 1.60	21.9 ± 1.37	21.6 ± 1.23

[a] Mean ± standard error for five animals; values are given as μg/mL.

Centrilobular hepatocellular hypertrophy was observed in exposed animals, and the severity generally increased with dose (Table 4). This lesion was characterized by minimal to mild enlargement (hypertrophy) of hepatocytes that were centrilobular in distribution. Hypertrophic hepatocytes had homogeneous or slightly granular eosinophilic cytoplasm. The nuclei were often enlarged and contained prominent basophilic chromatin clumps. A low incidence of focal hepatocellular necrosis occurred in several groups of female mice receiving oxazepam, but not in the control group (Table 4). The incidence and the severity of the lesion were not chemical related, and the highest incidence occurred in the females exposed to 2,500 ppm. Foci of hepatocellular necrosis also occurred in one control and several exposed male mice. Because of the generally low incidences and lack of dose-related increase in incidence or severity, the hepatocellular necrosis was not attributed to the ingestion of oxazepam.

There was a dose-related decreased incidence of cytoplasmic vacuolation of cells within the x-zone of the adrenal cortex in female mice (Table 4). The vacuolated cells adjacent to the medulla (x-zone) are transitory and normally disappear gradually in virgin female mice. The decreased number of vacuolated cells in females receiving oxazepam indicates an accelerated regression of the x-zone and maturation of the adrenal gland.

Dose Selection Rationale

Because the degree of increase in liver weight in mice at the 10,000 ppm concentration was considered potentially life threatening during a 2-year study, the exposure levels selected were 0, 2,500, and 5,000 ppm.

TABLE 4
Liver Weights and Incidences of Selected Nonneoplastic Lesions in Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam

Dose (ppm)	0	625	1,250	2,500	5,000	10,000
Male						
Liver[a]	10	10	10	10	10	10
Liver Weights						
Absolute	1.808 ± 0.080	2.354 ± 0.074**	2.275 ± 0.112*	2.761 ± 0.134**	3.035 ± 0.115**	3.528 ± 0.137**[b]
Relative	50.43 ± 1.35	61.23 ± 1.88	64.60 ± 2.75**	72.61 ± 3.92**	85.52 ± 2.61**	99.98 ± 4.27**[b]
Centrilobular Hypertrophy	0	9▲▲ (1.2)[c]	10▲▲ (1.3)	10▲▲ (1.1)	10▲▲ (1.7)	10▲▲ (1.6)
Hepatocellular Necrosis	1 (1.0)	1 (1.0)	0	1 (1.0)	0	1 (1.0)
Female						
Liver	10	10	10	10	10	10
Liver Weights						
Absolute	1.405 ± 0.028	1.731 ± 0.049**	1.910 ± 0.052**	2.328 ± 0.109**	2.610 ± 0.064**	3.084 ± 0.070**
Relative	47.32 ± 1.14	54.28 ± 1.47	61.16 ± 2.12**	72.42 ± 2.77**	84.16 ± 1.93**	100.63 ± 1.95**
Centrilobular Hypertrophy	0	10▲▲ (1.2)	10▲▲ (1.1)	10▲▲ (1.4)	10▲▲ (1.4)	10▲▲ (1.9)
Hepatocellular Necrosis	0	2 (1.0)	0	4 (1.0)	3 (1.0)	2 (1.0)
Adrenal Gland						
Cytoplasmic Vacuolization	10 (2.5)	2▲▲ (1.5)	4▲▲ (1.2)	1▲▲ (1.0)	1▲▲ (1.0)	0▲▲

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
▲▲ Significantly different (P≤0.01) from the control group by Fisher's exact test
[a] Number of mice with organ examined microscopically; 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
[c] Average severity of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; 5 = severe

57-WEEK STUDY

Survival

Estimates of survival probabilities for male and female Swiss-Webster mice are shown in Table 5 and in the Kaplan-Meier curves in Figure 2. The original design of this study provided for administration of dosed feed to male mice for 103 weeks and to females for 104 weeks, followed by a 1-week observation period. The original study design also included an interim evaluation at week 66. However, there were large numbers of moribund animals and deaths after 40 weeks of exposure. These deaths were considered due to heart failure secondary to pulmonary hypertension and edema, which resulted from systemic amyloidosis, a condition common in Swiss-Webster mice and apparently enhanced by oxazepam exposure. At 57 weeks, 19 males and 28 females receiving 2,500 ppm, and 10 males and 17 females receiving 5,000 ppm were surviving, and the study was terminated. No interim evaluations were conducted.

TABLE 5
Survival of Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

Dose (ppm)	0	2,500	5,000
Male			
Animals initially in study	60	60	60
Moribund	10	13	24
Natural deaths	5	28	26
Animals surviving to study termination	45	19	10
Percent probability of survival at end of study[a]	75	32	17
Mean survival (days)[b]	373	338	335
Survival analyses[c]	P<0.001	P<0.001	P<0.001
Female			
Animals initially in study	60	60	60
Moribund	9	13	11
Natural deaths	4	18	31
Animals surviving to study termination	47	28[e]	17
Missing[d]		1	1
Percent probability of survival at end of study	78	48	29
Mean survival (days)	382	351	353
Survival analyses	P<0.001	P<0.001	P<0.001

[a] Kaplan-Meier determinations based on the number of animals alive on first day of terminal sacrifice
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns.
[d] Censored from survival analyses
[e] Includes one animal that died during the last week of the study.

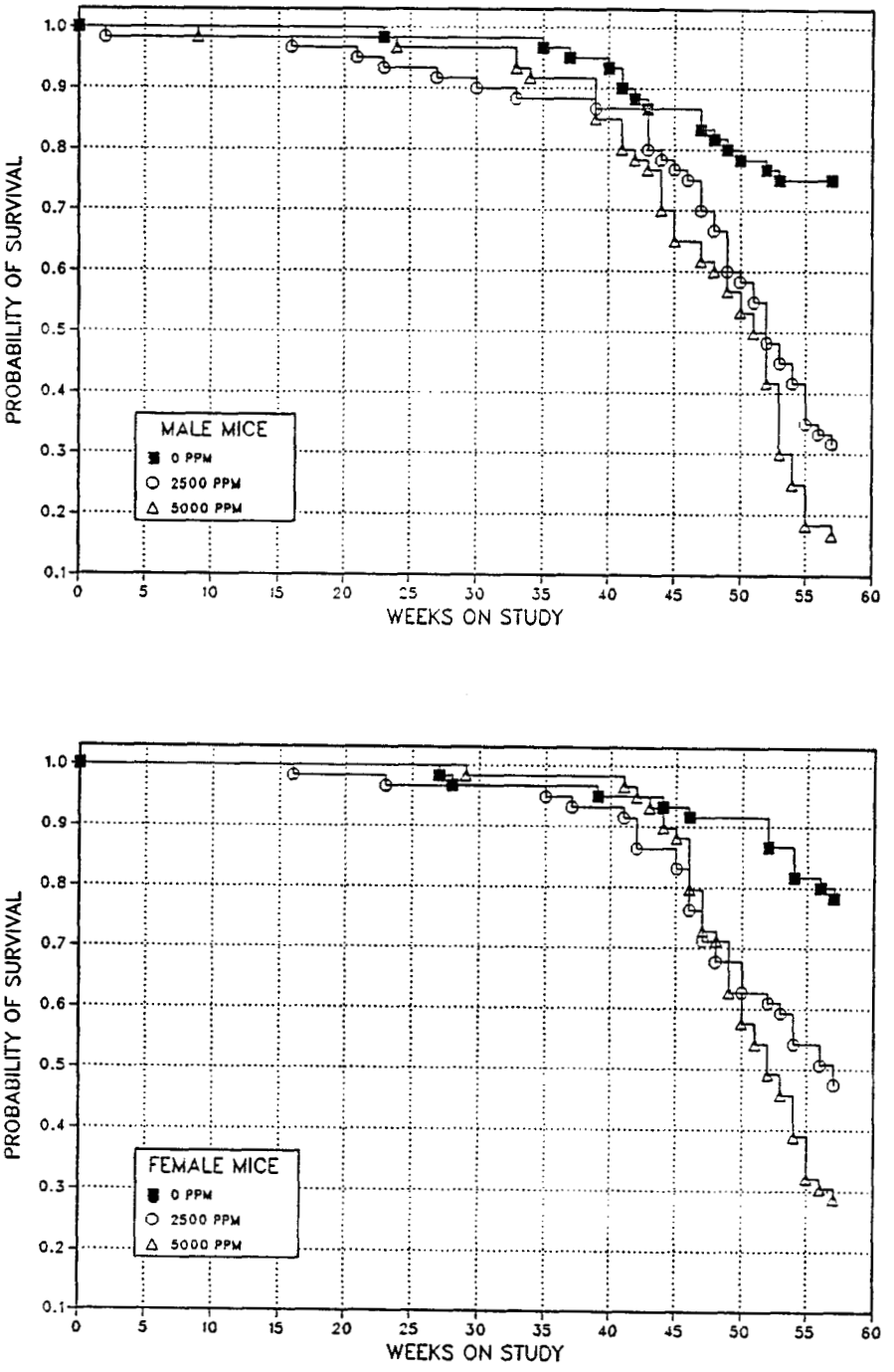


FIGURE 2
Kaplan-Meier Survival Curves for Swiss-Webster Mice Administered Oxazepam in Feed for 57 Weeks

Body Weights, Feed Consumption, Compound Consumption, and Clinical Findings

Mean body weights of exposed male mice were similar to those of the controls in the early weeks of the study. Beginning at week 17, however, mean body weights of exposed male mice were lower than those of the controls (Table 6 and Figure 3). Except for week 1, mean body weights of exposed females were greater than those of controls during the early part of the study. After week 29, the mean body weights of 5,000 ppm females were similar to those of controls, but those of 2,500 ppm females remained slightly greater than those of controls until the end of the study (Table 7 and Figure 3). Feed consumption by exposed males and females was slightly lower than that by the controls, and females in all groups, consumed slightly more feed than males throughout the study (Tables I1 and I2). Dietary levels of 2,500

TABLE 6
Mean Body Weights and Survival of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm Number of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm Number of Survivors	5,000 ppm Av. Wt. (g)	5,000 ppm Wt. (% of controls)	5,000 ppm Number of Survivors
1	26.7	60	26.5	99	60	26.5	99	60
2	27.9	60	28.6	103	60	28.7	103	60
3	29.4	60	30.6	104	59	31.1	106	60
4	30.0	60	31.5	105	59	31.9	106	60
5	30.8	60	31.8	103	59	32.4	105	60
6	31.9	60	32.4	102	59	33.4	105	60
7	32.8	60	33.2	101	59	34.0	104	60
8	33.4	60	33.2	99	59	34.0	102	60
9	33.2	60	33.7	102	59	34.2	103	60
10	34.3	60	34.5	101	59	35.2	103	59
11	35.2	60	35.1	100	59	35.8	102	59
12	35.1	60	34.8	99	59	35.4	101	59
13	36.0	60	36.0	100	59	36.2	101	59
17	38.4	60	37.5	98	58	37.4	97	59
21	40.3	60	38.7	96	58	38.2	95	59
25	41.6	59	39.8	96	56	39.2	94	58
29	42.2	59	40.8	97	55	39.4	93	58
33	42.8	59	40.7	95	54	39.2	92	58
37	43.0	58	41.0	95	53	39.4	92	55
41	42.2	55	40.3	96	52	39.2	93	50
45	41.7	52	39.4	95	47	37.8	91	42
49	41.5	49	38.6	93	40	37.4	90	36
53	41.7	46	37.9	91	28	36.4	87	24
57	41.1	45	37.4	91	20	37.2	91	11
Mean for weeks								
1-13	32.1		32.5	101		33.0	103	
14-52	41.5		39.6	95		38.6	93	
53-57	41.4		37.7	91		36.8	89	

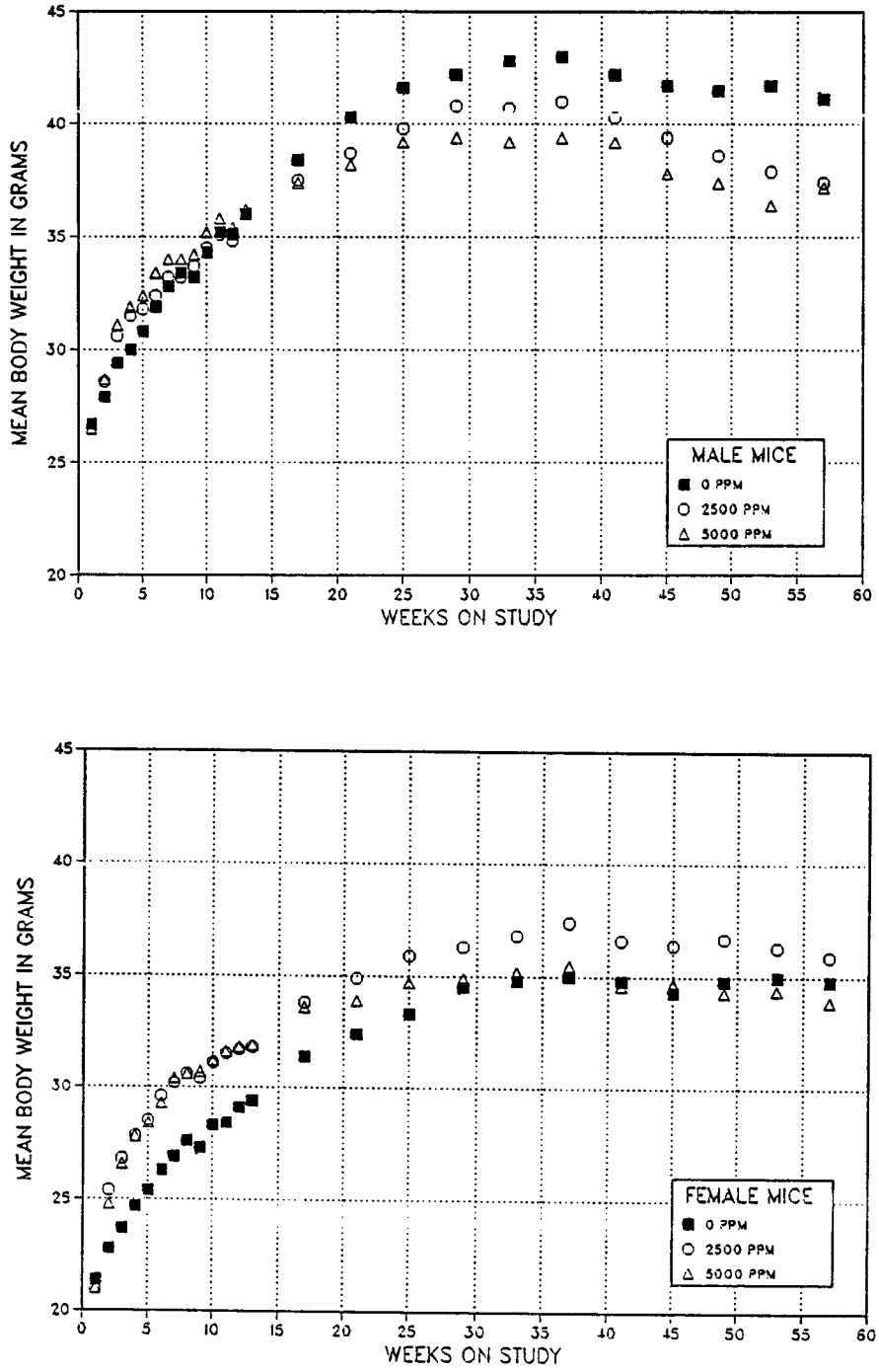


FIGURE 3
Growth Curves for Swiss-Webster Mice Administered Oxazepam in Feed for 57 Weeks

TABLE 7
Mean Body Weights and Survival of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm Number of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm Number of Survivors	5,000 ppm Av. Wt. (g)	5,000 ppm Wt. (% of controls)	5,000 ppm Number of Survivors
1	21.4	60	21.0	98	60	21.0	98	60
2	22.8	60	25.4	111	60	24.8	109	60
3	23.7	60	26.8	113	60	26.6	112	60
4	24.7	60	27.8	113	59	27.8	113	60
5	25.4	60	28.5	112	59	28.4	112	60
6	26.3	60	29.6	113	59	29.3	111	60
7	26.9	60	30.2	112	59	30.4	113	60
8	27.6	60	30.6	111	59	30.6	111	60
9	27.3	60	30.4	111	59	30.7	113	60
10	28.3	60	31.1	110	59	31.2	110	60
11	28.4	60	31.5	111	59	31.6	111	60
12	29.1	60	31.7	109	59	31.8	109	60
13	29.4	60	31.8	108	59	31.9	109	60
17	31.4	60	33.8	108	58	33.6	107	59
21	32.4	60	34.9	108	58	33.9	105	59
25	33.3	60	35.9	108	57	34.7	104	59
29	34.5	58	36.3	105	57	34.9	101	59
33	34.8	58	36.8	106	57	35.2	101	58
37	35.0	58	37.4	107	56	35.5	101	58
41	34.8	57	36.6	105	55	34.6	99	58
45	34.3	56	36.4	106	51	34.7	101	53
49	34.8	55	36.7	106	40	34.3	99	40
53	35.0	52	36.3	104	36	34.4	98	28
57	34.8	48	35.9	103	29	33.9	97	18
Mean for weeks								
1-13	26.3		29.0	110		28.9	110	
14-52	33.9		36.1	106		34.6	102	
53-57	34.9		36.1	103		34.2	98	

and 5,000 ppm oxazepam resulted in average daily compound consumption levels of 270 and 570 mg/kg for males and 320 and 670 mg/kg for females.

On study days 4 (females) and 5 (males), clinical findings of hypoactivity, slow respiration, partially closed eyelids, lethargy, and decreased spontaneous exploratory behavior were noted in most exposed animals. Except for hypoactivity, the incidences of these clinical findings had decreased by days 8 (females) and 9 (males). By days 15 and 16, the appearance and behavior of exposed animals were similar to controls.

Serum Oxazepam Concentrations

Serum oxazepam concentrations were similar in the males and females receiving 2,500 and 5,000 ppm (Table 8). No dose- or sex-related differences in serum oxazepam concentrations occurred after 57 weeks of exposure.

TABLE 8
Serum Oxazepam Concentrations in Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

Dose (ppm)	0	2,500	5,000
Male			
n	10	10	10
Serum oxazepam (μg/mL)	0 ± 0	6.65 ± 2.87	7.73 ± 4.76
Female			
n	9[b]	6[c]	10
Serum oxazepam (μg/mL)	0 ± 0	7.25 ± 1.44	6.89 ± 3.04

[a] Mean ± standard deviation
[b] An aliquot of serum sample from one animal was contaminated. The remaining volume of serum sample was insufficient for a repeat analysis.
[c] Analysis results from four animals were deleted due to instrument error. Insufficient specimen remained to repeat the analysis.

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of Swiss-Webster mice with neoplasms of the liver and nonneoplastic lesions of the liver, heart, lung, and other organs. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male Swiss-Webster mice and Appendix B for female Swiss-Webster mice.

Liver: The principal toxic effects associated with the ingestion of oxazepam in the feed occurred in the liver. The incidences of centrilobular hepatocellular hypertrophy in exposed males and females were significantly greater than those of the controls (Tables 9, 10, A4, and B4). In exposed mice, the hypertrophy was generally mild in severity while in the few affected control mice the severity was minimal. The hypertrophy was similar to that described in the 14-week study.

The incidences of eosinophilic foci, a putative preneoplastic lesion, and of hepatocellular adenoma were significantly greater than those of the controls in 2,500 and 5,000 ppm males and females. The incidence of hepatocellular carcinoma was significantly greater in 2,500 ppm males and 5,000 ppm males and females. The incidence of hepatocellular neoplasms and the number of mice with multiple hepatocellular neoplasms increased with increasing exposure level in both males and females (Tables A1 and B1).

The eosinophilic foci, hepatocellular adenomas, and hepatocellular carcinomas constitute a morphologic continuum of increasing size, progressive loss of normal hepatic architecture, increasing disorganization of hepatic plates, and increasing cellular pleomorphism and atypia. The eosinophilic foci were relatively discrete aggregates of often enlarged hepatocytes with homogeneous eosinophilic cytoplasm. While some foci were larger than a single hepatic lobule, the lobular pattern was retained and the organization of the hepatic plates was only minimally altered. Hepatocellular adenomas were discrete nodules larger than eosinophilic foci. Normal hepatic lobulation was not apparent and the hepatic plates were distorted to varying degrees within the adenomas. While the hepatocytes within the adenomas were often enlarged and eosinophilic, there was little or no pleomorphism or atypia. In

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

Dose (ppm)	0	2,500	5,000
Liver[a]	60	60	60
Centrilobular Hypertrophy	12 (1.3)	46** (1.8)[b]	47** (1.8)
Basophilic Focus	1	0	1
Eosinophilic Focus	0	22**	22**
Focus (any type)	1	22**	22**
Hepatocellular Adenoma			
Overall rate[c]	1/60 (2%)	35/60 (58%)	50/60 (83%)
Adjusted rate[d]	2.2%	88.7%	98.0%
Terminal rate[e]	1/45 (2%)	15/19 (79%)	9/10 (90%)
First incidence (days)	397 (T)	268	231
Logistic regression test[f]	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma			
Overall rate	0/60 (0%)	5/60 (8%)	19/60 (32%)
Adjusted rate	0.0%	21.7%	72.0%
Terminal rate	0/45 (0%)	3/19 (16%)	5/10 (50%)
First incidence (days)	—[g]	356	302
Life table test	P<0.001	P=0.003	P<0.001
Logistic regression test	P<0.001	P=0.010	P<0.001
Hepatocellular Adenoma or Carcinoma			
Overall rate	1/60 (2%)	35/60 (58%)	52/60 (87%)
Adjusted rate	2.2%	88.7%	98.1%
Terminal rate	1/45 (2%)	15/19 (79%)	9/10 (90%)
First incidence (days)	397 (T)	268	231
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001

** Significantly different (P≤0.01) from the control group by the logistic regression test
(T)Terminal sacrifice
[a] Number of animals with liver examined microscopically
[b] Average severity of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Number of lesion-bearing animals/number of animals necropsied or examined microscopically
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression analysis regards these lesions as nonfatal.
[g] Not applicable; no neoplasms in animal group

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

Dose (ppm)	0	2,500	5,000
Liver[a]	60	59	59
Centrilobular Hypertrophy	3 (1.3)	51** (1.6)[b]	53** (1.8)
Basophilic Focus	0	0	0
Eosinophilic Focus	0	20**	14**
Focus (any type)	0	20**	14**
Hepatocellular Adenoma			
Overall rate[c]	0/60 (0%)	22/59 (37%)	47/59 (80%)
Adjusted rate[d]	0.0%	52.6%	95.7%
Terminal rate[e]	0/47 (0%)	10/28 (36%)	15/17 (88%)
First incidence (days)	–[g]	291	284
Logistic regression test[f]	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma			
Overall rate	1/60 (2%)	1/59 (2%)	11/59 (19%)
Adjusted rate	2.1%	3.6%	51.6%
Terminal rate	1/47 (2%)	1/28 (4%)	8/17 (47%)
First incidence (days)	397 (T)	397 (T)	337
Life table test	P<0.001	P=0.642	P<0.001
Logistic regression test	P<0.001	P=0.642	P<0.001
Hepatocellular Adenoma or Carcinoma			
Overall rate	1/60 (2%)	23/59 (39%)	47/59 (80%)
Adjusted rate	2.1%	55.2%	95.7%
Terminal rate	1/47 (2%)	11/28 (39%)	15/17 (88%)
First incidence (days)	397 (T)	291	284
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001

** Significantly different (P≤0.01) from the control group by the logistic regression test
(T)Terminal sacrifice
[a] Number of animals with liver examined microscopically
[b] Average severity of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Number of lesion-bearing animals/number of animals necropsied or examined microscopically
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression analysis regards these lesions as nonfatal.
[g] Not applicable; no neoplasms in animal group

contrast, hepatocellular carcinomas had heterogeneous growth patterns with areas of distinct trabecular or adenoid arrangements. The neoplastic hepatocytes usually exhibited a greater degree of pleomorphism characterized by variation in size, staining quality of the cytoplasms, and size and shape of the nuclei.

Multiple Organs: Amyloid deposition is a common spontaneous condition in Swiss-Webster mice, and exposure to oxazepam appears to have exacerbated this condition, resulting in significant dose-related increases in the incidence and severity in multiple organs compared to those of the controls. The organs most commonly affected in both male and female mice in this study were the heart, glandular stomach, intestine, spleen, lymph nodes, thyroid and parathyroid glands, adrenal cortex, and uterus (Tables A4 and B4). Except in the heart, the severity of these lesions in these organs averaged minimal to mild in the controls and mild to moderate in the 5,000 ppm group.

In the heart, the severity of amyloid deposition in the myocardium ranged from moderate to marked in exposed mice and minimal to mild in controls. Dose-related increased incidences in myocardial amyloid deposition occurred in exposed males (43/60, 52/60, 52/60; Table A4). Additionally, the incidences of atrial thrombosis increased in a dose-related manner (males: 1/60, 34/60, 35/60; females: 2/60, 23/59, 31/59; Tables A4 and B4). These thrombi were quite large and distended or occluded the atria. The thrombi were usually associated with moderate to marked myocardial amyloid deposition.

In the lung, oxazepam exposure was also associated with increased incidences of inflammatory fibrosis in males (0 ppm, 0/60; 2,500 ppm, 27/60; 5,000 ppm, 25/60; Table A4) and females (0 ppm, 1/60; 2,500 ppm, 14/59; 5,000 ppm, 8/59; Table B4) and dose-related increased incidences of mononuclear cell infiltrates (males: 1/60, 31/60, 37/60; females: 2/60, 16/59, 26/59; Tables A4 and B4). Lung inflammation was primarily multifocal; however, the fibrosis and mononuclear cell infiltration were usually widely disseminated or diffuse. The severity of inflammatory fibrosis in affected animals was similar among exposed and control groups (males: 0, 2.3, 2.2; females: 2.0, 1.9, 2.3). The lung changes were consistent with pulmonary hypertension and were most likely due to heart failure secondary to amyloid deposition and secondary to pulmonary edema.

Uterus: The incidence of cystic endometrial hyperplasia decreased with increasing exposure level (13/60, 2/59, 0/57; Table B4).

Neurobehavioral Evaluation

During the 6-month neurobehavioral evaluation, forelimb grip strength was significantly decreased in male mice exposed to 5,000 ppm. Hindlimb grip strength at 6 months and forelimb and hindlimb grip strengths at 12 months were not affected by oxazepam exposure (Tables G8 and G9). Females in the 2,500 ppm group and males and females in the 5,000 ppm groups exhibited significantly decreased paw lick latencies in the thermal sensitivity tests at 6 months (Table G10). No significant differences in paw lick latencies were observed at 12 months in any exposed group. Motor activity was not affected by oxazepam exposure at 6 months but was reduced by 53% in the 5,000 ppm females at 12 months (Table G11). Startle response was not affected by oxazepam exposure.

B6C3F$_1$ MICE

14-WEEK STUDY

One male mouse in the 10,000 ppm group died during the study with a urinary tract infection (Table 11). Mean body weight gains of exposed groups were similar to those of the controls (Table 11). Feed consumption by all male and female exposed groups was lower than that by controls early in the study, but was similar during the latter part of the study (Table 11). Dietary levels of 625, 1,250, 2,500, 5,000, and 10,000 ppm resulted in average daily consumption values of 100, 200, 390, 890, and 1,810 mg/kg body weight in males and 130, 260, 450, 920, and 2,050 mg/kg in females. Serum oxazepam concentrations increased with exposure level in both males and females; however, as in the Swiss-Webster study, these increases were not proportional to the increase in dose (Table 12). Serum oxazepam concentrations were similar in males and females at each exposure level. Chemical-related clinical findings of drowsiness, lethargy, and decreased spontaneous locomotor activity were observed in all exposed groups. These findings occurred for only a few days beginning about day 2 of the study.

TABLE 11
Survival, Mean Body Weights, and Feed Consumption of B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 2	Week 14
Male							
0	10/10	23.9 ± 0.3	33.6 ± 0.7	9.7 ± 0.6		5.1	5.5
625	10/10	24.2 ± 0.5	35.3 ± 0.7	11.1 ± 0.4	105	3.9	4.9
1,250	10/10	24.0 ± 0.5	34.3 ± 0.6	10.3 ± 0.8	102	3.8	4.9
2,500	10/10	24.6 ± 0.3	34.1 ± 0.4	9.5 ± 0.3	102	3.8	4.6
5,000	10/10	23.5 ± 0.5	33.1 ± 0.5	9.5 ± 0.4	98	3.6	5.0
10,000	9/10[d]	23.6 ± 0.6	33.5 ± 0.5	9.5 ± 0.5	100	3.2	5.7
Female							
0	10/10	19.5 ± 0.2	29.7 ± 0.7	10.2 ± 0.7		5.7	6.8
625	10/10	20.2 ± 0.5	31.7 ± 0.7	11.5 ± 0.7	107	3.4	6.5
1,250	10/10	19.1 ± 0.4	30.5 ± 0.6	11.4 ± 0.7	103	3.3	6.3
2,500	10/10	19.8 ± 0.3	29.4 ± 0.6	9.6 ± 0.5	99	3.2	4.5
5,000	10/10	19.6 ± 0.4	30.3 ± 0.6	10.7 ± 0.6	102	3.3	5.1
10,000	10/10	19.9 ± 0.2	30.0 ± 0.5	10.1 ± 0.4	101	3.0	6.1

[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 study.
[c] Feed consumption is expressed as grams per animal per day; data for week 2 are based on 20 animals; data for week 14 are based on 10 animals.
[d] Week of death: 13

TABLE 12
Serum Oxazepam Concentrations in B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	0	625	1,250	2,500	5,000	10,000
Male						
2 weeks	0.00 ± 0.00	5.19 ± 0.55[b]	8.30 ± 0.85[c]	10.9 ± 0.57	16.9 ± 1.08	22.5 ± 1.92
12 weeks	0.00 ± 0.00	6.06 ± 0.31	8.46 ± 0.57	11.1 ± 0.33	16.5 ± 1.18	19.9 ± 1.18
Female						
2 weeks	0.00 ± 0.00	5.75 ± 0.58[b]	7.08 ± 0.76	11.8 ± 1.57	15.1 ± 1.17	22.7 ± 1.36
12 weeks	0.00 ± 0.00	5.38 ± 0.53	8.04 ± 0.66	11.3 ± 1.07	17.3 ± 1.67	23.8 ± 0.30

[a] Mean ± standard error for five animals; values are given as μg/mL.
[b] n=4
[c] n=3

Oxazepam produced a deficit in grip strength, which was more marked in males than in females (Tables G12 and G13). This deficit was only temporary, however, because it was observed at 2 weeks but not at 12 weeks. Increases in motor activity were observed at all exposure levels at both 2 weeks and 12 weeks (Table G15). This disinhibitory effect may be indicative of an anxiety-reducing effect of oxazepam. Somatosensory integrity was measured by the tactile startle response. Increases in initial startle reactivity were observed in 625 and 1,250 ppm males and in 2,500 ppm females at 2 weeks. Decreased reactivity was seen at 12 weeks and was particularly evident in the 2,500, 5,000, and 10,000 ppm females. An overall increased sensitivity of exposed mice to an auditory prepulse as part of the tactile startle response was noted (Tables G16, G17, and G18). In addition, decreased paw lick latencies were observed in the 625, 1,250, and 10,000 ppm females at 12 weeks (Table G14). Changes in startle response and thermal sensitivity may be due to an effect on the sensory component of the startle reflex circuit. Oxazepam effects on the sensory system in general, and specifically on arousal mechanisms, may have accounted for these changes.

Absolute and relative liver weights of all exposed males and females were notably greater than those of the controls (Tables 13 and F2). These increases were exposure related. Absolute thymus weights of 625, 1,250, 2,500, and 10,000 ppm males and relative thymus weights of 2,500 and 10,000 ppm males were significantly greater than those of the controls, and absolute and relative kidney weights of exposed females were variable, but were also significantly greater than those of the controls. There were no histopathologic differences in the thymus or kidney that could account for these increases.

Centrilobular hepatocellular hypertrophy occurred in exposed male and female B6C3F$_1$ mice (Table 13). Exposure-related increases in the severity of this lesion occurred in both males and females. These lesions were similar to those previously described for the Swiss-Webster mice. In both strains of mice, the presence of centrilobular hypertrophy correlated with exposure-related increases in the absolute and relative liver weights.

Dose Selection Rationale

Because the degree of increase in liver weight in mice at the 10,000 ppm concentration was considered potentially life threatening during a 2-year study, the doses of oxazepam selected for the 2-year study were 0, 2,500, and 5,000 ppm. An additional exposure level of 125 ppm was selected in an attempt to produce a group of mice with serum oxazepam levels in the 1 μg/mL range, similar to that produced in humans by a therapeutic dose of oxazepam.

TABLE 13
Liver Weights and Incidences of Nonneoplastic Lesions of the Liver in B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam

Dose (ppm)	0	625	1,250	2,500	5,000	10,000
Male						
Liver[a]	10	10	10	10	10	10
Liver Weights						
Absolute	1.634 ± 0.052	1.919 ± 0.044**[b]	2.150 ± 0.037**	2.186 ± 0.038**	2.545 ± 0.065**	2.966 ± 0.057**
Relative	47.24 ± 0.96	52.91 ± 0.67**[b]	61.42 ± 0.47**	62.50 ± 0.94**	74.83 ± 1.09**	88.09 ± 1.19**
Centrilobular Hypertrophy	0	10▲▲ (1.0)[c]	10▲▲ (2.0)	10▲▲ (2.0)	10▲▲ (2.1)	10▲▲ (3.0)
Female						
Liver	10	0	10	10	10	10
Liver Weights						
Absolute	1.387 ± 0.021	1.743 ± 0.031**[d]	1.871 ± 0.046**[b]	1.898 ± 0.044**	2.391 ± 0.072**	2.837 ± 0.061**[b]
Relative	46.59 ± 1.01	55.44 ± 1.02**[d]	61.54 ± 0.76**[b]	63.20 ± 1.19**	77.35 ± 1.16**	93.64 ± 1.03**[b]
Centrilobular Hypertrophy	0	10▲▲ (1.0)	10▲▲ (1.0)	10▲▲ (2.0)	10▲▲ (2.0)	10▲▲ (3.0)

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
▲▲ Significantly different (P≤0.01) from the control group by Fisher's exact test
[a] Number of mice with organ examined microscopically; 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
[c] Average severity of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; 5 = severe
[d] n=10

2-YEAR STUDY

Survival

Estimates of survival probabilities for male and female B6C3F$_1$ mice in the 2-year study are shown in Table 14 and in the Kaplan-Meier curves in Figure 4. Survival of males and females receiving 125 ppm was similar to controls. However, there were a large number of dead and moribund males and females in the 2,500 and 5,000 ppm groups. Only 30% of the males and 4% of the females exposed to 2,500 ppm survived until the end of the study; most deaths in these groups occurred after week 85 of the study (Tables 15 and 16). Mortality in 5,000 ppm males was greatly increased beginning at week 65, and there were no survivors by week 93 of the study. Mortality in 5,000 ppm females increased after week 57, and there were no survivors by week 89. Due to the increased mortality in the 2,500 and 5,000 ppm groups, the 1-week observation period at the end of the study was canceled except for mice being observed for neurobehavioral effects. The early deaths of exposed mice were considered due at least in part to hepatic neoplasia.

TABLE 14
Survival of B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

Dose (ppm)	0	125	2,500	5,000
Male				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Moribund	2	4	22	30
Natural deaths	3	2	13	20
Animals surviving to study termination	45	44	15	0
Percent probability of survival at end of study[a]	90	88	30	0
Mean survival (days)[b]	668	670	641	545
Survival analyses[c]	P<0.001	P=0.987	P<0.001	P<0.001
Female				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Moribund	8	7	22	15
Natural deaths	3	2	26	35
Animals surviving to study termination	39	41	2	0
Percent probability of survival at end of study	78	82	4	0
Mean survival (days)	664	667	631	498
Survival analyses	P<0.001	P=0.761N	P<0.001	P<0.001

[a] Kaplan-Meier determinations based on the number of animals alive on first day of terminal sacrifice
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A lower incidence in a dose group is indicated by N.

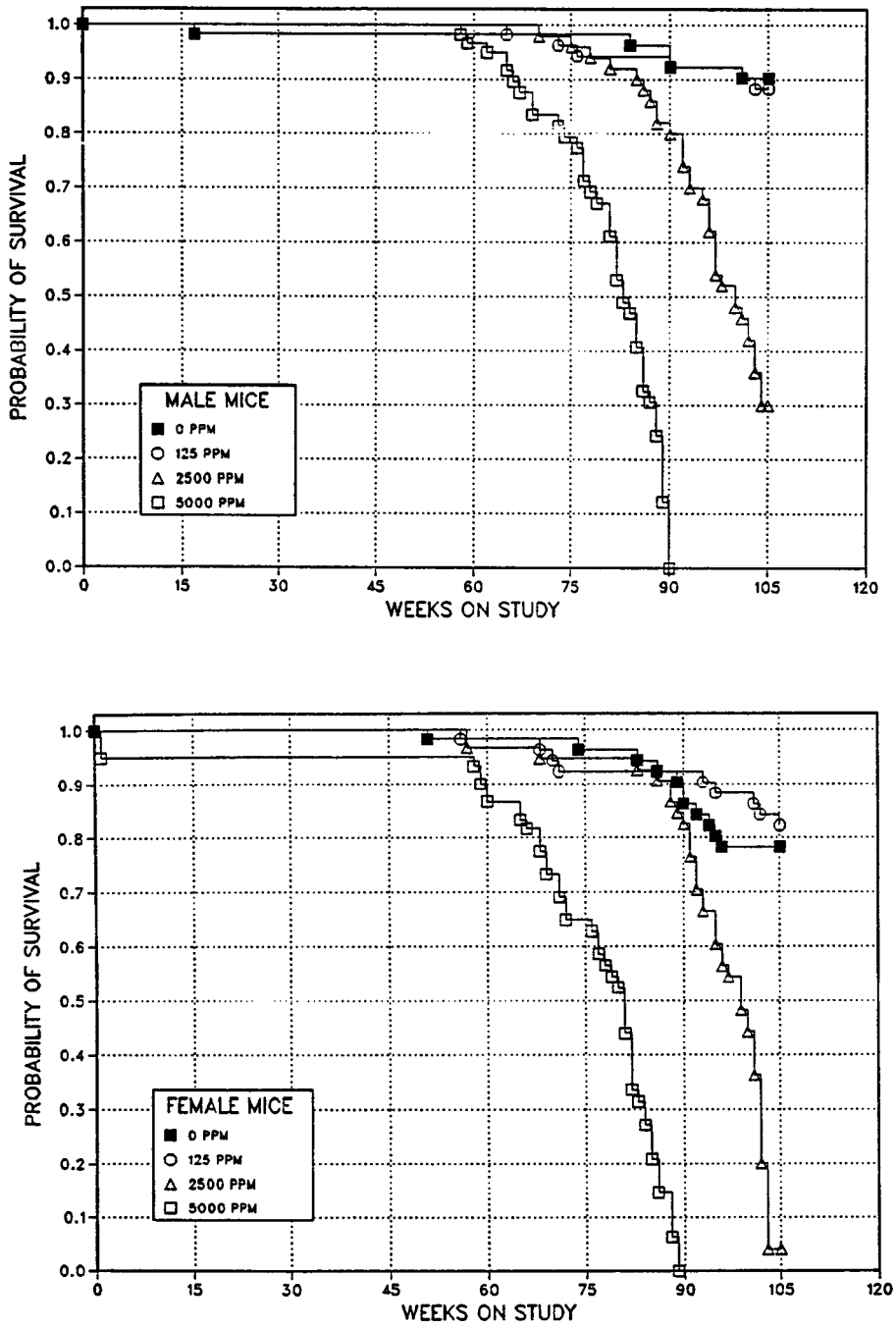


FIGURE 4
Kaplan-Meier Survival Curves for B6C3F$_1$ Mice Administered Oxazepam in Feed for 2 Years

TABLE 15
Mean Body Weights and Survival of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	125 ppm Av. Wt. (g)	125 ppm Wt. (% of controls)	125 ppm No. of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm No. of Survivors	5,000 ppm Av. Wt. (g)	5,000 ppm Wt. (% of controls)	5,000 ppm No. of Survivors
1	21.5	60	21.2	99	60	21.4	100	60	21.2	99	60
2	22.8	60	23.7	104	60	23.8	104	60	23.4	103	60
3	23.9	60	25.7	108	60	26.0	109	60	25.5	107	60
4	24.7	60	26.8	109	60	27.1	110	60	26.9	109	60
5	26.3	60	28.1	107	60	28.1	107	60	28.0	107	60
6	27.5	60	29.3	107	60	29.2	106	60	28.7	104	60
7	29.1	60	30.5	105	60	30.1	103	60	29.8	102	60
8	30.0	60	31.6	105	60	30.7	102	60	30.6	102	60
9	31.0	60	31.9	103	60	31.2	101	60	30.7	99	60
10	32.0	60	32.9	103	60	31.7	99	60	31.5	98	60
11	32.9	60	33.6	102	60	32.6	99	60	32.2	98	60
12	33.8	60	34.9	103	60	33.3	99	60	32.8	97	60
13	34.6	60	35.4	102	60	33.5	97	60	33.3	96	60
17	38.5	60	38.2	99	60	35.9	93	60	34.9	91	60
21	41.2	59	39.9	97	60	36.7	89	60	35.2	85	60
25	43.5	59	41.8	96	60	38.1	88	60	36.0	83	60
29	44.3	59	43.0	97	60	38.8	88	60	36.7	83	60
33	46.3	59	44.4	96	60	39.4	85	60	37.1	80	60
37	46.6	59	45.1	97	60	40.1	86	60	37.6	81	60
41	47.1	59	46.1	98	60	40.8	87	60	38.0	81	60
45	47.4	59	46.3	98	60	40.8	86	60	37.8	80	60
49	47.5	59	46.9	99	60	41.0	86	60	37.7	79	60
53	47.3	59	47.5	100	60	41.1	87	60	37.1	78	60
57	48.0	59	47.9	100	60	41.4	86	60	36.4	76	60
61	48.5	59	48.3	100	60	41.3	85	60	35.8	74	58
65	48.7	59	48.3	99	60	40.5	83	60	34.7	71	57
69[a]	48.3	49	48.2	100	49	39.7	82	50	33.9	70	43
73	48.8	49	48.4	99	49	38.4	79	49	33.6	69	41
77	48.8	49	48.9	100	47	37.1	76	48	33.4	68	38
81	48.6	49	48.6	100	47	36.1	74	47	33.0	68	33
85	49.4	48	49.6	100	47	35.4	72	46	32.8	66	22
89	49.4	48	48.9	99	47	34.5	70	41	32.6	66	10
93	49.1	46	48.7	99	46	33.1	67	36			
97	48.3	46	48.2	100	46	33.0	68	29			
101	47.7	46	47.8	100	45	33.2	70	24			
104	48.3	45	47.6	99	44	33.5	69	16			
Mean for weeks											
1-13	28.5		29.7	104		29.1	102		28.8	101	
14-52	44.7		43.5	97		39.1	87		36.8	82	
53-104	48.5		48.4	100		37.0	76		34.3	71	

[a] Interim evaluation occurred during week 66.

TABLE 16
Mean Body Weights and Survival of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	125 ppm Av. Wt. (g)	125 ppm Wt. (% of controls)	125 ppm No. of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm No. of Survivors	5,000 ppm Av. Wt. (g)	5,000 ppm Wt. (% of controls)	5,000 ppm No. of Survivors
1	18.1	60	18.1	100	60	18.2	101	60	18.0	99	60
2	19.1	60	20.7	108	60	21.0	110	60	20.5	107	57
3	20.8	60	22.0	106	60	22.6	109	60	21.8	105	57
4	21.4	60	22.5	105	60	22.8	107	60	23.0	108	57
5	22.1	60	23.7	107	60	24.0	109	60	23.9	108	57
6	23.3	60	25.1	108	60	25.1	108	60	24.7	106	57
7	24.4	60	26.9	110	60	26.5	109	60	26.1	107	57
8	25.5	60	27.6	108	60	27.3	107	60	26.3	103	57
9	26.5	60	28.4	107	60	27.6	104	60	26.7	101	57
10	27.3	60	29.2	107	60	28.2	103	60	27.2	100	57
11	28.2	60	30.3	107	60	28.9	103	60	27.8	99	57
12	29.4	60	31.0	105	60	29.8	101	60	28.4	97	57
13	30.3	60	31.8	105	60	30.3	100	60	28.5	94	57
17	35.5	60	35.9	101	60	33.4	94	60	31.5	89	57
21	38.7	60	38.4	99	60	34.6	89	60	32.5	84	57
25	41.7	60	40.3	97	60	36.6	88	60	33.6	81	57
29	43.9	60	42.0	96	60	37.7	86	60	34.4	78	57
33	45.9	60	43.2	94	60	38.4	84	60	35.2	77	57
37	47.8	60	44.2	93	60	39.4	82	60	36.0	75	57
41	48.7	60	45.3	93	60	40.3	83	60	36.8	76	57
45	49.9	60	45.3	91	60	39.9	80	60	36.2	73	57
49	50.8	60	46.2	91	60	40.3	79	60	36.9	73	57
53	51.0	59	46.3	91	60	40.6	80	60	36.4	71	57
57	51.9	59	46.5	90	59	40.6	78	60	35.8	69	57
61	53.2	59	47.0	88	59	40.9	77	58	35.1	66	52
65	53.4	59	46.6	87	59	41.0	77	58	34.2	64	52
69[a]	53.6	49	46.8	87	48	40.7	76	47	33.9	63	37
73	54.4	49	47.5	87	46	39.3	72	47	33.5	62	31
77	54.4	48	47.3	87	46	38.5	71	47	33.3	61	29
81	55.1	48	47.3	86	46	37.8	69	47	33.1	60	25
85	56.0	47	47.2	84	46	36.8	66	46	33.4	60	12
89	54.6	46	47.1	86	46	36.1	66	43			
93	55.2	42	46.8	85	46	35.2	64	34			
97	54.1	39	46.5	86	44	35.6	66	28			
101	53.2	39	45.5	86	43	35.0	66	21			
Mean for weeks											
1-13	24.3		25.9	107		25.6	105		24.8	102	
14-52	44.8		42.3	94		37.8	84		34.8	78	
53-101	53.9		46.8	87		38.3	71		34.3	64	

[a] Interim evaluation occurred during week 66.

Body Weights, Feed Consumption, Compound Consumption, and Clinical Findings

Mean body weights of males receiving 125 ppm were similar to those of the controls throughout the study. Mean body weights of males that received 2,500 and 5,000 ppm were more than 10% lower than those of the controls after week 21 (Table 15 and Figure 5). Mean body weights of females were more than 10% lower than those of controls beginning at week 61 in the 125 ppm group, week 21 in the 2,500 ppm group, and week 17 in the 5,000 ppm group (Table 16 and Figure 5). Feed consumption by exposed males and females was similar to that by controls (Tables I3 and I4). Dietary levels of 125, 2,500, and 5,000 ppm resulted in average daily oxazepam consumption levels of 12, 310, and 690 mg/kg for males and 15, 350, and 780 mg/kg for females.

In the 5,000 ppm groups, lethargy and sedation were observed in a few mice during the first week of the study. Several animals exposed to 5,000 ppm also exhibited decreased spontaneous exploratory behavior and impaired locomotor activity. As tolerance to the initial depressant effects of oxazepam developed, the incidence and severity of clinical findings in exposed mice decreased. Behavior and appearance of exposed mice were similar to controls by the end of the second study week.

Serum Oxazepam Concentrations

Serum oxazepam concentrations were measured at the 15-month interim evaluation and at the end of the study. Due to excessive mortality, no blood samples from males or females exposed to 5,000 ppm were available at the end of the study. Blood samples were collected from 2,500 ppm females and males during weeks 102 and 103. Serum oxazepam concentrations increased with increasing exposure level in both males and females at the 15-month interim evaluation and at the end of 2 years (Tables 17 and 18). Serum oxazepam concentrations were similar between males and females in each exposure group.

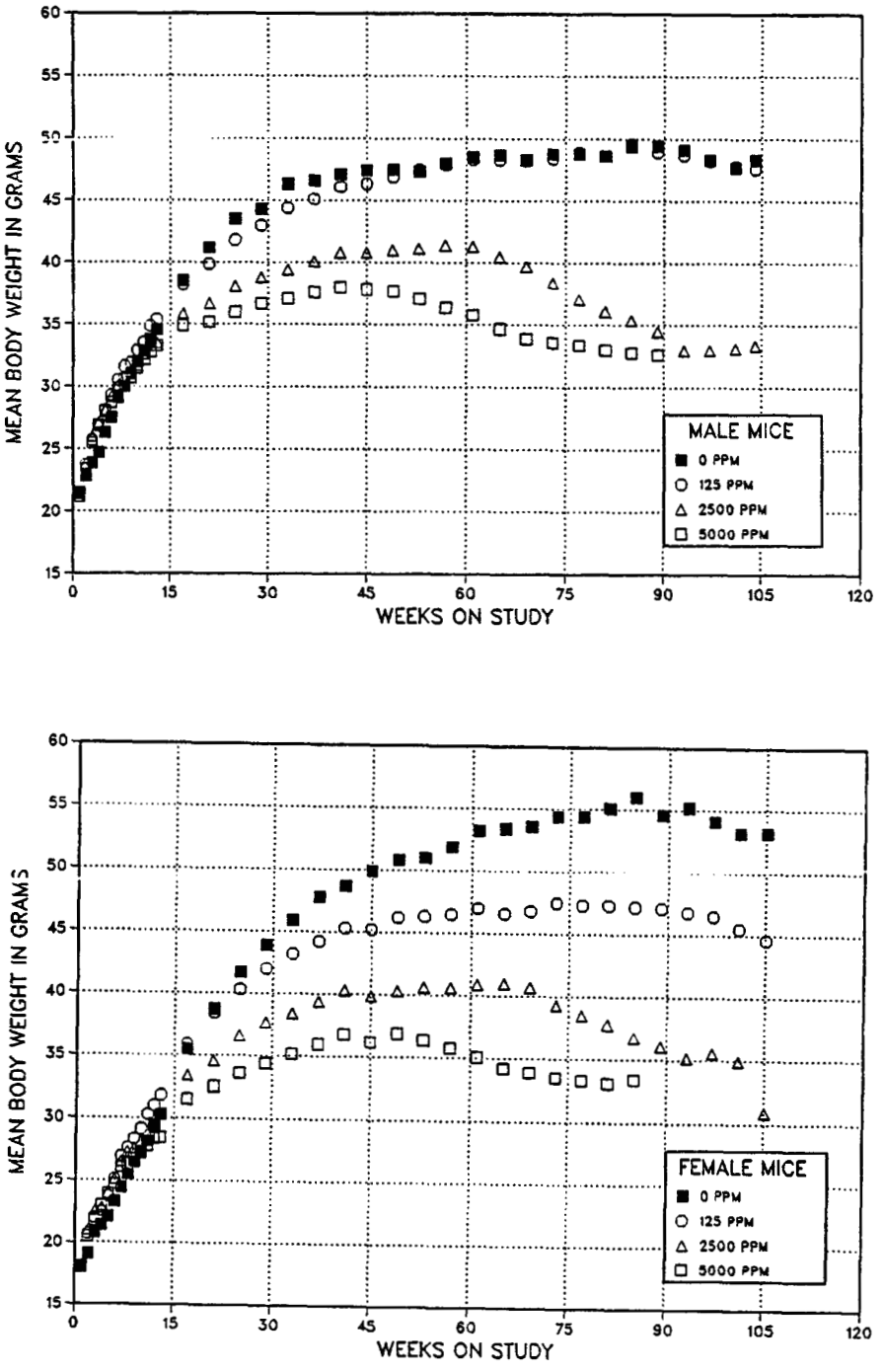


FIGURE 5
Growth Curves for B6C3F$_1$ Mice Administered Oxazepam in Feed for 2 Years

TABLE 17
Serum Oxazepam Concentrations in B6C3F$_1$ Mice at the 15-Month Interim Evaluation in the 2-Year Feed Study of Oxazepam[a]

Dose (ppm)	0	125	2,500	5,000
Male				
Serum oxazepam (μg/mL)	0 ± 0	1.19 ± 0.19	5.94 ± 1.00	7.09 ± 1.66
Female				
Serum oxazepam (μg/mL)	0 ± 0	1.19 ± 0.08	6.84 ± 1.84	10.16 ± 2.62

[a] Mean ± standard deviation for 10 animals

TABLE 18
Serum Oxazepam Concentrations in B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

Dose (ppm)	0	125	2,500	5,000
Male				
Serum oxazepam (μg/mL)	0 ± 0	1.03 ± 0.23	4.08 ± 1.20	–[b]
Female				
Serum oxazepam (μg/mL)	0 ± 0	1.00 ± 0.28	5.41 ± 1.95	–

[a] Mean ± standard deviation for 10 animals
[b] No samples collected due to 100% mortality

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions and neoplasms in the liver and thyroid gland and nonneoplastic lesions in the testis of $B6C3F_1$ mice. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male $B6C3F_1$ mice and Appendix D for female $B6C3F_1$ mice.

Liver: The administration of oxazepam in feed to $B6C3F_1$ mice was associated with a spectrum of lesions similar to that observed in the Swiss-Webster strain. At the 15-month interim evaluation, centrilobular hepatocellular hypertrophy occurred in all males in the 125, 2,500, and 5,000 ppm groups and in all females in the 2,500 and 5,000 ppm groups (Tables 19, 20, C5, and D5). At 2 years, incidences of centrilobular hepatocellular hypertrophy were significantly increased in the 2,500 and 5,000 ppm groups, and the incidence and severity increased with increasing dietary concentration.

The incidences of eosinophilic foci in 2,500 ppm males and females and in 5,000 ppm females and of hepatocellular adenoma or carcinoma (combined) in 2,500 and 5,000 ppm males and females were also significantly greater than in the controls at the 15-month interim evaluation. At 2 years, the incidences of hepatocellular foci (all types) in males were lower in the exposed groups than in the controls. The incidence of eosinophilic foci in 125 ppm females was significantly greater than controls, but the incidences in 2,500 and 5,000 ppm females were similar to controls. The unusual distribution of hepatocellular foci (a putative preneoplastic lesion) in the exposed groups was attributed to the pronounced dose-related development, multiplicity, and confluence of hepatocellular neoplasms in the 2,500 and 5,000 ppm groups.

In the 2-year study, all 2,500 and 5,000 ppm males, all 2,500 ppm females, and all but three 5,000 ppm females had one or more hepatocellular neoplasms and the incidences of hepatocellular neoplasms in these groups were significantly greater than those of the controls. In male and female mice, the incidences of both hepatocellular adenoma and hepatocellular carcinoma in the 2,500 and 5,000 ppm groups were significantly greater than those of the controls; the incidence of hepatocellular adenoma in 125 ppm females was also significantly greater than that of the controls. Hepatoblastoma, a rare phenotypic variant of hepatocellular carcinoma, occurred in all exposed groups of mice but not in the control groups. Moreover, the incidences of hepatoblastoma increased with increasing exposure level, and metastatic foci of hepatoblastoma and hepatocellular carcinoma were commonly observed in the lung (Tables C1 and D1).

The morphology of the liver neoplasms in $B6C3F_1$ mice was similar to that of neoplasms in Swiss-Webster mice. The cellular component identifying these neoplasms as hepatoblastomas was characterized by sheets of cells, occasionally forming rosettes, with a scant vascular stroma. The cells were small with scant basophilic cytoplasm and round hyperchromatic nuclei, similar to the hepatoblasts of the developing fetal liver. This cell population was almost always a component of a larger neoplasm with the morphologic characteristics of a typical carcinoma, although in a few, the predominant component had the characteristics of an adenoma.

Samples of liver neoplasms from each control and exposure group were collected for analysis of the occurrence of hepatocellular neoplasms with an activated H-*ras* oncogene, and the mutation spectrum of the H-*ras* gene in those neoplasms with an activated H-*ras* was determined (Appendix L). Nineteen neoplasms from the control groups, 37 from the 125 ppm group, and 20 each from the 2,500 and 5,000 ppm groups of males and females were analyzed. The neoplasms were sampled to provide an approximately even distribution of hepatocellular adenomas and carcinomas from each group. The mutation spectrum of the H-*ras* genes in neoplasms from exposed mice did not differ from the spectrum of mutations observed in neoplasms from controls, but the proportion of neoplasms with an activated H-*ras* decreased with increasing exposure level. While 58% of the neoplasms from control mice had an activated H-*ras*, only 1 of the 40 neoplasms from mice receiving 2,500 or 5,000 ppm oxazepam exhibited a similar molecular lesion. In the 125 ppm group, 35% of the neoplasms had an activated H-*ras* oncogene, suggesting that, although the incidence of liver neoplasms was not statistically increased in the 125 ppm group compared to that of the controls,

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

Dose (ppm)	0	125	2,500	5,000
15-Month Interim Evaluation				
Liver[a]	10	10	10	10
Centrilobular Hypertrophy	0	10** (1.1)[b]	10** (2.9)	10** (3.0)
Basophilic Focus	0	0	0	1
Clear Cell Focus	0	0	0	1
Eosinophilic Focus	0	1	9**	2
Focus (any type)	0	1	9**	3
Hepatoblastoma	0	0	0	1
Hepatocellular Adenoma	0	3	9**	9**
Hepatocellular Carcinoma	0	1	4*	9**
Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma	0	3	9**	10**
2-Year Study				
Liver	49	50	50	50
Centrilobular Hypertrophy	0	2 (2.0)	26** (2.4)	43** (3.0)
Basophilic Focus	2	1	0	0
Clear Cell Focus	13	6	1	0
Mixed Cell Focus	2	1	1	0
Eosinophilic Focus	18	12	8	8
Focus (any type)	27	16	8	8
Hepatoblastoma				
Overall rate[c]	0/49 (0%)	2/50 (4%)	21/50 (42%)	13/50 (26%)
Adjusted rate[d]	0.0%	4.5%	58.3%	52.9%
Terminal rate[e]	0/45 (0%)	2/44 (5%)	4/15 (27%)	0/0
First incidence (days)	–[g]	729 (T)	598	434
Life table test[f]	P<0.001	P=0.234	P<0.001	P<0.001
Logistic regression test[f]	P<0.001	P=0.234	P<0.001	P=0.014
Hepatocellular Adenoma				
Overall rate	17/49 (35%)	18/50 (36%)	34/50 (68%)	32/50 (64%)
Adjusted rate	37.8%	37.4%	87.3%	95.1%
Terminal rate	17/45 (38%)	14/44 (32%)	11/15 (73%)	0/0
First incidence (days)	729 (T)	453	486	401
Life table test	P<0.001	P=0.472	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.547	P<0.001	P=0.003
Hepatocellular Carcinoma				
Overall rate	9/49 (18%)	5/50 (10%)	45/50 (90%)	50/50 (100%)
Adjusted rate	18.7%	10.7%	95.7%	100.0%
Terminal rate	6/45 (13%)	3/44 (7%)	13/15 (87%)	0/0
First incidence (days)	586	453	540	401
Life table test	P<0.001	P=0.215N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.107N	P<0.001	P<0.001

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

Dose (ppm)	0	125	2,500	5,000
2-Year Study (continued)				
Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma[h]				
Overall rate	23/49 (47%)	19/50 (38%)	50/50 (100%)	50/50 (100%)
Adjusted rate	47.9%	39.5%	100.0%	100.0%
Terminal rate	20/45 (44%)	15/44 (34%)	15/15 (100%)	0/0
First incidence (days)	586	453	486	401
Life table test	P<0.001	P=0.315N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.205N	P<0.001	P<0.001

* Significantly different (P≤0.05) from the control group by Fisher's exact test (15-month interim evaluation)
** Significantly different (P≤0.01) from the control group by Fisher's exact test (15-month interim evaluation) or by the logistic regression test (2-year study)
(T)Terminal sacrifice
[a] Number of animals with liver examined microscopically
[b] Average severity of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Number of lesion-bearing animals/number of animals necropsied or examined microscopically
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposed 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 analysis regards these lesions as nonfatal. A lower incidence in an exposed group is indicated by N.
[g] Not applicable; no neoplasms in animal group
[h] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 485/1,366 (35.5% ± 14.3%); range 10%-68%

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

Dose (ppm)	0	125	2,500	5,000
15-Month Interim Evaluation				
Liver[a]	10	10	10	10
Centrilobular Hypertrophy	0	0	10** (2.7)[b]	10** (3.0)
Basophilic Focus	0	0	0	0
Clear Cell Focus	0	0	0	0
Eosinophilic Focus	1	0	8**	7**
Focus (any type)	1	0	8**	7**
Hepatoblastoma	0	0	0	2
Hepatocellular Adenoma	1	1	9**	10**
Hepatocellular Carcinoma	1	0	2	10**
Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma	2	1	9**	10**
2-Year Study				
Liver	50	50	50	50
Centrilobular Hypertrophy	0	2 (1.5)	11** (2.5)	29** (2.9)
Basophilic Focus	4	0	0	0
Clear Cell Focus	2	3	0	0
Mixed Cell Focus	0	0	0	1
Eosinophilic Focus	9	19*	2	5
Focus (any type)	14	21	2	5
Hepatoblastoma				
Overall rate[c]	0/50 (0%)	1/50 (2%)	8/50 (16%)	8/50 (16%)
Adjusted rate[d]	0.0%	2.3%	31.7%	57.8%
Terminal rate[e]	0/39 (0%)	0/41 (0%)	0/2 (0%)	0/0
First incidence (days)	–[g]	714	614	471
Life table test[f]	P<0.001	P=0.519	P<0.001	P<0.001
Logistic regression test[f]	P<0.001	P=0.502	P=0.007	P=0.003
Hepatocellular Adenoma				
Overall rate	25/50 (50%)	35/50 (70%)	35/50 (70%)	36/50 (72%)
Adjusted rate	59.3%	79.5%	96.7%	100.0%
Terminal rate	22/39 (56%)	32/41 (78%)	1/2 (50%)	0/0
First incidence (days)	598	471	393	403
Life table test	P<0.001	P=0.062	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.037	P=0.014	P=0.001
Hepatocellular Carcinoma				
Overall rate	9/50 (18%)	5/50 (10%)	49/50 (98%)	44/50 (88%)
Adjusted rate	21.6%	11.9%	100.0%	100.0%
Terminal rate	7/39 (18%)	4/41 (10%)	2/2 (100%)	0/0
First incidence (days)	598	714	393	410
Life table test	P<0.001	P=0.173N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.185N	P<0.001	P<0.001

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

Dose (ppm)	0	125	2,500	5,000
2-Year Study (continued)				
Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma[h]				
Overall rate	28/50 (56%)	36/50 (72%)	50/50 (100%)	47/50 (94%)
Adjusted rate	66.5%	81.8%	100.0%	100.0%
Terminal rate	25/39 (64%)	33/41 (80%)	2/2 (100%)	0/0
First incidence (days)	598	471	393	403
Life table test	P<0.001	P=0.126	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.084	P<0.001	P<0.001

* Significantly different (P≤0.05) from the control group by Fisher's exact test (15-month interim evaluation) or by the logistic regression test (2-year study)
** P≤0.01
(T)Terminal sacrifice
[a] Number of animals with liver examined microscopically
[b] Average severity of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Number of lesion-bearing animals/number of animals necropsied or examined microscopically
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposed 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 analysis regards these lesions as nonfatal. A lower incidence in an exposed group is indicated by N.
[g] Not applicable; no neoplasms in animal group
[h] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 223/1,363 (16.4% ± 10.7%); range 3%-42%

there was an increase in a similar subset of neoplasms (lacking an activated H-*ras*) that occurred with increased incidence at higher exposure levels.

Thyroid gland: At the 15-month interim evaluation, the incidences of follicular cell hyperplasia in male and female mice receiving 2,500 and 5,000 ppm were significantly greater than those of the controls (Tables 21, C5, and D5). At study termination, the incidences of follicular cell hyperplasia in all groups of mice receiving oxazepam were significantly greater than those of the controls. Follicular cell hyperplasia occurred in nearly all mice in the 2,500 and 5,000 ppm groups and in 44% of males and 68% of females in the 125 ppm groups, while only 8% of control males and 32% of control females were similarly affected. The lesion was focal or multifocal in distribution, and the extent and severity generally increased with increasing exposure level. In female mice, follicular cell adenomas occurred with a dose-related positive trend, and the incidences in the 2,500 and 5,000 ppm groups were significantly greater than that of the control group. However, the incidences of adenomas in the 2,500 and 5,000 ppm groups were only marginally greater than the range of follicular cell neoplasms in historical control females from recent NTP studies (0% to 9%, Table D4). Follicular cell adenomas were observed in one male in each exposure group, but not in the controls.

Testis: There was a dose-related increased incidence of testicular atrophy (bilateral) in male mice in the 2,500 and 5,000 ppm groups (1/50, 0/50, 25/50, 38/50; Table C5). The atrophy was primarily a decrease in or an absence of maturation of the spermatogenic cells within the tubules. The associated epididymides were void of mature spermatozoa and appeared shrunken. The shrunken epididymides of the exposed males often had small, focal, lymphocytic infiltrates (lymphocytic cellular infiltration: 2/50, 14/50, 33/50, 21/50; Table C5). The testicular atrophy occurred primarily in animals that were moribund or were found dead. It is not clear whether oxazepam was exerting a direct effect on germ-cell function or development, or whether this was related to an indirect effect of inanition due to oxazepam-induced liver neoplasms. The nature of the epididymal lymphocytic foci is not clear, but may relate to a normal phenomenon that is made more apparent when the epididymides are shrunken.

Uterus: The incidence of cystic endometrial hyperplasia in 2,500 and 5,000 ppm females was lower than that of the controls (46/50, 50/50, 19/50, 14/49; Table D5).

Neurobehavioral Evaluation

Forelimb grip strength at 12 months in males receiving 125, 2,500, or 5,000 ppm and at 18 months in males receiving 2,500 or 5,000 ppm oxazepam was significantly lower than that of the controls (Table G19). Hindlimb grip strength was not affected (Table G20). There were no significant differences in forelimb and hindlimb grip strengths in females. Motor activity was significantly increased at 6 and 12 months in males in the 2,500 and 5,000 ppm groups, in 125, 2,500, and 5,000 ppm females at 6 months, and in 2,500 and 5,000 ppm females at 12 months. However, motor activity was significantly decreased at 18 months in the 5,000 ppm groups, probably indicating the debilitating effect of oxazepam later in the study (Table G21). Response to a thermal stimulus as measured by paw lick latency was significantly decreased in 125 ppm males at 18 months (Table G22). There were no significant differences in startle response between exposed and control mice. No symptoms of withdrawal were noted during the 1-week, unexposed observation period at the end of the study.

TABLE 21
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland of B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

Dose (ppm)	0	125	2,500	5,000
Male				
15-Month Interim Evaluation				
Thyroid Gland[a]	10	10	10	10
Follicular Cell Hyperplasia[b]	0	0	5* (1.6)[c]	7** (1.9)
Follicular Cell Adenoma	0	0	0	0
2-Year Study				
Thyroid Gland	49	50	50	50
Follicular Cell Hyperplasia	4 (1.0)	22** (1.2)	49** (2.9)	47** (2.4)
Follicular Cell Adenoma	0	1	1	1
Female				
15-Month Interim Evaluation				
Thyroid Gland	10	10	10	10
Follicular Cell Hyperplasia	0	0	10** (1.7)	10** (2.2)
Follicular Cell Adenoma	0	0	0	0
2-Year Study				
Thyroid Gland	50	50	50	50
Follicular Cell Hyperplasia	16 (1.4)	34** (1.9)	49** (3.0)	44** (3.0)
Follicular Cell Adenoma[d]				
Overall rate[e]	0/50 (0%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate[f]	0.0%	9.8%	34.3%	46.1%
Terminal rate[g]	0/39 (0%)	4/41 (10%)	0/2 (0%)	0/0
First incidence (days)	–[i]	735 (T)	663	470
Logistic regression test[h]	P=0.007	P=0.070	P=0.019	P=0.017

* Significantly different (P≤0.05) from the control group by Fisher's exact test (15-month interim evaluation)
** Significantly different (P≤0.01) from the control group by Fisher's exact (15-month interim evaluation) or logistic regression tests (2-year study)
(T)Terminal sacrifice
[a] Number of mice with thyroid gland examined microscopically
[b] Number of lesion-bearing mice
[c] Average severity of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; 5 = severe
[d] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 32/1,348 (2.4% ± 2.8%); range 0%-9%
[e] Number of lesion-bearing animals/number of animals necropsied or examined microscopically
[f] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[g] Observed incidence in animals surviving until the end of the study
[h] In the control column are the P values associated with the trend test. In the exposed columns are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression regards these lesions as nonfatal.
[i] Not applicable; no neoplasms in animal group

GENETIC TOXICITY

Oxazepam (3 to 3,333 μg/plate) did not induce mutations in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, and TA1535 when tested in a preincubation protocol with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table E1). In cytogenetic tests with cultured Chinese hamster ovary cells, oxazepam did not induce sister chromatid exchanges or chromosomal aberrations, with or without S9 (Tables E2 and E3). Peripheral blood samples obtained from $B6C3F_1$ mice in the 14-week toxicity study were analyzed for frequency of micronucleated normochromatic erythrocytes; no increase in micronucleated normochromatic erythrocytes was observed at any of the exposure levels (Table E4).

SUPPLEMENTAL STUDIES

The marked liver neoplasm response to oxazepam exposure prompted the performance of supplementary short-term studies to further evaluate this effect. These studies were performed at the NIEHS facility under experimental conditions described in Appendixes M through P. The studies were conducted according to applicable health and safety requirements and NIEHS and NIH guidelines for the use of experimental animals. They were not done to the standards of record keeping required under Good Laboratory Practice Regulations.

Studies to Evaluate the Potential for Oxazepam to Stimulate Liver Cell Replication (Appendix M): Concentrations of 25, 125, 2,500, and 5,000 ppm oxazepam given in feed to male $B6C3F_1$ mice for up to 13 weeks caused a dose-related increase in the nuclear labeling index in studies that measured the incorporation of bromodeoxyuridine into replicating liver cells. This increase was statistically significant at all exposure levels except 25 ppm, and was limited to mice evaluated at 15 days. Cell replication rates were similar to that of controls in most groups evaluated at 30 days and after.

Studies to Evaluate the Potential for Oxazepam to Produce Hepatocellular Necrosis (Appendix N): Clinical pathology and light microscopy evaluations were performed at 15-day intervals in the study in which liver cell replication was examined. There was minimal evidence suggestive of hepatocyte necrosis either by light microscopy or in clinical chemistry measures. There was, however, evidence of cholestasis, likely due to physical obstruction of bile canaliculi by swollen hepatocytes.

Studies of Comparative Oxazepam Metabolism and Toxicokinetics in $B6C3F_1$ and Swiss-Webster Mice (Appendixes O and P): The metabolic fate and toxicokinetics of oxazepam were evaluated and compared to published data from studies in humans. Mice and humans both form glucuronides of oxazepam, and form 3- and 4-hydroxy- and methoxy-derivatives of the phenyl group. Glucuronidation was induced in the mouse with chronic administration. Nonetheless, oxidative metabolism of the phenyl group, likely through formation of an epoxide, appeared to be more prevalent in the mouse than is reported for humans. A very small amount of oxazepam was covalently bound to liver protein during metabolism in the mouse. The bioavailability of oxazepam from feed was about 40% compared to a reported bioavailability of 95% in humans taking a therapeutic dose. Elimination half-lives of the parent compound did not differ in the Swiss-Webster and $B6C3F_1$ mice and were similar to values reported for humans. Female mice generally attained slightly higher blood oxazepam levels for a given dose of oxazepam when compared to males, regardless of the route of administration.

DISCUSSION AND CONCLUSIONS

The toxicity and carcinogenicity studies of oxazepam in Swiss-Webster and B6C3F$_1$ mice were prompted by a long-standing concern over the findings of liver neoplasms in Swiss-Webster mice in studies in which relatively few animals were given oxazepam in the diet for less than 12 months (Fox and Lahcen, 1974). The Swiss-Webster mouse is not generally used in rodent carcinogenicity studies because of its short life span and because historical data to assist in the interpretation of findings are limited. Therefore, the current studies were performed with both strains of mice in an attempt to confirm the prior results and to allow for a comparison of potential neoplasm responses between the Swiss-Webster mouse and the more commonly used B6C3F$_1$ strain.

At the time of these studies, there were no data in the literature on which to base an estimated maximum exposure level for mice; however, Owen *et al.* (1970) had reported that 2 of 20 Sprague-Dawley rats died while receiving 5,000 ppm oxazepam in the diet for 6 weeks. Therefore, in the 14-week studies, 10,000 ppm was selected as the highest concentration. One male B6C3F$_1$ mouse in the 10,000 ppm group died because of a urinary tract infection, and one female Swiss-Webster mouse in the 10,000 ppm group died, presumably because of exposure to the chemical. The early deaths did not occur during the first week, when clinical findings indicating sedation and lethargy were at their maximum in males and females of each strain. The mice were able to adapt to the pharmacologic action of oxazepam by the second week of the study and appeared clinically normal thereafter.

In neurobehavioral assessments, oxazepam was found to produce two primary effects in the 14-week studies. The first effect was a transient reduction in grip strength that was considered to result from a nonspecific muscle relaxant or depressant effect. This was seen primarily in males of each strain and may be a manifestation of the sedative effects noted clinically during the early part of the study. An antianxiety effect, similar to that commonly seen in animal studies with other benzodiazepine drugs, was inferred from findings of facilitated motor activity, enhanced startle response, and decreased paw lick latencies (Crawley and Goodwin, 1980; Freeman and Thurmond, 1985). There were minor differences between the sexes and strains in some aspects of the neurobehavioral findings, but the general responses to the drug were similar and followed predicted patterns.

In general, exposed mice gained as much or somewhat more weight during the 14-week studies than did controls. Thus, body weight gain was not a factor in the selection of exposure levels for the chronic studies. The only consistent difference in organ weights was a dose-related increase in absolute and relative liver weights. The absolute and relative liver weights of males and females of each strain exposed to 10,000 ppm were nearly double those of the controls. Microscopically, centrilobular hepatocellular hypertrophy was observed in each strain with only minor evidence of focal necrosis in Swiss-Webster mice. Although no clinical chemistry studies were performed with these animals, in supplemental studies of a similar design with B6C3F$_1$ mice, outlined in Appendix N, there was evidence for cholestasis, which was considered a secondary effect of physical obstruction of bile canaliculi by the hypertrophic hepatocytes. There was no clinical or microscopic evidence to suggest significant toxicity to hepatocytes in the B6C3F$_1$ mice, and this was consistent with the findings in the core study.

Increased liver weights have frequently been reported in many rodent studies with benzodiazepines (Owen *et al.*, 1970; Kitagawa *et al.*, 1974; Scrollini *et al.*, 1975; Irikura *et al.*, 1977), and the histopathologic appearance of the livers is typically described as normal, although hepatocyte swelling was noted by Irikura *et al.* (1977). Diwan *et al.* (1986) reported hepatomegaly in B6C3F$_1$ mice receiving 1,500 ppm oxazepam or diazepam in the diet for 53 weeks. Total cytochrome-P-450 and the activity of aminopyrine *N*-demethylase were increased in livers of exposed animals. Proliferation of smooth endoplasmic reticulum and enhanced sterol metabolism

have been reported in studies of liver biopsies taken from humans that received therapeutic doses of diazepam (Jezequel, 1974; Orlandi *et al.*, 1975).

A high dose of 5,000 ppm was chosen for each strain of mice for the chronic studies because an increase in liver weight of more than about 70% was thought to be potentially life threatening. In the chronic studies, the survival of both strains and mean body weight gains of male and female $B6C3F_1$ mice exposed to 2,500 and 5,000 ppm were markedly lower than those of the controls. The body weight depression of all exposed female $B6C3F_1$ mice and of 2,500 and 5,000 ppm $B6C3F_1$ males could not be predicted based on the body weight gains in the 14-week studies. Survival of 125 ppm $B6C3F_1$ mice was similar to that of the controls. Mice exposed to 5,000 ppm also had clinical findings indicating sedation and lethargy early in the study but then appeared clinically normal. The only clear clinical findings attributed to oxazepam during the later part of the study were abdominal swelling, presumably due to liver hypertrophy and neoplasia, and a spectrum of findings typical of moribund animals as the liver neoplasms progressed.

Neurobehavioral evaluations were performed on both strains at 6-month intervals throughout the chronic studies. The purpose of these studies was to determine if there were any prominent changes in the character of the behaviors or the extent of the responses exhibited by the mice. Unfortunately, the early deaths prevented a rigorous evaluation of behavior beyond about 12 months, and effects on behavior secondary to neoplasia and reduced body weight also confounded interpretation of the studies. However, within these limitations, there were no noticeable changes in behavior patterns of mice from those noted in the 14-week studies, and no new or unusual behaviors developed during the chronic studies. One notable strain difference was observed and involved the disinhibitory effect of oxazepam on motor activity. Swiss-Webster mice appeared to adapt to this pharmacologic effect very early in the study, while the effect persisted in $B6C3F_1$ mice for at least 12 months.

There were several lesions attributed to oxazepam in the pathologic evaluation of the tissues of mice. One of these effects was limited to Swiss-Webster mice and involved increased incidences of systemic amyloid deposition in tissues. Swiss-Webster mice are very susceptible to the formation of amyloid deposits, which are thought to contribute to the relatively short average life span of this strain. However, the extent and severity of the lesions were increased in mice receiving oxazepam, and amyloid deposits in the heart likely contributed to pulmonary hypertension leading to edema and heart failure, which was considered the likely cause of death of many of these mice. Little is known about the similarities in etiology, if any, between amyloidosis in the Swiss-Webster mouse and the formation of amyloid plaques in the central nervous system (CNS) of humans with Alzheimer's disease. There have been no reports associating benzodiazepine use with Alzheimer's disease.

Testicular atrophy was observed in $B6C3F_1$ mice exposed to 2,500 and 5,000 ppm oxazepam. This occurred primarily in animals that were moribund or were found dead. It was not clear whether oxazepam was exerting a direct effect on germ cell function or development or whether this was an indirect effect of inanition due to oxazepam-induced liver neoplasms. There is support for both effects in the literature (i.e., treatment of Sprague-Dawley rats for 14 days with diazepam at 3 mg/kg resulted in a significant reduction in serum testosterone concentrations; Calvo *et al.*, 1991). Also, CD-1 mice held to 70% of control body weight by restricted feeding had tubular degeneration and atrophy in the testis (Chapin *et al.*, 1993).

A third effect was hepatocellular hypertrophy and increased incidences of liver neoplasms in male and female mice of each strain. Centrilobular hypertrophy was diagnosed in the majority of exposed Swiss-Webster mice and the increased incidences were dose related in the $B6C3F_1$ mice. In many instances this nonneoplastic lesion was difficult to diagnose in mice in the 2,500 and 5,000 ppm groups, because there was little or no liver which was not part of a neoplasm. Nonetheless, this lesion appeared similar to those observed in the 14-week studies.

The incidence of liver neoplasia was markedly increased by oxazepam exposure in male and female mice of each strain. In Swiss-Webster mice receiving 2,500 or 5,000 ppm, there were increases in the degree of neoplastic response that were related to dose. The number of mice with carcinomas rather than adenomas, and with multiple adenomas or carcinomas rather than single neoplasms, was higher in the 5,000 ppm groups than in the 2,500 ppm

groups. There were also a large number of 2,500 and 5,000 ppm B6C3F$_1$ mice that developed multiple adenomas and carcinomas, and these groups of mice also developed a significant number of hepatoblastomas, a relatively unusual phenotypic variant of hepatocellular carcinoma (Diwan *et al.*, 1992). The lower incidence of hepatoblastomas in the 5,000 ppm groups versus the 2,500 ppm groups may have been due to the shorter survival of the 5,000 ppm mice. The overall numerical expression of liver neoplasia in B6C3F$_1$ mice was somewhat higher than in the Swiss-Webster mice at comparable exposure levels, but this was probably influenced by the shorter duration of the Swiss-Webster mouse study.

Although there was not a statistically significant increase in the overall liver neoplasm incidence in the B6C3F$_1$ mice exposed to 125 ppm compared to the incidence in controls, the incidence in females (36/50, 72%) was high considering that these mice weighed 5 to 7 g less than the controls. As noted by Haseman (1992), there is a positive linear relationship between the maximum weekly average body weight and the ultimate incidence of liver neoplasms in control female B6C3F$_1$ mice. The typical incidence in control female B6C3F$_1$ mice that achieved a maximum body weight of 47.5 g (similar to the maximum body weight of the 125 ppm group) during 2-year studies is 25%. Also, two males and one female in the 125 ppm group had a hepatoblastoma. No hepatoblastomas were observed in the controls in this study, and historically, none were found in 1,366 control male mice and only one was found in 1,363 female control mice, again indicating that this is an unusual neoplasm type and that its occurrence is likely chemical related.

Diwan *et al.* (1989) noted that formation of hepatoblastomas in hybrid D2B6F$_1$ mice initiated with *N*-nitrosodiethylamine and given 0.05% phenobarbital in drinking water was increased over that in mice receiving only the initiator. They also showed that this response was different in different strains, or in hybrids of the same strains but from matings of the opposite sex. This finding is somewhat similar to the present studies with oxazepam. Historically, phenobarbital alone had been thought to cause increased incidences of hepatocellular adenomas and carcinomas, which had a low metastatic potential and would not result in increased mortality (McClain, 1990). Although the responses of the mouse liver to phenobarbital and oxazepam appear similar in many respects, the hepatic neoplasms and metastases induced by oxazepam were considered responsible for the high rate of early mortality seen in B6C3F$_1$ mice and may have contributed to early deaths in Swiss-Webster mice.

In exposed B6C3F$_1$ mice, there was a marked increase in follicular cell hyperplasia of the thyroid gland at all exposure levels, and follicular cell adenomas were significantly increased in the 2,500 and 5,000 ppm groups of female mice. A relationship between induction of hepatic microsomal enzymes and altered thyroid function leading to neoplasia has been demonstrated in the rat, and a hypothesis has been developed to account for these findings (McClain, 1989; McClain *et al.*, 1989). This involves induction of the glucuronidation activity in the liver for thyroxine, causing enhanced biliary excretion. This leads to a persistent increase in thyroid-stimulating hormone levels, which fosters increased thyroid follicular cell hyperplasia and neoplasia. Comparative metabolism studies, outlined in Appendix P, indicated that glucuronidation is a major metabolic pathway for oxazepam, and induction of this activity occurs with repeated dosing. Naive Swiss-Webster mice rely on glucuronidation for elimination of oxazepam to a somewhat lesser extent than do B6C3F$_1$ mice, but clearly, induction of glucuronidation activity is seen with each strain. Thus, it appears that further study of the effects of repeated exposure to oxazepam on thyroxine metabolism in these two strains of mice is needed to evaluate whether this hypothesis can account for the sex and strain specificity noted for follicular cell adenomas in these studies.

Prior studies of the potential carcinogenicity of benzodiazepines (reviewed in the Introduction) have provided somewhat mixed results, with no suggestion of a positive response comparable in magnitude to that seen in the liver in the current studies. Assays of the potential for benzodiazepines to cause gene mutation or other forms of genetic damage are typically negative (Carlo *et al.*, 1989), and this includes the findings in this report with oxazepam (Appendix E). However, there have been numerous reports that the benzodiazepines can act as neoplasm promoters in various tissues (see Introduction), and oxazepam was clearly shown to enhance the liver neoplasm response of B6C3F$_1$ mice given an initiating dose of *N*-nitrosodiethylamine (Diwan *et al.*, 1986). Therefore, a number of studies supplemental

to the current studies were performed in an attempt to more fully describe the effects of oxazepam on the liver in these strains of mice and to characterize some of the molecular changes in the neoplasms from these animals. These are described in more detail in Appendixes L through P.

Dietary administration of oxazepam to male B6C3F$_1$ mice was found to induce a transient increase in liver cell replication (Appendix M). The increase was clearly evident only at the 15-day time point in the 125, 2,500 and 5,000 ppm groups, although there was a suggestion of an increase at 25 ppm, a lower exposure level than any used in the chronic studies. This response is similar to that observed in phenobarbital studies. Smith *et al.* (1991) noted a liver cell labeling index of 30% in male CD-1 mice receiving 0.1% phenobarbital in the diet for 1 week versus 2% in controls. However, by 5 weeks of exposure the labeling indices were not different from those of the controls. Similar findings of a lack of evidence for sustained increased liver cell replication were noted by Ward *et al.* (1988) in their study of male B6C3F$_1$ mice exposed for 40 weeks to 500 ppm phenobarbital in drinking water.

The neoplasms observed in the livers of the B6C3F$_1$ mice in the 2-year study of oxazepam were sampled and analyzed for the frequency and mutation spectrum of activated H-*ras* oncogenes (Appendix L). Oncogenes are a large group of genes whose products play a role in the control of cell replication and differentiation, and when altered through point mutations or rearrangement, or when overexpressed, are thought to contribute to neoplasm formation (Travali *et al.*, 1990). The H-*ras* oncogene is frequently found activated in mouse liver neoplasms, and the frequency of the occurrence and the positions of point mutations found in these genes, which result in activation of the oncogene, have been characterized and used to distinguish between chemical-induced neoplasms and neoplasms that arise "spontaneously" in controls (Goodman *et al.*, 1991). While the mutation spectrum of the H-*ras* gene in neoplasms from exposed mice (primarily from the 125 ppm group) did not differ from the spectrum in neoplasms from control mice, the incidence of neoplasms with an activated H-*ras* gene declined dramatically with increasing exposure level. These findings are quite similar to those reported by Fox *et al.* (1990) in their study of the frequency of liver neoplasms with activated H-*ras* oncogenes in B6C3F$_1$ mice given phenobarbital. The absence of mutated H-*ras* genes supports the notion that oxazepam and phenobarbital are not potent mutagens and suggests that these agents foster an environment that favors the development of, or "promotes," neoplasms that do not express this genetic lesion. Approximately 20% to 30% of the liver neoplasms that develop in the control male B6C3F$_1$ mouse do not have an activated H-*ras* oncogene (Fox *et al.*, 1990). Nearly all of the neoplasms found in the livers of mice receiving 2,500 or 5,000 ppm lacked an activated H-*ras*, and 65% of the neoplasms analyzed in the 125 ppm group also lacked a mutated form of this gene. The implication of this is that although the liver neoplasm incidence was not statistically different between the controls and the 125 ppm group, oxazepam was able to increase the incidence of neoplasms formed that lacked an activated H-*ras* oncogene. With increased exposure, there is an apparent increased suppression of neoplasms that contain an activated oncogene.

As previously indicated, the intention of the 125 ppm exposure level was to produce a serum oxazepam concentration close to the therapeutic range for humans, which is about 0.3 to 1 μg/mL (Greenblatt *et al.*, 1980; Salzman *et al.*, 1983). Exposure to 125 ppm resulted in serum or plasma oxazepam concentrations of about 1 μg/mL in the 2-year B6C3F$_1$ study and in the toxicokinetic studies outlined in Appendix O. However, using different analysis methods, serum levels were determined to be from 2 to about 6 μg/mL in mice exposed to 125 ppm in the cell replication studies (Appendix M). The reason for this discrepancy is not clear. Nonetheless, the results of the cell replication and neoplasm oncogene studies indicate that oxazepam is capable of exerting an influence on neoplasm development in the liver at exposure levels which result in blood oxazepam concentrations that are not very different from those typically achieved in humans taking the drug.

This apparent similarity in exposure levels does not apply when considering the total exposure level required to achieve these plasma levels. The estimated average exposure level for the B6C3F$_1$ mice exposed to oxazepam in feed at 125 ppm was between 10 and 29 mg/kg per day over the course of the 2-year study. A typical adult human therapeutic dose is 15 to 30 mg (0.2 to 0.4 mg/kg) taken as needed (*Goodman and Gilman's*, 1990). While the half-life

for elimination from the blood is similar in humans and in mice, the total amount of drug handled by the liver is proportionately larger in mice consuming 125 ppm in the feed than in humans taking a therapeutic dose, even when considering the differences in bioavailability (Appendix O). The disproportionality between the amount of oxazepam consumed and the resulting blood concentrations is even more pronounced at the higher oxazepam concentrations used in these feed studies.

A comparison of the metabolic pathways for oxazepam for the Swiss-Webster and the $B6C3F_1$ mouse with that reported for humans is outlined in Figures P2 and P3. As discussed in Appendix P, the primary pathway in the human involves glucuronidation of the parent molecule and excretion in the urine. Glucuronidation of oxazepam or of oxy- or methoxy-derivatives is also a major pathway in the Swiss-Webster and $B6C3F_1$ mouse, and glucuronidation is enhanced with repeated exposure. There is evidence for oxidative metabolism of the phenyl group, particularly at the 3 and 4 positions, in the human as well as in the mouse, and this activity accounts for a larger fraction of the administered material in the mouse under the conditions studied. Formation of the 3- and 4-oxy derivatives suggests that a potentially reactive 3,4-epoxide may be an intermediate, and may account for the fact that some of the radiolabeled material cannot be extracted from liver proteins. Whether oxidative metabolism is in any way involved in the liver neoplasms seen in these studies has not been determined.

Many aspects of the neoplasms and other responses of mice to oxazepam resemble those reported with phenobarbital. Benzodiazepines and phenobarbital are known to modulate nervous impulses in the CNS through interactions with independent receptors on GABAergic neurons (see Introduction). There are benzodiazepine receptors in the peripheral tissues including the liver (Olsen *et al.*, 1986). Whether activation of the peripheral benzodiazepine receptor influences the neoplastic process, or if phenobarbital may also activate this receptor or influence the same biological processes, may be fruitful areas of research.

Phenobarbital is currently considered an agent that is "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC), based primarily on evidence of increased hepatocellular neoplasms in experimental studies with rats and mice (IARC, 1987). There has been no clear evidence linking liver neoplasms in humans with phenobarbital exposure, although some authors have attributed an increase in CNS neoplasms in selected patient populations to the drug, while other studies have rejected this link (Clemmesen and Hjalgrim-Jensen, 1978; Gold *et al.*, 1978; Olsen *et al.*, 1989). The IARC has reported that there is inadequate evidence to determine whether diazepam is carcinogenic to humans or to experimental animals (IARC, 1987).

CONCLUSIONS

Under the conditions of these feed studies, there was *clear evidence of carcinogenic activity** of oxazepam in male and female Swiss-Webster mice based on increased incidences of hepatocellular adenoma and carcinoma. There was *clear evidence of carcinogenic activity* of oxazepam in male and female $B6C3F_1$ mice based on increased incidences of hepatoblastoma and hepatocellular adenoma and carcinoma. Increased incidences of hyperplasia of thyroid gland follicular cells in male and female $B6C3F_1$ mice and of follicular cell adenomas in female $B6C3F_1$ mice were also related to oxazepam exposure.

Administration of oxazepam to Swiss-Webster mice resulted in centrilobular hepatocellular hypertrophy and increased incidences and severity of systemic amyloidosis. Administration of oxazepam to $B6C3F_1$ mice also resulted in centrilobular hepatocellular hypertrophy.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this report appear on page 12.

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APPENDIX A
SUMMARY OF LESIONS IN MALE SWISS-WEBSTER MICE IN THE 57-WEEK FEED STUDY OF OXAZEPAM

TABLE A1
Summary of the Incidence of Neoplasms in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

	0 ppm	2,500 ppm	5,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
Early deaths			
Moribund	10	13	24
Natural deaths	5	28	26
Survivors			
Terminal sacrifice	45	19	10
Animals examined microscopically	60	60	60
Alimentary System			
Intestine small, duodenum	(60)	(57)	(58)
Adenoma		1 (2%)	
Intestine small, jejunum	(59)	(59)	(56)
Liver	(60)	(60)	(60)
Hepatoblastoma		1 (2%)	
Hepatocellular carcinoma		4 (7%)	13 (22%)
Hepatocellular carcinoma, multiple		1 (2%)	6 (10%)
Hepatocellular adenoma	1 (2%)	14 (23%)	8 (13%)
Hepatocellular adenoma, multiple		21 (35%)	42 (70%)
Histiocytic sarcoma	1 (2%)		
Sarcoma, metastatic, skeletal muscle	1 (2%)		
Pancreas	(60)	(60)	(60)
Sarcoma, metastatic, skeletal muscle	1 (2%)		
Salivary glands	(60)	(60)	(60)
Carcinoma	1 (2%)		
Histiocytic sarcoma	1 (2%)		
Stomach, glandular	(60)	(59)	(60)
Cardiovascular System			
Heart	(60)	(60)	(60)
Sarcoma, metastatic, skeletal muscle	1 (2%)		
Endocrine System			
Adrenal cortex	(60)	(60)	(60)
Adenoma	1 (2%)		
Capsule, adenoma		1 (2%)	
Adrenal medulla	(60)	(58)	(56)
Thyroid gland	(60)	(60)	(60)
Cardiovascular System			
None			
Genital System			
Epididymis	(60)	(58)	(60)
Histiocytic sarcoma	1 (2%)		
Testes	(60)	(60)	(60)

TABLE A1
Summary of the Incidence of Neoplasms in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Hematopoietic System			
Bone marrow	(60)	(60)	(60)
Lymph node	(7)	(7)	(4)
Inguinal, sarcoma, metastatic, skeletal muscle	1 (14%)		
Lumbar, histiocytic sarcoma	1 (14%)		
Mediastinal, histiocytic sarcoma	1 (14%)		
Pancreatic, sarcoma, metastatic, skeletal muscle	1 (14%)		
Renal, histiocytic sarcoma	1 (14%)		
Lymph node, mandibular	(57)	(56)	(55)
Histiocytic sarcoma	1 (2%)		
Lymph node, mesenteric	(59)	(55)	(57)
Histiocytic sarcoma	1 (2%)		
Sarcoma, metastatic, skeletal muscle	1 (2%)		
Spleen	(60)	(60)	(60)
Histiocytic sarcoma	1 (2%)		
Sarcoma, metastatic, skeletal muscle	1 (2%)		
Thymus	(59)	(57)	(51)
Integumentary System			
Skin	(60)	(60)	(59)
Hemangiosarcoma	1 (2%)	1 (2%)	
Neurofibrosarcoma			1 (2%)
Musculoskeletal System			
Skeletal muscle	(60)	(60)	(60)
Sarcoma	1 (2%)		
Nervous System			
Brain	(60)	(60)	(60)
Respiratory System			
Lung	(60)	(60)	(60)
Alveolar/bronchiolar adenoma	10 (17%)	5 (8%)	7 (12%)
Alveolar/bronchiolar adenoma, multiple	2 (3%)	1 (2%)	
Alveolar/bronchiolar carcinoma	5 (8%)	3 (5%)	
Alveolar/bronchiolar carcinoma, multiple		1 (2%)	
Histiocytic sarcoma	1 (2%)		
Sarcoma, metastatic, skeletal muscle	1 (2%)		
Nose	(60)	(60)	(60)
Special Senses System			
None			

TABLE A1
Summary of the Incidence of Neoplasms in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Urinary System			
Kidney	(60)	(60)	(60)
Histiocytic sarcoma	1 (2%)		
Sarcoma, metastatic, skeletal muscle	1 (2%)		
Urinary bladder	(60)	(59)	(59)
Histiocytic sarcoma	1 (2%)		
Systemic Lesions			
Multiple organs[b]	(60)	(60)	(60)
Histiocytic sarcoma	1 (2%)		
Lymphoma malignant	1 (2%)		
Lymphoma malignant lymphocytic	2 (3%)	2 (3%)	2 (3%)
Lymphoma malignant mixed	3 (5%)		3 (5%)
Lymphoma malignant undifferentiated cell	4 (7%)	5 (8%)	1 (2%)
Neoplasm Summary			
Total animals with primary neoplasms[c]	26	42	54
Total primary neoplasms	33	61	83
Total animals with benign neoplasms	14	36	51
Total benign neoplasms	14	43	57
Total animals with malignant neoplasms	17	17	25
Total malignant neoplasms	19	18	26
Total animals with metastatic neoplasms	1		
Total metastatic neoplasm	9		

[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 A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm

Number of Days on Study	1 6 1	2 4 4	2 5 7	2 7 9	2 8 1	2 8 1	2 9 3	2 9 5	3 2 7	3 2 7	3 3 1	3 3 8	3 4 4	3 5 8	3 7 1	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	2 2 8	2 0 8	1 9 2	2 3 8	1 9 0	1 9 8	1 8 4	2 1 3	1 8 2	2 1 2	2 3 6	2 0 6	2 9 1	1 8 7	1 9 3	1 8 1	1 8 3	1 8 5	1 8 6	1 8 8	1 8 9	1 9 4	1 9 5	1 9 6	1 9 7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																						X			
Histiocytic sarcoma									X																
Sarcoma, metastatic, skeletal muscle													X												
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle													X												
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Histiocytic sarcoma									X																
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue							+																		
Tooth							+																		
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle													X												
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X												
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	M	M	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	1 9 9	2 0 0	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 0 7	2 0 9	2 1 0	2 1 1	2 1 4	2 1 5	2 1 6	2 1 7	2 1 8	2 1 9	2 2 0	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5	2 2 6	2 2 7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Histiocytic sarcoma																									
Sarcoma, metastatic, skeletal muscle																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Histiocytic sarcoma																									
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Tooth																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	2 2 9	2 3 0	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 3 7	2 3 9	2 4 0	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	60
Gallbladder	+	+	+	+	+	+	+	M	+	+	57
Intestine large, colon	+	+	+	+	+	M	+	+	+	+	59
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	59
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	60
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	59
Liver	+	+	+	+	+	+	+	+	+	+	60
Hepatocellular adenoma											1
Histiocytic sarcoma											1
Sarcoma, metastatic, skeletal muscle											1
Pancreas	+	+	+	+	+	+	+	+	+	+	60
Sarcoma, metastatic, skeletal muscle											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Carcinoma							X				1
Histiocytic sarcoma											1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	60
Tongue											1
Tooth											1
Cardiovascular System											
Blood vessel	+	+	+	+	+	+	+	+	+	+	60
Heart	+	+	+	+	+	+	+	+	+	+	60
Sarcoma, metastatic, skeletal muscle											1
Endocrine System											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	60
Adenoma											1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	60
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	60
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	54
Pituitary gland	+	+	+	+	+	+	+	+	M	M	58
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
General Body System											
Tissue NOS									+		1
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	60
Histiocytic sarcoma											1
Preputial gland	+	+	+	+	+	+	+	+	+	+	60
Prostate	+	+	+	+	+	+	+	+	+	+	60
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	60
Testes	+	+	+	+	+	+	+	+	+	+	60

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	1 6 1	2 4 4	2 5 7	2 7 9	2 8 1	2 8 1	2 9 3	2 9 5	3 2 7	3 2 7	3 3 1	3 3 8	3 4 4	3 5 8	3 7 1	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	2 2 8	2 0 8	1 9 2	2 3 8	1 9 0	1 9 8	1 8 4	2 1 3	1 8 2	2 1 2	2 3 6	2 0 6	1 9 1	1 8 7	1 9 3	1 8 1	1 8 3	1 8 5	1 8 6	1 8 8	1 8 9	1 9 4	1 9 5	1 9 6	1 9 7
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+			+				+		+		+												
Inguinal, sarcoma, metastatic, skeletal muscle													X												
Lumbar, histiocytic sarcoma									X																
Mediastinal, histiocytic sarcoma									X																
Pancreatic, sarcoma, metastatic, skeletal muscle													X												
Renal, histiocytic sarcoma									X																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Histiocytic sarcoma									X																
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Sarcoma, metastatic, skeletal muscle													X												
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Sarcoma, metastatic, skeletal muscle													X												
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	+	M	M	M	M	M	M	+	+	M	+	M	M	M	M	M	M	M	+	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma													X												
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+					+	+	+	+		+			+										
Spinal cord		+			+		+	+	+	+		+													
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma						X																			
Histiocytic sarcoma									X																
Sarcoma, metastatic, skeletal muscle													X												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	1 9 9	2 0 0	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 0 7	2 0 9	2 1 0	2 1 1	2 1 4	2 1 5	2 1 6	2 1 7	2 1 8	2 1 9	2 2 0	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5	2 2 6	2 2 7
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																	+								
Inguinal, sarcoma, metastatic, skeletal muscle																									
Lumbar, histiocytic sarcoma																									
Mediastinal, histiocytic sarcoma																									
Pancreatic, sarcoma, metastatic, skeletal muscle																									
Renal, histiocytic sarcoma																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Sarcoma, metastatic, skeletal muscle																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Sarcoma, metastatic, skeletal muscle																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	+	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																									
Spinal cord	+																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X		X									X		X		X	X	X			
Alveolar/bronchiolar adenoma, multiple														X											
Alveolar/bronchiolar carcinoma					X														X		X	X			
Histiocytic sarcoma																									
Sarcoma, metastatic, skeletal muscle																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	2 2 9	2 3 0	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 3 7	2 3 9	2 4 0	Total Tissues/ Tumors
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	60
Lymph node	+										7
Inguinal, sarcoma, metastatic, skeletal muscle											1
Lumbar, histiocytic sarcoma											1
Mediastinal, histiocytic sarcoma											1
Pancreatic, sarcoma, metastatic, skeletal muscle											1
Renal, histiocytic sarcoma											1
Lymph node, mandibular	+	+	+	M	+	+	+	+	M	+	57
Histiocytic sarcoma											1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	59
Histiocytic sarcoma											1
Sarcoma, metastatic, skeletal muscle											1
Spleen	+	+	+	+	+	+	+	+	+	+	60
Histiocytic sarcoma											1
Sarcoma, metastatic, skeletal muscle											1
Thymus	+	+	+	+	+	+	+	+	+	+	59
Integumentary System											
Mammary gland	M	M	+	M	M	M	+	M	M	M	9
Skin	+	+	+	+	+	+	+	+	+	+	60
Hemangiosarcoma											1
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	60
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	60
Sarcoma											1
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Peripheral nerve											7
Spinal cord											8
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma			X			X			X		10
Alveolar/bronchiolar adenoma, multiple							X				2
Alveolar/bronchiolar carcinoma											5
Histiocytic sarcoma											1
Sarcoma, metastatic, skeletal muscle											1
Nose	+	+	+	+	+	+	+	+	+	+	60
Trachea	+	+	+	+	+	+	+	+	+	+	60

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	1 6 1	2 4 4	2 5 7	2 7 9	2 8 1	2 8 1	2 9 3	2 9 5	3 2 7	3 2 7	3 3 1	3 3 8	3 4 4	3 5 8	3 7 1	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	2 2 8	2 0 8	1 9 2	2 3 8	1 9 0	1 9 8	1 8 4	2 1 3	1 8 2	2 1 2	2 3 6	2 0 6	1 9 1	1 8 7	1 9 3	1 8 1	1 8 3	1 8 5	1 8 6	1 8 8	1 8 9	1 9 4	1 9 5	1 9 6	1 9 7
Special Senses System																									
Ear		+					+			+															
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Sarcoma, metastatic, skeletal muscle													X												
Urethra	+																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Lymphoma malignant			X																						
Lymphoma malignant lymphocytic				X								X													
Lymphoma malignant mixed		X								X	X														
Lymphoma malignant undifferentiated cell type					X							X		X	X										

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Carcass ID Number	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	9	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
	9	0	1	2	3	4	5	7	9	0	1	4	5	6	7	8	9	0	1	2	3	4	5	6	7
Special Senses System																									
Ear																									
Eye																	+								+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Sarcoma, metastatic, skeletal muscle																									
Urethra																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymphoma malignant																									
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed																									
Lymphoma malignant undifferentiated cell type																									

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	2 2 9	2 3 0	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 3 7	2 3 9	2 4 0	Total Tissues/ Tumors
Special Senses System											
Ear									+		4
Eye											2
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Histiocytic sarcoma											1
Sarcoma, metastatic, skeletal muscle											1
Urethra											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	60
Histiocytic sarcoma											1
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Histiocytic sarcoma											1
Lymphoma malignant											1
Lymphoma malignant lymphocytic											2
Lymphoma malignant mixed											3
Lymphoma malignant undifferentiated cell type											4

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm

Number of Days on Study	0 1 4	1 0 7	1 4 5	1 5 9	1 8 3	2 0 6	2 2 5	2 6 8	2 9 5	2 9 6	2 9 6	2 9 7	3 0 5	3 1 2	3 1 7	3 2 7	3 2 8	3 2 9	3 3 1	3 3 6	3 3 9	3 4 0	3 4 1	3 5 0
Carcass ID Number	2 9 5	2 7 0	2 9 0	2 6 4	2 4 8	2 5 4	2 4 3	2 7 3	2 5 1	2 4 1	2 4 7	2 9 4	2 5 6	2 7 8	2 8 9	2 8 7	2 8 6	2 9 7	2 9 9	2 9 5	2 5 7	2 5 3	2 8 6	2 4 6	2 6 6
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	A	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Intestine small, jejunum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma																								
Hepatocellular carcinoma																								
Hepatocellular carcinoma, multiple																								
Hepatocellular adenoma								X			X					X		X	X		X		X	X
Hepatocellular adenoma, multiple																	X					X		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue							+																	
Cardiovascular System																								
Blood vessel	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																								
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+
Pituitary gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																								
None																								
Genital System																								
Epididymis	+	+	+	+	+	+		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis								+		+		+		+			+	+	+			+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm
(continued)

Number of Days on Study	3 5 1	3 5 6	3 5 9	3 6 0	3 6 1	3 6 2	3 6 5	3 6 9	3 7 5	3 7 6	3 7 9	3 8 2	3 8 3	3 8 5	3 8 7	3 9 3	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	2 6 2	2 9 1	2 4 5	2 8 3	2 9 6	2 5 2	2 5 9	2 8 1	2 6 1	2 5 0	2 8 5	2 6 6	2 8 0	2 4 2	2 7 5	2 9 3	2 4 4	2 4 9	2 5 5	2 5 8	2 6 0	2 6 3	2 6 5	2 6 7	2 7 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma		X																							
Hepatocellular carcinoma		X										X								X					
Hepatocellular carcinoma, multiple																					X				
Hepatocellular adenoma											X		X				X				X				
Hepatocellular adenoma, multiple		X	X			X	X	X		X		X		X				X		X		X		X	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																			+						
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	M	+	M	+	+	M
Pituitary gland	+	+	+	+	M	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis	+			+		+	+	+		+	+	+	+		+	+	+								
Preputial gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	2 7 2	2 7 4	2 7 7	2 7 9	2 8 2	2 8 4	2 8 8	2 9 2	2 9 7	3 0 0	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	60
Gallbladder	+	+	+	+	+	+	+	+	+	+	58
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	60
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	58
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, duodenum	+	+	M	+	+	+	+	+	+	+	57
Adenoma				X							1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	58
Liver	+	+	+	+	+	+	+	+	+	+	60
Hepatoblastoma											1
Hepatocellular carcinoma					X						4
Hepatocellular carcinoma, multiple											1
Hepatocellular adenoma							X			X	14
Hepatocellular adenoma, multiple	X	X		X	X	X		X	X		21
Pancreas	+	+	+	+	+	+	+	+	+	+	60
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	59
Tongue											2
Cardiovascular System											
Blood vessel	+	+	+	+	+	+	+	+	+	+	59
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	60
Capsule, adenoma					X						1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	58
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	60
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	52
Pituitary gland	+	+	+	+	+	+	+	+	+	+	53
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
General Body System											
None											
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	58
Penis											23
Preputial gland	+	+	+	+	+	+	+	+	+	+	59
Prostate	+	+	+	+	+	+	+	+	+	+	58
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	59
Testes	+	+	+	+	+	+	+	+	+	+	60

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm
(continued)

Number of Days on Study	0 1 4	1 0 7	1 4 5	1 5 9	1 8 3	2 0 6	2 2 5	2 6 8	2 9 5	2 9 6	2 9 6	2 9 7	3 0 5	3 1 2	3 1 7	3 2 7	3 2 8	3 2 9	3 3 1	3 3 6	3 3 9	3 4 0	3 4 1	3 4 1	3 5 0
Carcass ID Number	2 9 5	2 7 0	2 9 0	2 6 4	2 4 8	2 5 4	2 4 3	2 7 3	2 5 1	2 4 1	2 4 7	2 9 4	2 5 6	2 7 8	2 8 9	2 7 6	2 8 7	2 9 9	2 9 8	2 5 7	2 5 3	2 8 6	2 4 6	2 6 9	2 6 8
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node										+					+	+		+							
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Lymph node, mesenteric	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	M	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+
Integumentary System																									
Mammary gland	+	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+						+		+	+			+					+							
Spinal cord	+								+	+			+					+							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma								X																	
Alveolar/bronchiolar carcinoma, multiple																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear											+														
Eye																									
Harderian gland																	+								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic															X	X									
Lymphoma malignant undifferentiated cell type			X				X			X		X													

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm
(continued)

Number of Days on Study	3 5 1	3 5 1 6	3 5 1 9	3 6 0	3 6 1	3 6 2	3 6 5	3 6 9	3 7 5	3 7 6	3 7 9	3 8 2	3 8 3	3 8 5	3 8 7	3 9 3	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	2 6 2	2 9 1	2 4 5	2 8 3	2 9 6	2 5 2	2 5 9	2 8 1	2 6 1	2 5 0	2 8 5	2 6 6	2 8 0	2 4 2	2 7 5	2 9 3	2 4 4	2 4 9	2 5 5	2 5 8	2 6 0	2 6 3	2 6 5	2 6 7	2 7 1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+		+									+													
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	+	M	M	M	M	M	M	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve												+													
Spinal cord												+													
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X									X						X	X
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma										X															
Alveolar/bronchiolar carcinoma, multiple											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye								+																	
Harderian gland																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									
Lymphoma malignant undifferentiated cell type	X																								

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	2 7 2	2 7 4	2 7 7	2 7 9	2 8 2	2 8 4	2 8 8	2 9 2	2 9 7	3 0 0	Total Tissues/ Tumors
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	60
Lymph node											7
Lymph node, mandibular		+	+	+	+	+	+	+	+	+	56
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	55
Spleen	+	+	+	+	+	+	+	+	+	+	60
Thymus	+	+	+	+	+	+	+	+	+	+	57
Integumentary System											
Mammary gland	+	M	M	M	M	M	M	M	M	M	6
Skin	+	+	+	+	+	+	+	+	+	+	60
Hemangiosarcoma											1
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	60
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	60
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Peripheral nerve											7
Spinal cord											6
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma				X							5
Alveolar/bronchiolar adenoma, multiple	X										1
Alveolar/bronchiolar carcinoma	X										3
Alveolar/bronchiolar carcinoma, multiple											1
Nose	+	+	+	+	+	+	+	+	+	+	60
Trachea	+	+	+	+	+	+	+	+	+	+	60
Special Senses System											
Ear											1
Eye											1
Harderian gland											1
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Urinary bladder	+	+	+	+	+	+	+	+	+	+	59
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Lymphoma malignant lymphocytic											2
Lymphoma malignant undifferentiated cell type											5

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm

Number of Days on Study	0 5 7	1 6 5	2 2 5	2 3 1	2 3 7	2 7 1	2 7 1	2 7 2	2 7 3	2 8 1	2 8 5	2 8 6	2 9 2	2 9 6	3 0 2	3 0 2	3 0 5	3 0 6	3 1 0	3 1 1	3 1 4	3 2 8	3 2 8	3 3 4	3 4 1
Carcass ID Number	3 1 9	3 5 1	3 2 0	3 5 7	3 4 8	3 3 5	3 3 8	3 5 6	3 0 8	3 1 0	3 3 9	3 3 6	3 2 3	3 6 0	3 2 1	3 2 9	3 5 3	3 3 2	3 2 8	3 3 4	3 2 7	3 4 3	3 5 0	3 3 0	3 0 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+		+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	A	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma															X									X	
Hepatocellular carcinoma, multiple																							X		
Hepatocellular adenoma								X						X						X					
Hepatocellular adenoma, multiple				X		X	X				X	X	X		X		X	X	X		X	X	X	X	X
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+		+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis															+	+				+	+	+		+	
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm
(continued)

Number of Days on Study	3 4 1	3 4 4	3 4 5	3 5 1	3 5 5	3 5 8	3 6 1	3 6 2	3 6 3	3 6 4	3 6 5	3 6 8	3 6 9	3 7 0	3 7 0	3 7 1	3 7 1	3 7 3	3 7 4	3 7 5	3 7 9	3 8 3	3 8 5	3 8 5	3 9 5
Carcass ID Number	3 1 4	3 2 5	3 1 6	3 0 2	3 3 7	3 4 6	3 0 7	3 4 2	3 0 3	3 5 8	3 1 8	3 5 9	3 0 6	3 1 2	3 4 7	3 0 9	3 1 3	3 1 7	3 2 4	3 0 5	3 4 4	3 2 2	3 4 1	3 4 9	3 5 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	M	+	+	M
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	M	+	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma		X	X		X					X		X			X	X									X
Hepatocellular carcinoma, multiple				X							X									X					
Hepatocellular adenoma	X						X				X														
Hepatocellular adenoma, multiple		X	X		X	X		X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	M	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis		+		+	+		+	+	+				+	+			+	+	+		+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	3 0 4	3 1 1	3 1 5	3 2 6	3 3 1	3 3 3	3 4 0	3 4 5	3 5 4	3 5 5	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	60
Gallbladder	+	+	+	+	+	+	+	+	+	+	56
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	59
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	59
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	55
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	58
Intestine small, jejunum	+	+	+	+	+	+	M	+	+	+	56
Intestine small, ileum	M	+	+	+	+	+	+	+	+	+	54
Liver	+	+	+	+	+	+	+	+	+	+	60
Hepatocellular carcinoma			X			X				X	13
Hepatocellular carcinoma, multiple				X					X		6
Hepatocellular adenoma						X	X				8
Hepatocellular adenoma, multiple		X	X	X	X			X	X	X	42
Pancreas	+	+	+	+	+	+	+	+	+	+	60
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	60
Cardiovascular System											
Blood vessel	+	+	+	+	+	+	+	+	+	+	59
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	60
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	56
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	60
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	52
Pituitary gland	+	+	+	+	+	+	+	+	+	+	57
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
General Body System											
None											
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	60
Penis	+				+						24
Preputial gland	+	+	M	+	+	+	+	M	+	+	58
Prostate	+	M	+	+	+	+	+	+	+	+	59
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	60
Testes	+	+	+	+	+	+	+	+	+	+	60

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm
(continued)

Number of Days on Study	0 5 7	1 6 5	2 2 5	2 3 1	2 3 7	2 7 1	2 7 1	2 7 2	2 7 3	2 8 1	2 8 5	2 8 6	2 9 2	2 9 6	3 0 2	3 0 2	3 0 5	3 0 6	3 1 0	3 1 1	3 1 4	3 2 8	3 2 8	3 3 4	3 4 1
Carcass ID Number	3 1 9	3 5 1	3 2 0	3 5 7	3 4 8	3 3 5	3 3 8	3 5 6	3 0 8	3 1 0	3 3 9	3 3 6	3 2 3	3 6 0	3 2 1	3 2 9	3 5 3	3 3 2	3 2 8	3 3 4	3 2 7	3 4 3	3 5 0	3 3 0	3 0 1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node								+			+										+				·
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	M	M	M	+	+	M	+	+	+	M	+	M	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Neurofibrosarcoma							X																		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+			+					+							+	+								
Spinal cord	+		+	+					+								+								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X				X				X					X				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear	+			+					+	+											+				
Eye					+																				
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic								X													X				
Lymphoma malignant mixed											X														
Lymphoma malignant undifferentiated cell type																									

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm
(continued)

Number of Days on Study	3 4 1	3 4 4	3 4 5	3 5 1	3 5 5	3 5 8	3 6 1	3 6 2	3 6 3	3 6 4	3 6 5	3 6 8	3 6 9	3 7 0	3 7 0	3 7 1	3 7 1	3 7 3	3 7 4	3 7 5	3 7 9	3 8 3	3 8 5	3 8 5	3 9 5
Carcass ID Number	3 1 4	3 2 5	3 1 6	3 0 2	3 3 7	3 4 6	3 0 7	3 4 2	3 0 3	3 5 8	3 1 8	3 5 9	3 0 6	3 1 2	3 4 7	3 0 9	3 1 3	3 1 7	3 2 4	3 0 5	3 4 4	3 4 2	3 4 1	3 4 9	3 5 2
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																									
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	+	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neurofibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+												+		+							+		
Spinal cord		+												+		+							+		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma															X								X	X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																								+	
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed													X												
Lymphoma malignant undifferentiated cell type																									

TABLE A2
Individual Animal Tumor Pathology of Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	3 0 4	3 1 1	3 1 5	3 2 6	3 3 1	3 3 3	3 4 0	4 4 5	4 5 4	4 5 5	Total Tissues/ Tumors
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	60
Lymph node	+										4
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	55
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	57
Spleen	+	+	+	+	+	+	+	+	+	+	60
Thymus	+	M	+	+	+	+	+	+	+	M	51
Integumentary System											
Mammary gland	+	M	+	M	M	M	M	M	M	M	5
Skin	+	+	+	+	+	+	+	+	+	+	59
Neurofibrosarcoma											1
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	60
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	60
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Peripheral nerve											9
Spinal cord											9
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma											7
Nose	+	+	+	+	+	+	+	+	+	+	60
Trachea	+	+	+	+	+	+	+	+	+	+	60
Special Senses System											
Ear											6
Eye											1
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Urinary bladder	+	+	+	+	+	+	+	+	+	+	59
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Lymphoma malignant lymphocytic											2
Lymphoma malignant mixed						X					3
Lymphoma malignant undifferentiated cell type	X										1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

	0 ppm	2,500 ppm	5,000 ppm
Liver: Hepatocellular Adenoma			
Overall rate[a]	1/60 (2%)	35/60 (58%)	50/60 (83%)
Adjusted rate[b]	2.2%	88.7%	98.0%
Terminal rate[c]	1/45 (2%)	15/19 (79%)	9/10 (90%)
First incidence (days)	397 (T)	268	231
Life table test[d]	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test[d]		P<0.001	P<0.001
Liver: Hepatocellular Carcinoma			
Overall rate	0/60 (0%)	5/60 (8%)	19/60 (32%)
Adjusted rate	0.0%	21.7%	72.0%
Terminal rate	0/45 (0%)	3/19 (16%)	5/10 (50%)
First incidence (days)	–[e]	356	302
Life table test	P<0.001	P=0.003	P<0.001
Logistic regression test	P<0.001	P=0.010	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.029	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	1/60 (2%)	35/60 (58%)	52/60 (87%)
Adjusted rate	2.2%	88.7%	98.1%
Terminal rate	1/45 (2%)	15/19 (79%)	9/10 (90%)
First incidence (days)	397 (T)	268	231
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	12/60 (20%)	6/60 (10%)	7/60 (12%)
Adjusted rate	26.7%	28.9%	26.0%
Terminal rate	12/45 (27%)	5/19 (26%)	0/10 (0%)
First incidence (days)	397 (T)	369	272
Life table test	P=0.123	P=0.482	P=0.164
Logistic regression test	P=0.353N	P=0.570	P=0.329N
Cochran-Armitage test	P=0.117N		
Fisher exact test		P=0.100N	P=0.159N
Lung: Alveolar/bronchiolar Carcinoma			
Overall rate	5/60 (8%)	4/60 (7%)	0/60 (0%)
Adjusted rate	10.5%	14.2%	0.0%
Terminal rate	4/45 (9%)	1/19 (5%)	0/10 (0%)
First incidence (days)	281	268	–
Life table test	P=0.266N	P=0.407	P=0.217N
Logistic regression test	P=0.043N	P=0.551N	P=0.051N
Cochran-Armitage test	P=0.030N		
Fisher exact test		P=0.500N	P=0.029N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	14/60 (23%)	9/60 (15%)	7/60 (12%)
Adjusted rate	30.2%	35.7%	26.0%
Terminal rate	13/45 (29%)	5/19 (26%)	0/10 (0%)
First incidence (days)	281	268	272
Life table test	P=0.200	P=0.291	P=0.286
Logistic regression test	P=0.158N	P=0.415N	P=0.155N
Cochran-Armitage test	P=0.056N		
Fisher exact test		P=0.177N	P=0.074N
All Organs: Malignant Lymphoma or Histiocytic Sarcoma			
Overall rate	10/60 (17%)	7/60 (12%)	6/60 (10%)
Adjusted rate	17.6%	13.9%	28.3%
Terminal rate	0/45 (0%)	0/19 (0%)	2/10 (20%)
First incidence (days)	244	145	272
Life table test	P=0.415N	P=0.435N	P=0.519N
Logistic regression test	P=0.005N	P=0.036N	P=0.007N
Cochran-Armitage test	P=0.169N		
Fisher exact test		P=0.301N	P=0.211N
All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rate	9/60 (15%)	7/60 (12%)	6/60 (10%)
Adjusted rate	16.0%	13.9%	28.3%
Terminal rate	0/45 (0%)	0/19 (0%)	2/10 (20%)
First incidence (days)	244	145	272
Life table test	P=0.508N	P=0.526N	P=0.603
Logistic regression test	P=0.014N	P=0.080N	P=0.021N
Cochran-Armitage test	P=0.243N		
Fisher exact test		P=0.395N	P=0.291N
All Organs: Benign Neoplasms			
Overall rate	14/60 (23%)	36/60 (60%)	51/60 (85%)
Adjusted rate	30.4%	91.5%	98.0%
Terminal rate	13/45 (29%)	16/19 (84%)	9/10 (90%)
First incidence (days)	344	268	231
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
All Organs: Malignant Neoplasms			
Overall rate	17/60 (28%)	17/60 (28%)	25/60 (42%)
Adjusted rate	29.6%	44.1%	80.3%
Terminal rate	5/45 (11%)	4/19 (21%)	6/10 (60%)
First incidence (days)	244	145	271
Life table test	P<0.001	P=0.170	P<0.001
Logistic regression test	P=0.321	P=0.203N	P=0.555N
Cochran-Armitage test	P=0.072		
Fisher exact test		P=0.580N	P=0.090

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam
(continued)

	0 ppm	2,500 ppm	5,000 ppm
All Organs: Benign or Malignant Neoplasms			
Overall rate	26/60 (43%)	42/60 (70%)	54/60 (90%)
Adjusted rate	45.4%	92.6%	100.0%
Terminal rate	14/45 (31%)	16/19 (84%)	10/10 (100%)
First incidence (days)	244	145	231
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.005	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.003	P<0.001

(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 control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 A4
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

	0 ppm	2,500 ppm	5,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
Early deaths			
Moribund	10	13	24
Natural deaths	5	28	26
Survivors			
Terminal sacrifice	45	19	10
Animals examined microscopically	60	60	60
Alimentary System			
Intestine large, colon	(59)	(60)	(59)
Amyloid deposition	3 (5%)	29 (48%)	29 (49%)
Lumen, hemorrhage			1 (2%)
Intestine large, rectum	(59)	(58)	(59)
Amyloid deposition		5 (9%)	9 (15%)
Intestine large, cecum	(59)	(59)	(55)
Amyloid deposition	6 (10%)	24 (41%)	29 (53%)
Ulcer		1 (2%)	1 (2%)
Lumen, hemorrhage			1 (2%)
Intestine small, duodenum	(60)	(57)	(58)
Amyloid deposition	39 (65%)	49 (86%)	50 (86%)
Ulcer	1 (2%)		
Intestine small, jejunum	(59)	(59)	(56)
Amyloid deposition	31 (53%)	49 (83%)	48 (86%)
Ulcer	1 (2%)		1 (2%)
Intestine small, ileum	(59)	(58)	(54)
Amyloid deposition	46 (78%)	48 (83%)	43 (80%)
Inflammation, granulomatous	1 (2%)		
Liver	(60)	(60)	(60)
Amyloid deposition	22 (37%)	31 (52%)	28 (47%)
Basophilic focus	1 (2%)		1 (2%)
Eosinophilic focus		22 (37%)	22 (37%)
Fatty change			1 (2%)
Hematopoietic cell proliferation	1 (2%)		2 (3%)
Infarct		1 (2%)	
Infiltration cellular, lymphocyte		1 (2%)	
Inflammation, chronic		2 (3%)	1 (2%)
Inflammation, subacute	1 (2%)		1 (2%)
Necrosis	10 (17%)	13 (22%)	8 (13%)
Centrilobular, hypertrophy	12 (20%)	46 (77%)	47 (78%)
Oval cell, hyperplasia		1 (2%)	
Pancreas	(60)	(60)	(60)
Amyloid deposition		1 (2%)	
Duct, cyst			1 (2%)
Salivary glands	(60)	(60)	(60)
Amyloid deposition	32 (53%)	44 (73%)	44 (73%)
Stomach, forestomach	(60)	(60)	(60)
Acanthosis		1 (2%)	
Amyloid deposition			4 (7%)
Hyperkeratosis		3 (5%)	1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam (continued)

	0 ppm	2,500 ppm	5,000 ppm
Alimentary System (continued)			
Stomach, glandular	(60)	(59)	(60)
Amyloid deposition	8 (13%)	29 (49%)	22 (37%)
Mineralization		3 (5%)	1 (2%)
Epithelium, hyperplasia			1 (2%)
Cardiovascular System			
Heart	(60)	(60)	(60)
Amyloid deposition	43 (72%)	52 (87%)	52 (87%)
Degeneration	2 (3%)		
Inflammation, subacute	1 (2%)		
Atrium, thrombosis	1 (2%)	34 (57%)	35 (58%)
Valve, thrombosis	1 (2%)	2 (3%)	
Ventricle, thrombosis		1 (2%)	
Endocrine System			
Adrenal cortex	(60)	(60)	(60)
Amyloid deposition	17 (28%)	37 (62%)	41 (68%)
Hyperplasia	4 (7%)		
Capsule, hyperplasia	3 (5%)		
Adrenal medulla	(60)	(58)	(56)
Hyperplasia	2 (3%)		
Islets, pancreatic	(60)	(60)	(60)
Hyperplasia	4 (7%)		
Parathyroid gland	(54)	(52)	(52)
Amyloid deposition	10 (19%)	39 (75%)	42 (81%)
Thyroid gland	(60)	(60)	(60)
Amyloid deposition	19 (32%)	48 (80%)	48 (80%)
Inflammation, chronic			1 (2%)
General Body System			
None			
Genital System			
Preputial gland	(60)	(59)	(58)
Cyst			1 (2%)
Inflammation, suppurative	5 (8%)	3 (5%)	1 (2%)
Seminal vesicle	(60)	(59)	(60)
Fibrosis	1 (2%)		
Testes	(60)	(60)	(60)
Edema	1 (2%)		
Germinal epithelium, atrophy	1 (2%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam (continued)

	0 ppm	2,500 ppm	5,000 ppm
Hematopoietic System			
Lymph node	(7)	(7)	(4)
Inguinal, hyperplasia, lymphoid		1 (14%)	
Mediastinal, hyperplasia, lymphoid		1 (14%)	
Pancreatic, inflammation, granulomatous		1 (14%)	
Renal, hyperplasia, lymphoid	2 (29%)		
Lymph node, mandibular	(57)	(56)	(55)
Amyloid deposition	8 (14%)	29 (52%)	29 (53%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	
Lymph node, mesenteric	(59)	(55)	(57)
Amyloid deposition	25 (42%)	36 (65%)	35 (61%)
Cyst	2 (3%)		1 (2%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	2 (4%)
Infiltration cellular, plasma cell			1 (2%)
Necrosis		1 (2%)	
Spleen	(60)	(60)	(60)
Amyloid deposition	16 (27%)	32 (53%)	30 (50%)
Hematopoietic cell proliferation	3 (5%)	2 (3%)	
Hyperplasia, lymphoid	7 (12%)	4 (7%)	3 (5%)
Necrosis			1 (2%)
Capsule, fibrosis		1 (2%)	
Thymus	(59)	(57)	(51)
Amyloid deposition			1 (2%)
Vein, thrombosis			1 (2%)
Integumentary System			
Skin	(60)	(60)	(59)
Edema	1 (2%)		
Hyperkeratosis	2 (3%)		
Ulcer	3 (5%)	1 (2%)	
Musculoskeletal System			
None			
Nervous System			
Peripheral nerve	(7)	(7)	(9)
Axon, degeneration	2 (29%)	1 (14%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam (continued)

	0 ppm	2,500 ppm	5,000 ppm
Respiratory System			
Lung	(60)	(60)	(60)
Fibrosis		27 (45%)	25 (42%)
Inflammation, chronic	6 (10%)	3 (5%)	2 (3%)
Inflammation, granulomatous	1 (2%)		
Inflammation, subacute	1 (2%)	2 (3%)	11 (18%)
Thrombosis	1 (2%)	1 (2%)	1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	4 (7%)	3 (5%)
Alveolus, infiltration cellular, mononuclear cell	1 (2%)	31 (52%)	37 (62%)
Alveolus, metaplasia, osseous	1 (2%)		
Alveolus, metaplasia, squamous			1 (2%)
Special Senses System			
Ear	(4)	(1)	(6)
External ear, ulcer	1 (25%)		
Eye	(2)	(1)	(1)
Degeneration			1 (100%)
Urinary System			
Kidney	(60)	(60)	(60)
Cyst			1 (2%)
Hydronephrosis	1 (2%)		
Infarct	2 (3%)	1 (2%)	1 (2%)
Inflammation, granulomatous	2 (3%)		
Inflammation, suppurative		1 (2%)	1 (2%)
Artery, thrombosis		1 (2%)	
Glomerulus, amyloid deposition	42 (70%)	35 (58%)	42 (70%)
Interstitium, amyloid deposition	3 (5%)	14 (23%)	10 (17%)
Renal tubule, necrosis		1 (2%)	
Urethra	(1)		
Transitional epithelium, hyperplasia	1 (100%)		
Urinary bladder	(60)	(59)	(59)
Calculus gross observation			1 (2%)
Calculus micro observation only			1 (2%)
Hemorrhage		1 (2%)	
Inflammation, suppurative			2 (3%)
Transitional epithelium, hyperplasia		1 (2%)	2 (3%)

[a] Number of animals examined microscopically at site and number of animals with lesion

APPENDIX B
SUMMARY OF LESIONS IN FEMALE SWISS-WEBSTER MICE IN THE 57-WEEK FEED STUDY OF OXAZEPAM

TABLE B1
Summary of the Incidence of Neoplasms in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

	0 ppm	2,500 ppm	5,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
Early deaths			
Moribund	9	13	11
Natural deaths	4	18	31
Survivors			
Died last week of study		1	
Terminal sacrifice	47	27	17
Missing		1	1
Animals examined microscopically	60	59	59
Alimentary System			
Gallbladder	(60)	(58)	(56)
Intestine large, cecum	(58)	(56)	(51)
Intestine small, duodenum	(59)	(56)	(56)
Adenoma		1 (2%)	
Intestine small, jejunum	(59)	(59)	(56)
Carcinoma			1 (2%)
Liver	(60)	(59)	(59)
Hepatocellular carcinoma	1 (2%)	1 (2%)	6 (10%)
Hepatocellular carcinoma, multiple			5 (8%)
Hepatocellular adenoma		9 (15%)	8 (14%)
Hepatocellular adenoma, multiple		13 (22%)	39 (66%)
Histiocytic sarcoma			1 (2%)
Pancreas	(60)	(59)	(59)
Salivary glands	(60)	(59)	(59)
Stomach, glandular	(59)	(59)	(57)
Tooth	(2)		(2)
Adamantinoma NOS	1 (50%)		
Cardiovascular System			
Heart	(60)	(59)	(59)
Endocrine System			
Adrenal cortex	(60)	(59)	(59)
Adenoma		1 (2%)	
Adrenal medulla	(59)	(58)	(57)
Pituitary gland	(57)	(58)	(58)
Thyroid gland	(60)	(59)	(59)
Follicular cell, adenoma	1 (2%)	1 (2%)	
General Body System			
None			

TABLE B1
Summary of the Incidence of Neoplasms in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Genital System			
Ovary	(59)	(58)	(55)
Hemangioma	1 (2%)		1 (2%)
Uterus	(60)	(59)	(57)
Polyp stromal	1 (2%)		1 (2%)
Sarcoma stromal	1 (2%)		
Hematopoietic System			
Bone marrow	(60)	(59)	(59)
Lymph node	(8)	(10)	(7)
Pancreatic, histiocytic sarcoma			1 (14%)
Lymph node, mandibular	(58)	(57)	(58)
Histiocytic sarcoma			1 (2%)
Lymph node, mesenteric	(60)	(57)	(56)
Histiocytic sarcoma			1 (2%)
Spleen	(60)	(59)	(59)
Histiocytic sarcoma			1 (2%)
Thymus	(56)	(57)	(55)
Histiocytic sarcoma			1 (2%)
Integumentary System			
None			
Musculoskeletal System			
Skeletal muscle	(60)	(59)	(59)
Sarcoma stromal, metastatic, uterus	1 (2%)		
Nervous System			
Brain	(60)	(59)	(59)
Respiratory System			
Lung	(60)	(59)	(59)
Alveolar/bronchiolar adenoma	6 (10%)	4 (7%)	6 (10%)
Alveolar/bronchiolar carcinoma	5 (8%)	3 (5%)	1 (2%)
Nose	(60)	(59)	(59)
Special Senses System			
None			
Urinary System			
Kidney	(60)	(59)	(59)
Urinary bladder	(59)	(59)	(59)

TABLE B1
Summary of the Incidence of Neoplasms in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Systemic Lesions			
Multiple organs[b]	(60)	(59)	(59)
Histiocytic sarcoma			1 (2%)
Lymphoma malignant lymphocytic		1 (2%)	
Lymphoma malignant mixed	6 (10%)	8 (14%)	1 (2%)
Lymphoma malignant undifferentiated cell	3 (5%)	4 (7%)	5 (8%)
Neoplasm Summary			
Total animals with primary neoplasms[c]	24	37	49
Total primary neoplasms	26	46	75
Total animals with benign neoplasms	9	26	47
Total benign neoplasms	9	29	55
Total animals with malignant neoplasms	16	16	17
Total malignant neoplasms	16	17	20
Total animals with metastatic neoplasms	1		
Total metastatic neoplasm	1		
Total animals with neoplasms of uncertain origin 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 B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm

Number of Days on Study	1 8 4	1 9 3	2 7 2	3 0 6	3 2 2	3 5 8	3 6 1	3 6 2	3 7 2	3 7 2	3 7 5	3 9 2	3 9 6	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	0 0 9	0 3 8	0 4 7	0 6 0	0 1 0	0 3 2	0 2 3	0 1 9	0 0 3	0 1 5	0 4 2	0 5 6	0 1 8	0 0 1	0 0 2	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 1 1	0 1 2	0 1 3	0 1 4	0 1 6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Mesentery											+								+						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth											+									+					
Adamantinoma NOS											X														
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																						X			
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Hemangioma																									X
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal					X																				
Sarcoma stromal											X														

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	0 1 7	0 2 0	0 2 1	0 2 2	0 2 4	0 2 5	0 2 6	0 2 7	0 2 8	0 2 9	0 3 0	0 3 1	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 9	0 4 0	0 4 1	0 4 3	0 4 4	0 4 5	0 4 6	0 4 8
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Mesentery									+																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Adamantinoma NOS																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+
Pituitary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																									
Sarcoma stromal																									

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	0 4 9	0 5 0	0 5 1	0 5 2	0 5 3	0 5 4	0 5 5	0 5 7	0 5 8	0 5 9	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	60
Gallbladder	+	+	+	+	+	+	+	+	+	+	60
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	59
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	59
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	58
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	58
Liver	+	+	+	+	+	+	+	+	+	+	60
Hepatocellular carcinoma							X				1
Mesentery											3
Pancreas	+	+	+	+	+	+	+	+	+	+	60
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	59
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	59
Tooth											2
Adamantinoma NOS											1
Cardiovascular System											
Blood vessel	+	+	+	+	+	+	+	+	+	+	60
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	60
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	59
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	60
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	55
Pituitary gland	+	+	+	+	+	+	+	+	+	M	57
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
Follicular cell, adenoma											1
General Body System											
None											
Genital System											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	60
Ovary	+	+	+	+	+	+	+	+	+	+	59
Hemangioma											1
Uterus	+	+	+	+	+	+	+	+	+	+	60
Polyp stromal											1
Sarcoma stromal											1

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	1 8 4	1 9 3	2 7 2	3 0 6	3 2 2	3 5 8	3 6 1	3 6 2	3 7 2	3 7 2	3 7 5	3 9 2	3 9 6	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	0 0 1 9	0 3 8	0 4 7	0 6 0	0 1 0	0 3 2	0 2 3	0 1 9	0 0 3	0 1 5	0 4 2	0 5 6	0 1 8	0 0 1	0 0 2	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 1 1	0 1 2	0 1 3	0 1 4	0 1 6
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+		+	+	+		+		+		+												
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal, metastatic, uterus											X														
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve				+	+		+			+															
Spinal cord					+		+																		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																X									
Alveolar/bronchiolar carcinoma																				X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear							+																		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed	X	X			X	X						X	X												
Lymphoma malignant undifferentiated cell type			X				X		X																

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	0 1 7	0 2 0	0 2 1	0 2 2	0 2 4	0 2 5	0 2 6	0 2 7	0 2 8	0 2 9	0 3 0	0 3 1	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 9	0 4 0	0 4 1	0 4 3	0 4 4	0 4 5	0 4 6	0 4 8
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal, metastatic, uterus																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																									
Spinal cord																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma	X											X				X									
Alveolar/bronchiolar carcinoma			X	X						X										X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																									
Lymphoma malignant undifferentiated cell type																									

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 0 ppm
(continued)

Number of Days on Study	3 3 3 3 3 3 3 3 3 3 9 9 9 9 9 9 9 9 9 9 7 7 7 7 7 7 7 7 7 7	
Carcass ID Number	0 0 0 0 0 0 0 0 0 0 4 5 5 5 5 5 5 5 5 5 9 0 1 2 3 4 5 7 8 9	Total Tissues/ Tumors
Hematopoietic System		
Bone marrow	+ + + + + + + + + +	60
Lymph node		8
Lymph node, mandibular	+ + M + + + + + + +	58
Lymph node, mesenteric	+ + + + + + + + + +	60
Spleen	+ + + + + + + + + +	60
Thymus	+ + + + + + M + + M	56
Integumentary System		
Mammary gland	+ + + + + + + + + +	58
Skin	+ + + + + + + + + +	60
Musculoskeletal System		
Bone	+ + + + + + + + + +	60
Skeletal muscle	+ + + + + + + + + +	60
Sarcoma stromal, metastatic, uterus		1
Nervous System		
Brain	+ + + + + + + + + +	60
Peripheral nerve		4
Spinal cord		2
Respiratory System		
Lung	+ + + + + + + + + +	60
Alveolar/bronchiolar adenoma	X X	6
Alveolar/bronchiolar carcinoma		5
Nose	+ + + + + + + + + +	60
Trachea	+ + + + + + + + + +	60
Special Senses System		
Ear		1
Urinary System		
Kidney	+ + + + + + + + + +	60
Urinary bladder	+ + + + + + + + + +	59
Systemic Lesions		
Multiple organs	+ + + + + + + + + +	60
Lymphoma malignant mixed		6
Lymphoma malignant undifferentiated cell type		3

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm

Number of Days on Study	0 1 1 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 1 0 6 3 5 8 9 9 9 0 1 2 2 2 2 2 2 3 3 4 4 5 6 6 7 6 0 9 6 1 1 4 4 9 5 0 0 1 2 6 6 8 0 6 4 9 0 3 7
Carcass ID Number	1 1 0 1 0 0 1 0 0 0 1 0 1 1 0 0 1 0 1 0 1 0 1 0 0 0 1 6 1 7 7 1 6 8 8 1 7 0 0 9 6 1 7 0 8 1 8 0 6 8 0 2 7 6 2 5 8 8 6 3 0 7 2 4 0 6 1 1 3 9 5 7 7 2 4
Alimentary System	
Esophagus	+ +
Gallbladder	+ + + + + + + + + + + + + + + M + + + + + + + + +
Intestine large, colon	+ + + + + A + + + + + + + + + + + + + + + + + + +
Intestine large, rectum	+ + + + + A + + + + + + + + + + + + + + + + + + +
Intestine large, cecum	+ + + + + A + + + + + + + + + + + + + + + M + A
Intestine small, duodenum	+ + + + + A + + + + + + + A + + + + M + + + + +
Adenoma	X
Intestine small, jejunum	+ +
Intestine small, ileum	+ + + + + A + + + + A + + + + + + + + + + + + +
Liver	+ +
Hepatocellular carcinoma	
Hepatocellular adenoma	X X X X
Hepatocellular adenoma, multiple	X X X X
Mesentery	
Pancreas	+ +
Salivary glands	+ +
Stomach, forestomach	+ +
Stomach, glandular	+ +
Cardiovascular System	
Blood vessel	+ +
Heart	+ +
Endocrine System	
Adrenal cortex	+ +
Adenoma	
Adrenal medulla	+ +
Islets, pancreatic	+ +
Parathyroid gland	+ + + + + + + M M + M M + M + + M + + M + + +
Pituitary gland	+ + + + + M + + + + + + + + + + + + + + + + + + +
Thyroid gland	+ +
Follicular cell, adenoma	
General Body System	
None	
Genital System	
Clitoral gland	+ +
Ovary	+ M + + + +
Uterus	+ +

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	3 7 2	3 7 4	3 7 6	3 8 9	3 9 2	3 9 3	3 9 4	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	0 6 9	0 9 1	0 9 4	0 9 3	0 7 9	1 1 7	0 9 6	0 6 1	0 6 3	0 6 4	0 6 5	0 7 0	0 7 3	0 7 4	0 7 6	0 7 8	0 8 0	0 8 1	0 8 2	0 8 5	0 8 8	0 9 2	0 9 5	0 9 7	0 9 8
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+		+	+	+	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma								X																	
Hepatocellular adenoma		X														X				X					
Hepatocellular adenoma, multiple				X		X					X		X		X										X
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									X
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	0 9 9	1 0 1	1 0 5	1 0 6	1 0 8	1 0 9	1 1 3	1 1 4	1 1 9	1 2 0	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	59
Gallbladder	+	+	+	+	+	+	+	+	+	+	58
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	58
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	56
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	56
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	56
Adenoma											1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	56
Liver	+	+	+	+	+	+	+	+	+	+	59
Hepatocellular carcinoma											1
Hepatocellular adenoma				X			X				9
Hepatocellular adenoma, multiple			X					X			13
Mesentery										+	1
Pancreas	+	+	+	+	+	+	+	+	+	+	59
Salivary glands	+	+	+	+	+	+	+	+	+	+	59
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	59
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	59
Cardiovascular System											
Blood vessel	+	+	+	+	+	+	+	+	+	+	59
Heart	+	+	+	+	+	+	+	+	+	+	59
Endocrine System											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	59
Adenoma											1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	58
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	59
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	58
Thyroid gland	+	+	+	+	+	+	+	+	+	+	59
Follicular cell, adenoma									X		1
General Body System											
None											
Genital System											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	58
Ovary	+	+	+	+	+	+	+	+	+	+	58
Uterus	+	+	+	+	+	+	+	+	+	+	59

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	0 1 1 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
	1 0 6 3 5 8 9 9 9 0 1 2 2 2 2 2 2 3 3 4 4 5 6 6
	7 6 0 9 6 1 1 4 4 9 5 0 0 1 2 6 6 8 0 6 4 9 0 3 7
Carcass ID Number	1 1 0 1 0 0 1 0 0 0 1 0 1 1 0 0 1 0 1 0 1 0 1 0 0
	0 1 6 1 7 7 1 6 8 8 1 7 0 0 9 6 1 7 0 8 1 8 0 6 8
	0 2 7 6 2 5 8 8 6 3 0 7 2 4 0 6 1 1 3 9 5 7 7 2 4
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ +
Lymph node, mandibular	+ +
Lymph node, mesenteric	+ +
Spleen	+ +
Thymus	+ M +
Integumentary System	
Mammary gland	+ + M + + + + + + + + + + + + + M M + + + + + M M
Skin	+ +
Musculoskeletal System	
Bone	+ +
Skeletal muscle	+ +
Nervous System	
Brain	+ +
Peripheral nerve	+ + + + +
Spinal cord	+ + + + +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X
Alveolar/bronchiolar carcinoma	X
Nose	+ +
Trachea	+ +
Special Senses System	
Ear	+
Urinary System	
Kidney	+ +
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Lymphoma malignant lymphocytic	X
Lymphoma malignant mixed	X X X X X X X X
Lymphoma malignant undifferentiated cell type	X X X

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	3 7 2	3 7 4	3 7 6	3 8 9	3 9 2	3 9 3	3 9 4	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	0 6 9	0 9 1	0 9 4	0 9 3	0 7 9	1 1 7	0 9 6	0 6 1	0 6 3	0 6 4	0 6 5	0 7 0	0 7 3	0 7 4	0 7 6	0 7 8	0 8 0	0 8 1	0 8 2	0 8 5	0 8 8	0 9 2	0 9 5	0 9 7	0 9 8
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																				+						
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve							+																			
Spinal cord							+																			
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X			X												
Alveolar/bronchiolar carcinoma									X			X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Ear																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																										
Lymphoma malignant mixed																										
Lymphoma malignant undifferentiated cell type																				X						

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	0 9 9	1 0 1	1 0 5	1 0 6	1 0 8	1 0 9	1 1 3	1 1 4	1 1 9	1 2 0	Total Tissues/ Tumors
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	59
Lymph node											10
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	57
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	57
Spleen	+	+	+	+	+	+	+	+	+	+	59
Thymus	+	+	+	+	+	+	+	+	+	+	57
Integumentary System											
Mammary gland	+	+	M	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	59
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	59
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	59
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	59
Peripheral nerve											5
Spinal cord											6
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	59
Alveolar/bronchiolar adenoma					X						4
Alveolar/bronchiolar carcinoma											3
Nose	+	+	+	+	+	+	+	+	+	+	59
Trachea	+	+	+	+	+	+	+	+	+	+	59
Special Senses System											
Ear											1
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	59
Urinary bladder	+	+	+	+	+	+	+	+	+	+	59
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	59
Lymphoma malignant lymphocytic											1
Lymphoma malignant mixed					X						8
Lymphoma malignant undifferentiated cell type											4

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm

Number of Days on Study	111	197	284	294	295	305	306	314	316	318	320	321	321	325	325	327	328	336	337	337	339	340	342	349	350
Carcass ID Number	156	158	132	169	172	145	121	153	144	146	179	137	142	123	141	161	152	160	128	165	130	171	175	162	127
Alimentary System																									
Esophagus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder		+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine large, colon		+	A	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum		+	A	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum		+	A	+	+	M	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	A	+	+
Intestine small, duodenum		+	A	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum		+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum		+	A	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+
Liver		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																				X			X		
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma			X						X			X					X		X						
Hepatocellular adenoma, multiple					X						X		X	X	X			X		X	X	X	X		X
Histiocytic sarcoma															X										
Mesentery																									
Pancreas		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach		+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	
Stomach, glandular		+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	
Tooth							+												+						
Cardiovascular System																									
Blood vessel		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Heart		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla		+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland		+	+	+	M	+	M	M	+	+	+	+	+	M	+	+	M	M	M	+	M	M	M	+	M
Pituitary gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Clitoral gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Ovary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+
Hemangioma																									
Uterus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+
Polyp stromal																									

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	3 5 0	3 5 5	3 5 7	3 6 0	3 6 3	3 6 4	3 6 5	3 6 6	3 7 5	3 7 6	3 7 6	3 7 6	3 7 9	3 8 1	3 8 1	3 8 5	3 9 0	3 9 5	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	1 3 4	1 4 8	1 5 7	1 2 2	1 5 9	1 7 3	1 6 4	1 4 7	1 2 5	1 4 0	1 5 1	1 5 4	1 2 9	1 3 1	1 6 7	1 6 6	1 3 5	1 5 5	1 2 4	1 2 6	1 3 3	1 3 6	1 3 8	1 3 9	1 4 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Carcinoma																				X					
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																			X		X				
Hepatocellular carcinoma, multiple												X										X		X	
Hepatocellular adenoma					X													X							X
Hepatocellular adenoma, multiple	X	X		X		X	X	X	X	X	X	X	X		X	X	X		X	X	X	X	X	X	
Histiocytic sarcoma																									
Mesentery																	+								+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Hemangioma									X																
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																			X						

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	
Carcass ID Number	1 4 9	1 5 0	1 6 3	1 6 8	1 7 0	1 7 4	1 7 6	1 7 7	1 7 8	1 8 0	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	59
Gallbladder	+	+	+	+	+	+	+	+	+	+	56
Intestine large, colon	+	+	+	+	+	+	M	+	+	+	55
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	56
Intestine large, cecum	+	+	+	M	+	+	+	+	+	+	51
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	56
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	56
Carcinoma											1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	54
Liver	+	+	+	+	+	+	+	+	+	+	59
Hepatocellular carcinoma	X	X									6
Hepatocellular carcinoma, multiple						X			X		5
Hepatocellular adenoma											8
Hepatocellular adenoma, multiple	X	X	X	X	X	X		X	X		39
Histiocytic sarcoma											1
Mesentery							+				3
Pancreas	+	+	+	+	+	+	+	+	+	+	59
Salivary glands	+	+	+	+	+	+	+	+	+	+	59
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	57
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	57
Tooth											2
Cardiovascular System											
Blood vessel	+	+	+	+	+	+	+	+	+	+	58
Heart	+	+	+	+	+	+	+	+	+	+	59
Endocrine System											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	59
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	57
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	59
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	58
Thyroid gland	+	+	+	+	+	+	+	+	+	+	59
General Body System											
None											
Genital System											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	57
Ovary	+	+	+	+	+	+	+	+	+	+	55
Hemangioma											1
Uterus	+	+	+	+	+	+	+	+	+	+	57
Polyp stromal											1

Table B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	117	197	284	294	295	305	306	314	316	318	320	321	321	325	325	327	328	336	337	337	339	340	342	349	350
Carcass ID Number	156	158	132	169	172	145	121	153	144	146	179	137	142	123	141	161	152	160	128	165	130	171	175	162	127
Hematopoietic System																									
Bone marrow		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+		+										+	+										
Pancreatic, histiocytic sarcoma															X										
Lymph node, mandibular		+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Lymph node, mesenteric		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Spleen		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Thymus		+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Integumentary System																									
Mammary gland		+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	M	+	+	M	M	+	+	+	M
Skin		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve														+								+			
Spinal cord														+								+			+
Respiratory System																									
Lung		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X	X										
Alveolar/bronchiolar carcinoma																							X		
Nose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear									+							+									
Eye																									
Urinary System																									
Kidney		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Lymphoma malignant mixed																									
Lymphoma malignant undifferentiated cell type		X		X					X					X											

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	3 5 0	3 5 5	3 5 7	3 6 0	3 6 3	3 6 4	3 6 5	3 6 6	3 7 5	3 7 6	3 7 6	3 7 6	3 7 9	3 8 1	3 8 1	3 8 5	3 9 0	3 9 5	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7	3 9 7
Carcass ID Number	1 3 4	1 4 8	1 5 7	1 2 2	1 5 9	1 7 3	1 6 4	1 4 7	1 2 5	1 4 0	1 5 1	1 5 4	1 2 9	1 3 1	1 6 7	1 6 6	1 3 5	1 5 5	1 2 4	1 2 6	1 3 3	1 3 6	1 3 8	1 3 9	1 4 3
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node												+										+		+	
Pancreatic, histiocytic sarcoma																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Histiocytic sarcoma																									
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	M	M	+	M	M	+	+	+	M	+	+	+	+	+	M	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve									+			+					+								
Spinal cord									+			+					+								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																X		X							
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye																								+	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymphoma malignant mixed																						X			
Lymphoma malignant undifferentiated cell type												X													

TABLE B2
Individual Animal Tumor Pathology of Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	3 3 3 3 3 3 3 3 3 3 9 9 9 9 9 9 9 9 9 9 7 7 7 7 7 7 7 7 7 7	
Carcass ID Number	1 1 1 1 1 1 1 1 1 1 4 5 6 6 7 7 7 7 7 8 9 0 3 8 0 4 6 7 8 0	Total Tissues/ Tumors
Hematopoietic System		
Bone marrow	+ + + + + + + + + +	59
Lymph node		7
Pancreatic, histiocytic sarcoma		1
Lymph node, mandibular	+ + + + + + + + + +	58
Histiocytic sarcoma		1
Lymph node, mesenteric	+ + + + M + + + M M	56
Histiocytic sarcoma		1
Spleen	+ + + + + + + + + +	59
Histiocytic sarcoma		1
Thymus	+ + + + + + + + + +	55
Histiocytic sarcoma		1
Integumentary System		
Mammary gland	M + + + M + + + + +	44
Skin	+ + + + + + + + + +	59
Musculoskeletal System		
Bone	+ + + + + + + + + +	59
Skeletal muscle	+ + + + + + + + + +	59
Nervous System		
Brain	+ + + + + + + + + +	59
Peripheral nerve		5
Spinal cord		6
Respiratory System		
Lung	+ + + + + + + + + +	59
Alveolar/bronchiolar adenoma	X X	6
Alveolar/bronchiolar carcinoma		1
Nose	+ + + + + + + + + +	59
Trachea	+ + + + + + + + + +	59
Special Senses System		
Ear		2
Eye		1
Urinary System		
Kidney	+ + + + + + + + + +	59
Urinary bladder	+ + + + + + + + + +	59
Systemic Lesions		
Multiple organs	+ + + + + + + + + +	59
Histiocytic sarcoma		1
Lymphoma malignant mixed		1
Lymphoma malignant undifferentiated cell type		5

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

	0 ppm	2,500 ppm	5,000 ppm
Liver: Hepatocellular Adenoma			
Overall rate[a]	0/60 (0%)	22/59 (37%)	47/59 (80%)
Adjusted rate[b]	0.0%	52.6%	95.7%
Terminal rate[c]	0/47 (0%)	10/28 (36%)	15/17 (88%)
First incidence (days)	–[e]	291	284
Life table test[d]	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test[d]		P<0.001	P<0.001
Liver: Hepatocellular Carcinoma			
Overall rate	1/60 (2%)	1/59 (2%)	11/59 (19%)
Adjusted rate	2.1%	3.6%	51.6%
Terminal rate	1/47 (2%)	1/28 (4%)	8/17 (47%)
First incidence (days)	397 (T)	397 (T)	337
Life table test	P<0.001	P=0.642	P<0.001
Logistic regression test	P<0.001	P=0.642	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.748	P=0.002
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	1/60 (2%)	23/59 (39%)	47/59 (80%)
Adjusted rate	2.1%	55.2%	95.7%
Terminal rate	1/47 (2%)	11/28 (39%)	15/17 (88%)
First incidence (days)	397 (T)	291	284
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	6/60 (10%)	4/59 (7%)	6/59 (10%)
Adjusted rate	12.8%	12.4%	24.2%
Terminal rate	6/47 (13%)	3/28 (11%)	2/17 (12%)
First incidence (days)	397 (T)	294	325
Life table test	P=0.103	P=0.593	P=0.105
Logistic regression test	P=0.385	P=0.515N	P=0.393
Cochran-Armitage test	P=0.552		
Fisher exact test		P=0.382N	P=0.607
Lung: Alveolar/bronchiolar Carcinoma			
Overall rate	5/60 (8%)	3/59 (5%)	1/59 (2%)
Adjusted rate	10.6%	8.9%	2.6%
Terminal rate	5/47 (11%)	2/28 (7%)	0/17 (0%)
First incidence (days)	397 (T)	294	342
Life table test	P=0.317N	P=0.620N	P=0.413N
Logistic regression test	P=0.111N	P=0.458N	P=0.213N
Cochran-Armitage test	P=0.074N		
Fisher exact test		P=0.368N	P=0.107N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	11/60 (18%)	6/59 (10%)	7/59 (12%)
Adjusted rate	23.4%	19.4%	26.2%
Terminal rate	11/47 (23%)	5/28 (18%)	2/17 (12%)
First incidence (days)	397 (T)	294	325
Life table test	P=0.266	P=0.510N	P=0.259
Logistic regression test	P=0.407N	P=0.346N	P=0.506N
Cochran-Armitage test	P=0.183N		
Fisher exact test		P=0.156N	P=0.234N
All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rate	9/60 (15%)	13/59 (22%)	6/59 (10%)
Adjusted rate	15.4%	26.2%	16.1%
Terminal rate	0/47 (0%)	2/28 (7%)	1/17 (6%)
First incidence (days)	184	106	197
Life table test	P=0.543	P=0.117	P=0.581N
Logistic regression test	P=0.010N	P=0.260N	P=0.036N
Cochran-Armitage test	P=0.278N		
Fisher exact test		P=0.226	P=0.303N
All Organs: Malignant Lymphoma or Histiocytic Sarcoma			
Overall rate	9/60 (15%)	13/59 (22%)	7/59 (12%)
Adjusted rate	15.4%	26.2%	17.9%
Terminal rate	0/47 (0%)	2/28 (7%)	1/17 (6%)
First incidence (days)	184	106	197
Life table test	P=0.447	P=0.117	P=0.516
Logistic regression test	P=0.017N	P=0.260N	P=0.056N
Cochran-Armitage test	P=0.370N		
Fisher exact test		P=0.226	P=0.409N
All Organs: Benign Neoplasms			
Overall rate	9/60 (15%)	26/59 (44%)	47/59 (80%)
Adjusted rate	18.5%	61.2%	95.7%
Terminal rate	8/47 (17%)	13/28 (46%)	15/17 (88%)
First incidence (days)	322	291	284
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
All Organs: Malignant Neoplasms			
Overall rate	16/60 (27%)	16/59 (27%)	17/59 (29%)
Adjusted rate	27.7%	34.7%	61.0%
Terminal rate	6/47 (13%)	5/28 (18%)	9/17 (53%)
First incidence (days)	184	106	197
Life table test	P=0.037	P=0.230	P=0.025
Logistic regression test	P=0.390N	P=0.236N	P=0.427N
Cochran-Armitage test	P=0.437		
Fisher exact test		P=0.560	P=0.477

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam
(continued)

	0 ppm	2,500 ppm	5,000 ppm
All Organs: Benign or Malignant Neoplasms			
Overall rate	24/60 (40%)	37/59 (63%)	49/59 (83%)
Adjusted rate	41.8%	73.8%	95.8%
Terminal rate	14/47 (30%)	16/28 (57%)	15/17 (88%)
First incidence (days)	184	106	197
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.037	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.011	P<0.001

(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, 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 control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 B4
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

	0 ppm	2,500 ppm	5,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
Early deaths			
Moribund	9	13	11
Natural deaths	4	18	31
Survivors			
Died last week of study		1	
Terminal sacrifice	47	27	17
Missing		1	1
Animals examined microscopically	60	59	59
Alimentary System			
Intestine large, colon	(59)	(58)	(55)
Amyloid deposition	6 (10%)	24 (41%)	30 (55%)
Hemorrhage		1 (2%)	
Intestine large, rectum	(59)	(56)	(56)
Amyloid deposition	1 (2%)	4 (7%)	12 (21%)
Intestine large, cecum	(58)	(56)	(51)
Amyloid deposition	6 (10%)	23 (41%)	34 (67%)
Hemorrhage		1 (2%)	
Ulcer		2 (4%)	
Intestine small, duodenum	(59)	(56)	(56)
Amyloid deposition	45 (76%)	40 (71%)	47 (84%)
Ulcer	1 (2%)	2 (4%)	
Intestine small, jejunum	(59)	(59)	(56)
Amyloid deposition	28 (47%)	42 (71%)	49 (88%)
Ulcer	3 (5%)	2 (3%)	
Intestine small, ileum	(58)	(56)	(54)
Amyloid deposition	52 (90%)	43 (77%)	46 (85%)
Liver	(60)	(59)	(59)
Amyloid deposition	28 (47%)	31 (53%)	35 (59%)
Eosinophilic focus		20 (34%)	14 (24%)
Hematopoietic cell proliferation	5 (8%)	4 (7%)	2 (3%)
Hepatodiaphragmatic nodule		1 (2%)	
Infarct			1 (2%)
Inflammation, chronic	1 (2%)		1 (2%)
Inflammation, granulomatous		1 (2%)	
Inflammation, subacute	4 (7%)	4 (7%)	
Necrosis	10 (17%)	8 (14%)	7 (12%)
Centrilobular, hypertrophy	3 (5%)	51 (86%)	53 (90%)
Mesentery	(3)	(1)	(3)
Inflammation, granulomatous	1 (33%)	1 (100%)	1 (33%)
Inflammation, subacute	1 (33%)		
Inflammation, suppurative	1 (33%)		1 (33%)
Fat, necrosis			1 (33%)
Pancreas	(60)	(59)	(59)
Amyloid deposition	1 (2%)	3 (5%)	1 (2%)
Ectopic tissue		1 (2%)	
Salivary glands	(60)	(59)	(59)
Amyloid deposition	35 (58%)	36 (61%)	40 (68%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam (continued)

	0 ppm	2,500 ppm	5,000 ppm
Alimentary System (continued)			
Stomach, forestomach	(59)	(59)	(57)
Amyloid deposition		1 (2%)	3 (5%)
Hemorrhage		1 (2%)	
Hyperkeratosis		1 (2%)	5 (9%)
Stomach, glandular	(59)	(59)	(57)
Amyloid deposition	2 (3%)	17 (29%)	19 (33%)
Mineralization		2 (3%)	3 (5%)
Cardiovascular System			
Blood vessel	(60)	(59)	(58)
Mineralization		1 (2%)	
Heart	(60)	(59)	(59)
Amyloid deposition	50 (83%)	44 (75%)	53 (90%)
Degeneration	1 (2%)		
Atrium, thrombosis	2 (3%)	23 (39%)	31 (53%)
Coronary artery, inflammation, chronic		1 (2%)	
Valve, inflammation, chronic		1 (2%)	
Valve, thrombosis	1 (2%)		
Endocrine System			
Adrenal cortex	(60)	(59)	(59)
Amyloid deposition	24 (40%)	32 (54%)	41 (69%)
Hematopoietic cell proliferation	2 (3%)	2 (3%)	3 (5%)
Infiltration cellular, mononuclear cell		1 (2%)	
Capsule, hyperplasia			1 (2%)
Adrenal medulla	(59)	(58)	(57)
Hyperplasia	1 (2%)	1 (2%)	
Parathyroid gland	(55)	(50)	(45)
Amyloid deposition	24 (44%)	32 (64%)	33 (73%)
Thyroid gland	(60)	(59)	(59)
Amyloid deposition	22 (37%)	38 (64%)	46 (78%)
Follicular cell, hyperplasia	1 (2%)		
General Body System			
None			
Genital System			
Clitoral gland	(60)	(58)	(57)
Inflammation, suppurative	1 (2%)		2 (4%)
Ovary	(59)	(58)	(55)
Amyloid deposition	24 (41%)	13 (22%)	15 (27%)
Angiectasis	1 (2%)		1 (2%)
Cyst	7 (12%)	5 (9%)	1 (2%)
Arteriole, inflammation, chronic	3 (5%)	4 (7%)	4 (7%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam (continued)

	0 ppm	2,500 ppm	5,000 ppm
Genital System (continued)			
Uterus	(60)	(59)	(57)
Amyloid deposition	26 (43%)	28 (47%)	40 (70%)
Dilatation			1 (2%)
Infiltration cellular, polymorphonuclear	1 (2%)		
Inflammation, suppurative	1 (2%)		
Thrombosis	1 (2%)		
Endometrium, hyperplasia, cystic	13 (22%)	2 (3%)	
Hematopoietic System			
Lymph node	(8)	(10)	(7)
Axillary, hematopoietic cell proliferation		1 (10%)	
Inguinal, hyperplasia, lymphoid	1 (13%)		1 (14%)
Mediastinal, hematopoietic cell proliferation		1 (10%)	
Renal, hematopoietic cell proliferation		1 (10%)	
Lymph node, mandibular	(58)	(57)	(58)
Amyloid deposition	3 (5%)	23 (40%)	35 (60%)
Hematopoietic cell proliferation		1 (2%)	
Infiltration cellular, plasma cell	1 (2%)		
Lymph node, mesenteric	(60)	(57)	(56)
Amyloid deposition	16 (27%)	30 (53%)	35 (63%)
Cyst	1 (2%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation		1 (2%)	
Hyperplasia, lymphoid	5 (8%)	6 (11%)	
Inflammation, suppurative	1 (2%)		
Spleen	(60)	(59)	(59)
Amyloid deposition	12 (20%)	20 (34%)	39 (66%)
Fibrosis			1 (2%)
Hematopoietic cell proliferation	5 (8%)	4 (7%)	2 (3%)
Hyperplasia, lymphoid	14 (23%)	5 (8%)	1 (2%)
Thymus	(56)	(57)	(55)
Amyloid deposition		1 (2%)	
Hyperplasia, lymphoid	1 (2%)		
Integumentary System			
Skin	(60)	(59)	(59)
Hyperkeratosis	1 (2%)	1 (2%)	1 (2%)
Ulcer	2 (3%)		
Musculoskeletal System			
Skeletal muscle	(60)	(59)	(59)
Hemorrhage			1 (2%)
Inflammation, chronic	1 (2%)		
Nervous System			
Peripheral nerve	(4)	(5)	(5)
Axon, degeneration	2 (50%)	1 (20%)	

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam (continued)

	0 ppm	2,500 ppm	5,000 ppm
Respiratory System			
Lung	(60)	(59)	(59)
Fibrosis	1 (2%)	14 (24%)	8 (14%)
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)	
Inflammation, chronic	3 (5%)	2 (3%)	6 (10%)
Inflammation, granulomatous			1 (2%)
Inflammation, subacute	4 (7%)	4 (7%)	3 (5%)
Thrombosis		1 (2%)	
Alveolar epithelium, hyperplasia	6 (10%)	3 (5%)	2 (3%)
Alveolus, infiltration cellular, mononuclear cell	2 (3%)	16 (27%)	26 (44%)
Nose	(60)	(59)	(59)
Amyloid deposition			1 (2%)
Inflammation, suppurative			1 (2%)
Special Senses System			
None			
Urinary System			
Kidney	(60)	(59)	(59)
Cyst		1 (2%)	
Infarct		1 (2%)	
Necrosis			1 (2%)
Glomerulus, amyloid deposition	48 (80%)	39 (66%)	39 (66%)
Interstitium, amyloid deposition	5 (8%)	5 (8%)	7 (12%)

[a] Number of animals examined microscopically at site and number of animals with lesion

APPENDIX C
SUMMARY OF LESIONS IN MALE B6C3F$_1$ MICE IN THE 2-YEAR FEED STUDY OF OXAZEPAM

TABLE C1
Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	2	4	22	30
Natural deaths	3	2	13	20
Survivors				
Terminal sacrifice	45	44	15	
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatoblastoma				1 (10%)
Hepatocellular carcinoma		1 (10%)	4 (40%)	1 (10%)
Hepatocellular carcinoma, multiple				8 (80%)
Hepatocellular adenoma		3 (30%)	1 (10%)	
Hepatocellular adenoma, multiple			8 (80%)	9 (90%)
Cardiovascular System				
None				
Endocrine System				
None				
General Body System				
None				
Genital System				
Testes	(10)	(10)	(10)	(10)
Interstitial cell, adenoma		1 (10%)		
Hematopoietic System				
None				
Integumentary System				
None				
Musculoskeletal System				
None				

TABLE C1
Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500	5,000 ppm
15-Month Interim Evaluation (continued)				
Nervous System				
None				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma	2 (20%)	1 (10%)		
Hepatocellular carcinoma, metastatic, liver				1 (10%)
Special Senses System				
None				
Urinary System				
None				
2-Year Study				
Alimentary System				
Gallbladder	(47)	(50)	(44)	(24)
Histiocytic sarcoma		1 (2%)		
Intestine large, colon	(49)	(50)	(50)	(49)
Intestine small, duodenum	(49)	(50)	(49)	(50)
Polyp adenomatous	1 (2%)			
Intestine small, jejunum	(49)	(50)	(50)	(50)
Carcinoma	1 (2%)	1 (2%)		
Liver	(49)	(50)	(50)	(50)
Hemangiosarcoma, multiple	1 (2%)	1 (2%)		
Hemangiosarcoma, metastatic, skin			1 (2%)	
Hepatoblastoma		2 (4%)	20 (40%)	12 (24%)
Hepatoblastoma, multiple			1 (2%)	1 (2%)
Hepatocellular carcinoma	8 (16%)	3 (6%)	3 (6%)	5 (10%)
Hepatocellular carcinoma, multiple	1 (2%)	2 (4%)	42 (84%)	45 (90%)
Hepatocellular adenoma	10 (20%)	9 (18%)	6 (12%)	5 (10%)
Hepatocellular adenoma, multiple	7 (14%)	9 (18%)	28 (56%)	27 (54%)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Sarcoma, metastatic, kidney			1 (2%)	
Mesentery	(2)	(2)	(2)	(2)
Histiocytic sarcoma	1 (50%)	1 (50%)		
Fat, hepatocellular carcinoma, metastatic, liver			1 (50%)	
Pancreas	(49)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver			1 (2%)	
Histiocytic sarcoma	1 (2%)	1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma	1 (2%)		1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500	5,000 ppm
2-Year Study (continued)				
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(50)
Adenoma			1 (2%)	
Capsule, adenoma	6 (12%)	4 (8%)		
Capsule, hepatoblastoma, metastatic, liver			1 (2%)	
Capsule, histiocytic sarcoma	1 (2%)			
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign		1 (2%)		
Pituitary gland	(48)	(50)	(42)	(37)
Pars distalis, adenoma	1 (2%)			
Thyroid gland	(49)	(50)	(50)	(50)
Follicular cell, adenoma		1 (2%)	1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Prostate	(49)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver			1 (2%)	
Histiocytic sarcoma	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Lymph node	(2)	(1)	(1)	(2)
Lumbar, histiocytic sarcoma	1 (50%)			
Popliteal, histiocytic sarcoma		1 (100%)		
Renal, hepatoblastoma, metastatic, liver			1 (100%)	
Lymph node, mandibular	(44)	(45)	(42)	(42)
Carcinoma, metastatic, harderian gland		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Lymph node, mesenteric	(48)	(49)	(45)	(41)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Sarcoma, metastatic, kidney			1 (2%)	
Lymph node, mediastinal		(1)		(1)
Histiocytic sarcoma		1 (100%)		
Spleen	(49)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Hemangiosarcoma, metastatic, skin			1 (2%)	
Histiocytic sarcoma	1 (2%)	1 (2%)		
Thymus	(43)	(43)	(37)	(31)
Histiocytic sarcoma	1 (2%)	1 (2%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500	5,000 ppm
2-Year Study (continued)				
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, hemangiosarcoma		1 (2%)	1 (2%)	
Subcutaneous tissue, melanoma NOS			1 (2%)	
Musculoskeletal System				
Skeletal muscle	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	9 (18%)	11 (22%)	5 (10%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)	6 (12%)		
Alveolar/bronchiolar carcinoma	2 (4%)	1 (2%)		
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Carcinoma, metastatic, harderian gland		1 (2%)		
Hemangiosarcoma		1 (2%)		
Hepatoblastoma, metastatic, liver		1 (2%)	7 (14%)	5 (10%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	3 (6%)	10 (20%)	16 (32%)
Histiocytic sarcoma		1 (2%)		
Sarcoma, metastatic, kidney			1 (2%)	
Special Senses System				
Ear		(1)		
External ear, fibrosarcoma		1 (100%)		
Eye	(2)	(2)		
Carcinoma, metastatic, harderian gland	1 (50%)	1 (50%)		
Ciliary body, adenoma		1 (50%)		
Harderian gland	(3)	(2)		(1)
Adenoma	1 (33%)	1 (50%)		1 (100%)
Carcinoma	1 (33%)	1 (50%)		
Bilateral, adenoma	1 (33%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Sarcoma			1 (2%)	
Urinary bladder	(49)	(50)	(50)	(49)
Histiocytic sarcoma	1 (2%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500	5,000 ppm
2-Year Study (continued)				
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Lymphoma malignant lymphocytic				1 (2%)
Lymphoma malignant mixed	2 (4%)	1 (2%)	1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]				
15-Month interim evaluation	2	5	9	10
2-Year study	37	35	50	50
Total primary neoplasms				
15-Month interim evaluation	2	6	13	19
2-Year study	57	63	112	99
Total animals with benign neoplasms				
15-Month interim evaluation	2	5	9	9
2-Year study	30	30	37	33
Total benign neoplasms				
15-Month interim evaluation	2	5	9	9
2-Year study	39	43	42	35
Total animals with malignant neoplasms				
15-Month interim evaluation		1	4	10
2-Year study	17	18	47	50
Total malignant neoplasms				
15-Month interim evaluation		1	4	10
2-Year study	18	20	69	64
Total animals with metastatic neoplasms				
15-Month interim evaluation				1
2-Year study	2	4	21	21
Total metastatic neoplasms				
15-Month interim evaluation				1
2-Year study	2	7	27	21
Total animals with uncertain neoplasms - benign or malignant				
2-Year study			1	
Total uncertain neoplasms				
2-Year study			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 C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm

Number of Days on Study	1 1 7	5 8 6	6 2 4	6 2 7	7 0 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 2 1	0 2 0	0 0 3	0 5 3	0 4 8	0 0 1	0 0 2	0 0 5	0 0 6	0 1 1	0 1 2	0 1 9	0 2 6	0 2 8	0 3 0	0 3 2	0 3 4	0 3 5	0 4 5	0 4 9	0 5 2	0 5 5	0 5 8	0 6 0	0 6 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma								X																	
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, multiple							X																		
Hepatocellular carcinoma				X	X													X				X		X
Hepatocellular carcinoma, multiple		X																							
Hepatocellular adenoma																X	X				X			X
Hepatocellular adenoma, multiple						X	X											X				X	X		
Histiocytic sarcoma																						X			
Mesentery																						+			
Histiocytic sarcoma																						X			
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																						X			
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Squamous cell papilloma																					X				
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth													+												
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma						X		X										X							
Capsule, histiocytic sarcoma																							X		
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 0 7	0 0 8	0 1 0	0 1 4	0 1 6	0 1 7	0 2 2	0 2 3	0 2 4	0 2 7	0 3 3	0 3 7	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 6	0 4 7	0 5 0	0 5 1	0 5 4	0 5 6	0 5 7	0 5 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp adenomatous																					X					1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, multiple																										1
Hepatocellular carcinoma				X							X				X											8
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma			X			X										X		X				X			X	10
Hepatocellular adenoma, multiple	X																				X					7
Histiocytic sarcoma																										1
Mesentery								+																		2
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma					X																					1
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Capsule, adenoma	X					X						X														6
Capsule, histiocytic sarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma							X																			1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										

Table C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	117	186	264	267	707	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730
Carcass ID Number	021	020	003	053	048	001	002	005	006	011	012	019	026	028	030	032	034	035	045	049	052	055	058	060	004
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Seminal vesicle	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																							+		
Lumbar, histiocytic sarcoma																							X		
Lymph node, mandibular	M	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+
Histiocytic sarcoma																							X		
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X				X	X									X							X
Alveolar/bronchiolar adenoma, multiple										X															
Alveolar/bronchiolar carcinoma												X													
Hepatocellular carcinoma, metastatic, liver		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye			+		+																				
Carcinoma, metastatic, harderian gland			X																						
Harderian gland			+		+											+									
Adenoma																X									
Carcinoma			X																						
Bilateral, adenoma					X																				

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 0 7	0 0 8	0 1 0	0 1 4	0 1 6	0 1 7	0 2 2	0 2 3	0 2 4	0 2 7	0 3 3	0 3 7	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 6	0 4 7	0 5 0	0 5 1	0 5 4	0 5 6	0 5 7	0 5 9	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+																								2
Lumbar, histiocytic sarcoma																										1
Lymph node, mandibular	+	M	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	44
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Thymus	+	M	+	M	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	M	+	+	43
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X								X						X				X						9
Alveolar/bronchiolar adenoma, multiple											X															2
Alveolar/bronchiolar carcinoma																	X									2
Hepatocellular carcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										2
Carcinoma, metastatic, harderian gland																										1
Harderian gland																										3
Adenoma																										1
Carcinoma																										1
Bilateral, adenoma																										1

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	1	5	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	1	8	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3
	7	6	4	7	7	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	0
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	2	0	5	4	0	0	0	0	1	1	1	2	2	3	3	3	3	4	4	5	5	5	6	0
	1	0	3	3	8	1	2	5	6	1	2	9	6	8	0	2	4	5	5	9	2	5	8	0	4
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Urinary bladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Lymphoma malignant mixed																X									

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 0 7	0 0 8	0 1 0	0 1 4	0 1 6	0 1 7	0 2 2	0 2 3	0 2 4	0 2 7	0 3 3	0 3 7	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 6	0 4 7	0 5 0	0 5 1	0 5 4	0 5 6	0 5 7	0 5 9	Total Tissues/ Tumors
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Histiocytic sarcoma																										1	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Histiocytic sarcoma																										1	
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Histiocytic sarcoma																										1	
Lymphoma malignant mixed																X										2	

TABLE C2
Individual Animal Tumor Pathology of B6C3F₁ Male Mice in the 2-Year Feed Study of Oxazepam: 125 ppm

Number of Days on Study	453	508	527	625	701	715	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	080	110	082	098	064	073	062	069	074	075	076	077	083	084	089	090	091	094	095	096	097	101	102	108	120
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, multiple																									
Hepatoblastoma														X				X							
Hepatocellular carcinoma	X																								
Hepatocellular carcinoma, multiple						X								X											
Hepatocellular adenoma	X				X	X			X			X											X		
Hepatocellular adenoma, multiple			X							X				X	X		X	X							
Histiocytic sarcoma																									
Mesentery																									
Histiocytic sarcoma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																	X								
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																					X				
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 6 1	0 6 3	0 6 5	0 6 6	0 6 7	0 7 1	0 7 8	0 8 1	0 8 5	0 8 8	0 9 2	0 9 3	0 9 9	0 9 0	1 0 3	1 0 4	1 0 5	1 0 7	1 1 2	1 1 3	1 1 4	1 1 5	1 1 6	1 1 7	1 1 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma								X																		1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma			X																							1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, multiple																			X							1
Hepatoblastoma																										2
Hepatocellular carcinoma	X			X																						3
Hepatocellular carcinoma, multiple																										2
Hepatocellular adenoma									X						X							X				9
Hepatocellular adenoma, multiple	X												X						X							9
Histiocytic sarcoma								X																		1
Mesentery	+							+																		2
Histiocytic sarcoma								X																		1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma								X																		1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma										X							X						X			4
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign						X																				1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	4 5 3	5 0 8	5 2 7	6 2 5	7 0 1	7 1 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 8 0	1 1 0	0 8 2	0 9 8	0 6 4	0 7 3	0 6 2	0 6 9	0 7 4	0 7 5	0 7 6	0 7 7	0 8 3	0 8 4	0 8 9	0 9 0	0 9 1	0 9 4	0 9 5	0 9 6	0 9 7	1 0 1	1 0 2	1 0 8	1 2 0
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma				X																					
Lymph node																									
Popliteal, histiocytic sarcoma																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, harderian gland					X																				
Histiocytic sarcoma																									
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymph node, mediastinal																									
Histiocytic sarcoma																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																							X		
Histiocytic sarcoma																									
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+
Histiocytic sarcoma																									
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma										X															
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+																							
Spinal cord		+																							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X	X				X	X	X		X										X			
Alveolar/bronchiolar adenoma, multiple					X																				
Alveolar/bronchiolar carcinoma																									
Alveolar/bronchiolar carcinoma, multiple																					X				
Carcinoma, metastatic, harderian gland					X																				
Hemangiosarcoma		X																							
Hepatoblastoma, metastatic, liver														X											
Hepatocellular carcinoma, metastatic, liver						X								X											
Histiocytic sarcoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 6 1	0 6 3	0 6 5	0 6 6	0 6 7	0 7 1	0 7 8	0 8 1	0 8 5	0 8 8	0 9 2	0 9 3	0 9 9	0 9 0	1 0 3	1 0 4	1 0 5	1 0 7	1 1 2	1 1 3	1 1 4	1 1 5	1 1 6	1 1 7	1 1 9	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Lymph node								+																		1
Popliteal, histiocytic sarcoma								X																		1
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	45
Carcinoma, metastatic, harderian gland																										1
Histiocytic sarcoma								X																		1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma								X																		1
Lymph node, mediastinal								+																		1
Histiocytic sarcoma								X																		1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma								X																		1
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	M	+	+	43
Histiocytic sarcoma								X																		1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X		X				X				X												11
Alveolar/bronchiolar adenoma, multiple													X		X	X	X		X							6
Alveolar/bronchiolar carcinoma															X											1
Alveolar/bronchiolar carcinoma, multiple																										1
Carcinoma, metastatic, harderian gland																										1
Hemangiosarcoma																										1
Hepatoblastoma, metastatic, liver																										1
Hepatocellular carcinoma, metastatic, liver	X																									3
Histiocytic sarcoma								X																		1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	4 5 5 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	5 0 2 2 0 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
	3 8 7 5 1 5 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9
Carcass ID Number	0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1
	8 1 8 9 6 7 6 6 7 7 7 7 8 8 8 9 9 9 9 9 9 0 0 0 2
	0 0 2 8 4 3 2 9 4 5 6 7 3 4 9 0 1 4 5 6 7 1 2 8 0
Special Senses System	
Ear	
External ear, fibrosarcoma	
Eye	+
Carcinoma, metastatic, harderian gland	X
Ciliary body, adenoma	
Harderian gland	+
Adenoma	
Carcinoma	X
Urinary System	
Kidney	+ +
Histiocytic sarcoma	
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Histiocytic sarcoma	
Lymphoma malignant mixed	

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	00661	00663	00665	00666	00667	00671	00678	00681	00685	00688	00692	00693	00699	00700	00703	00704	00705	00707	01012	01013	01014	01015	01016	01017	01019	Total Tissues/ Tumors
Special Senses System																										
Ear							+																			1
External ear, fibrosarcoma							X																			1
Eye															+											2
Carcinoma, metastatic, harderian gland																										1
Ciliary body, adenoma															X											1
Harderian gland																								+		2
Adenoma																								X		1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma								X																		1
Urinary Bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma								X																		1
Lymphoma malignant mixed																							X			1

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm

Number of Days on Study	4 8 6	5 2 3	5 4 0	5 6 7	5 9 4	5 9 8	6 0 8	6 1 5	6 1 6	6 2 8	6 3 8	6 3 9	6 4 2	6 4 5	6 4 9	6 4 4	6 6 5	6 6 6	6 6 7	6 7 9	6 7 3	6 7 3	6 7 9	6 7 9	6 8 2	6 9 7
Carcass ID Number	1 2 1	1 5 9	1 3 2	1 3 8	1 2 2	1 4 1	1 4 2	1 3 5	1 6 7	1 6 5	1 8 0	1 5 4	1 5 2	1 6 9	1 2 4	1 4 8	1 6 3	1 4 9	1 2 6	1 3 6	1 5 7	1 6 4	1 7 8	1 7 4	1 7 6	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Gallbladder	+	+	+	+	+	+	+	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hemangiosarcoma, metastatic, skin													X													
Hepatoblastoma							X	X		X		X	X		X	X	X		X	X		X		X		
Hepatoblastoma, multiple						X																				
Hepatocellular carcinoma								X							X											
Hepatocellular carcinoma, multiple			X	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	
Hepatocellular adenoma											X									X			X	X		
Hepatocellular adenoma, multiple	X	X	X	X	X	X	X			X			X	X	X		X	X			X	X				
Sarcoma, metastatic, kidney																										
Mesentery																									+	
Fat, hepatocellular carcinoma, metastatic, liver																									X	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hepatoblastoma, metastatic, liver																			X							
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Squamous cell papilloma																										
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma																										
Capsule, hepatoblastoma, metastatic, liver																	X									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pituitary gland	+	+	M	+	M	+	+	M	+	+	M	M	+	+	M	+	M	+	+	+	+	+	+	+	+	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Follicular cell, adenoma																										
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	7 0 0	7 0 3	7 1 0	7 1 4	7 2 0	7 2 0	7 2 0	7 2 2	7 2 2	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 3 3 9	1 3 3 0	1 5 0 8	1 6 0 0	1 7 0 3	1 7 3 5	1 7 5 9	1 3 3 7	1 5 5 6	1 6 6 6	1 2 3 3	1 2 3 8	1 3 3 3	1 4 0 0	1 4 4 3	1 4 4 4	1 5 5 1	1 5 3 3	1 5 5 5	1 6 1 1	1 6 2 2	1 7 7 0	1 7 7 1	1 7 7 2	1 7 7 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, skin																										1
Hepatoblastoma	X				X	X	X								X		X							X	X	20
Hepatoblastoma, multiple																										1
Hepatocellular carcinoma																						X				3
Hepatocellular carcinoma, multiple	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X		X	X	X	42
Hepatocellular adenoma										X													X			6
Hepatocellular adenoma, multiple		X						X	X		X		X	X	X	X			X		X	X			X	X	28
Sarcoma, metastatic, kidney									X																	1
Mesentery									+																	2
Fat, hepatocellular carcinoma, metastatic, liver																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma, metastatic, liver																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma												X														1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																	X									1
Capsule, hepatoblastoma, metastatic, liver																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma						X																				1
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	4 8 6	5 2 3	5 4 0	5 6 7	5 9 4	5 9 8	6 0 8	6 1 5	6 1 6	6 2 8	6 3 8	6 3 9	6 4 2	6 4 5	6 4 6	6 4 5	6 6 6	6 6 7	6 6 9	6 7 3	6 7 3	6 7 9	6 7 9	6 8 2	6 9 7
Carcass ID Number	1 2 1	1 5 9	1 3 2	1 3 8	1 2 2	1 4 1	1 4 2	1 3 5	1 6 7	1 6 5	1 8 0	1 5 4	1 5 2	1 6 9	2 4 4	4 6 8	6 4 3	4 2 9	6 4 6	2 3 6	3 5 7	6 6 4	7 7 8	7 7 4	1 7 6
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma, metastatic, liver																				X					
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																				+					
Renal, hepatoblastoma, metastatic, liver																				X					
Lymph node, mandibular	+	+	+	M	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	M	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+
Sarcoma, metastatic, kidney																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, skin													X												
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	M	M	M	M	+	+	M
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma													X												
Subcutaneous tissue, melanoma NOS																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+																			+				
Spinal cord		+																			+				
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma	X																								
Hepatoblastoma, metastatic, liver						X	X			X		X				X									
Hepatocellular carcinoma, metastatic, liver				X					X						X	X									X
Sarcoma, metastatic, kidney																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
None																									

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	7 0 0	7 0 3	7 1 0	7 1 4	7 2 0	7 2 0	7 2 0	7 2 2	7 2 2	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 3 9	1 3 0	1 5 8	1 6 0	1 7 3	1 7 5	1 7 9	1 3 7	1 5 6	1 6 6	1 2 3	1 2 8	1 3 3	1 4 0	1 4 3	1 4 4	1 5 1	1 5 3	1 5 5	1 6 1	1 6 2	1 7 0	1 7 1	1 7 2	1 7 7	Total Tissues/ Tumors
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Hepatoblastoma, metastatic, liver																										1	
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Lymph node																										1	
Renal, hepatoblastoma, metastatic, liver																										1	
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	42	
Lymph node, mesenteric	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45	
Sarcoma, metastatic, kidney									X																	1	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Hemangiosarcoma, metastatic, skin																										1	
Thymus	+	M	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	37	
Integumentary System																											
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Subcutaneous tissue, hemangiosarcoma																										1	
Subcutaneous tissue, melanoma NOS			X																							1	
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Peripheral nerve																										2	
Spinal cord																										2	
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Alveolar/bronchiolar adenoma													X	X								X			X	5	
Hepatoblastoma, metastatic, liver	X																							X		7	
Hepatocellular carcinoma, metastatic, liver		X	X	X							X										X					10	
Sarcoma, metastatic, kidney									X																	1	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Special Senses System																											
None																											

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	4	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	8	2	4	6	9	9	0	1	1	2	3	3	4	4	4	6	6	6	6	7	7	7	7	8	9
	6	3	0	7	4	8	8	5	6	8	8	9	2	5	6	5	6	7	9	3	3	9	9	2	7
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	5	3	3	2	4	4	3	6	6	8	5	5	6	2	4	6	4	2	3	5	6	7	7	7
	1	9	2	8	2	1	2	5	7	5	0	4	2	9	4	8	3	9	6	6	7	4	8	4	6
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed															X										

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	7 0 0	7 0 3	7 1 0	7 1 4	7 2 0	7 2 0	7 2 0	7 2 2	7 2 2	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 3 9	1 3 0	1 5 8	1 6 0	1 7 3	1 7 5	1 7 9	1 3 7	1 5 6	1 6 6	2 2 3	2 3 8	3 3 3	4 4 0	4 4 3	4 4 4	5 5 1	5 5 3	5 5 5	6 1 1	6 2 2	7 0 0	7 1 1	7 2 2	7 7 7	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma									X																	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed																										1

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 5,000 ppm

Number of Days on Study	4 0 1	4 1 3	4 3 4	4 5 0	4 5 1	4 6 1	4 6 4	4 7 9	4 7 9	5 0 7	5 1 4	5 3 1	5 3 5	5 3 7	5 3 9	5 4 2	5 4 7	5 6 6	5 6 7	5 6 7	5 6 8	5 6 9	5 6 9	5 6 9	5 7 5
Carcass ID Number	2 3 3	2 0 0	2 1 3	2 2 4	2 3 1	2 3 2	2 0 1	1 8 5	2 1 5	1 8 7	2 0 4	1 8 3	2 3 4	2 2 3	1 9 6	2 3 2	2 3 8	2 0 5	2 0 5	2 2 6	1 9 5	2 2 1	2 2 7	2 2 9	1 9 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	+	M	+	+	M	+	M	+	+	M	+	M	+	M	+	+	M	+	+	M	+	+	M
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma			X			X			X								X		X						
Hepatoblastoma, multiple																							X		
Hepatocellular carcinoma	X														X								X		
Hepatocellular carcinoma, multiple		X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X
Hepatocellular adenoma																				X		X			
Hepatocellular adenoma, multiple	X			X	X	X	X	X	X			X	X		X	X	X				X		X	X	X
Mesentery																+							+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	+	+	M	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	M	I	M	+	M	+	M	M	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	5 7 5	5 8 7	5 8 9	5 9 3	5 9 5	5 9 6	5 9 6	5 9 6	5 9 8	6 0 9	6 1 2	6 1 4	6 1 5	6 1 7	6 1 7	6 2 2	6 2 2	6 2 3	6 2 3	6 2 4	6 2 4	6 2 5	6 2 5	6 2 8	6 2 8	
Carcass ID Number	1 9 4	2 0 2	1 0 0	2 3 9	2 4 0	1 8 1	2 1 8	2 3 0	2 1 2	1 9 9	2 2 0	2 0 6	2 0 9	2 0 8	2 0 9	2 1 4	2 1 5	3 1 1	1 3 7	3 9 8	1 1 6	1 8 2	2 0 3	1 9 3	2 0 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	M	M	+	M	+	M	+	M	M	M	+	M	M	M	M	M	+	M	M	M	+	+	+	M	+	24
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma		X		X			X					X			X		X			X						12
Hepatoblastoma, multiple																										1
Hepatocellular carcinoma				X																X						5
Hepatocellular carcinoma, multiple	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	45
Hepatocellular adenoma						X	X													X						5
Hepatocellular adenoma, multiple	X							X		X					X	X	X		X		X	X	X	X		27
Mesentery																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	42
Pituitary gland	+	M	+	+	+	+	+	M	+	+	M	+	M	+	+	M	+	M	+	+	+	+	+	+	+	37
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																									X	1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	4 0 1	4 1 3	4 3 4	4 5 0	4 5 1	4 6 1	4 6 4	4 7 9	4 7 9	5 0 7	5 1 4	5 3 1	5 3 5	5 3 7	5 3 9	5 4 2	5 4 7	5 6 6	5 6 7	5 6 7	5 6 8	5 6 9	5 6 9	5 6 9	5 7 5
Carcass ID Number	2 3 3	2 0 0	2 1 3	2 2 4	2 3 1	2 3 2	2 0 1	1 8 5	2 1 5	1 8 7	2 0 4	1 8 3	2 3 4	2 2 3	1 9 6	2 2 2	2 3 8	2 2 5	2 0 5	2 2 6	1 9 5	2 2 1	2 2 7	2 9 9	1 9 1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node												+	+												
Lymph node, mandibular	+	+	+	+	M	+	M	+	+	+	M	+	+	M	+	+	M	+	+	+	+	M	+	+	+
Lymph node, mesenteric	M	M	+	+	M	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal																+									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	M	+	M	+	M	M
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Hepatoblastoma, metastatic, liver						X											X		X						
Hepatocellular carcinoma, metastatic, liver							X			X													X		X
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																X									

TABLE C2
Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	5 7 5	5 8 7	5 8 9	5 9 3	5 9 5	5 9 6	5 9 6	5 9 6	5 9 8	6 0 9	6 1 2	6 1 4	6 1 5	6 1 7	6 1 7	6 2 2	6 2 2	6 2 3	6 2 3	6 2 4	6 2 4	6 2 5	6 2 5	6 2 8	6 2 8	
Carcass ID Number	1 9 4	2 0 2	1 9 0	2 3 9	2 4 0	1 8 1	2 1 8	2 3 0	2 1 2	1 9 9	2 2 0	2 0 6	2 0 9	2 0 8	2 0 9	1 1 4	2 3 5	1 3 1	2 1 7	1 3 7	1 9 8	2 1 6	1 8 2	2 0 3	1 9 3	2 0 7	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	42
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	41
Lymph node, mediastinal																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	M	M	M	M	I	+	+	+	+	+	M	M	M	+	+	+	M	M	M	+	+	M	+	+	31
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																						X				1
Hepatoblastoma, metastatic, liver															X					X						5
Hepatocellular carcinoma, metastatic, liver		X					X	X	X	X	X		X	X					X			X		X	X	16
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland						+																				1
Adenoma						X																				1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1

TABLE C3
Statistical Analysis of Primary Neoplasms in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	6/49 (12%)	4/50 (8%)	1/49 (2%)	0/50 (0%)
Adjusted rate[b]	13.3%	9.1%	6.7%	0.0%
Terminal rate[c]	6/45 (13%)	4/44 (9%)	1/15 (7%)	0/0
First incidence (days)	729 (T)	729 (T)	729 (T)	–[e]
Life table test[d]	P=0.456N	P=0.384N	P=0.409N	–
Logistic regression test[d]	P=0.456N	P=0.384N	P=0.409N	–
Cochran-Armitage test[d]	P=0.006N			
Fisher exact test[d]		P=0.357N	P=0.056N	P=0.012N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.3%	4.4%	0.0%	5.0%
Terminal rate	1/45 (2%)	1/44 (2%)	0/15 (0%)	0/0
First incidence (days)	624	701	–	596
Life table test	P=0.540	P=0.508N	P=0.251N	P=0.432
Logistic regression test	P=0.154N	P=0.470N	P=0.113N	P=0.463N
Cochran-Armitage test	P=0.158N			
Fisher exact test		P=0.500N	P=0.121N	P=0.309N
Liver: Hepatoblastoma				
Overall rate	0/49 (0%)	2/50 (4%)	21/50 (42%)	13/50 (26%)
Adjusted rate	0.0%	4.5%	58.3%	52.9%
Terminal rate	0/45 (0%)	2/44 (5%)	4/15 (27%)	0/0
First incidence (days)	–	729 (T)	598	434
Life table test	P<0.001	P=0.234	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.234	P<0.001	P=0.014
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.253	P<0.001	P<0.001
Liver: Hepatocellular Adenoma				
Overall rate	17/49 (35%)	18/50 (36%)	34/50 (68%)	32/50 (64%)
Adjusted rate	37.8%	37.4%	87.3%	95.1%
Terminal rate	17/45 (38%)	14/44 (32%)	11/15 (73%)	0/0
First incidence (days)	729 (T)	453	486	401
Life table test	P<0.001	P=0.472	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.547	P<0.001	P=0.003
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.530	P<0.001	P=0.003
Liver: Hepatocellular Carcinoma				
Overall rate	9/49 (18%)	5/50 (10%)	45/50 (90%)	50/50 (100%)
Adjusted rate	18.7%	10.7%	95.7%	100.0%
Terminal rate	6/45 (13%)	3/44 (7%)	13/15 (87%)	0/0
First incidence (days)	586	453	540	401
Life table test	P<0.001	P=0.215N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.107N	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.183N	P<0.001	P<0.001

TABLE C3
Statistical Analysis of Primary Neoplasms in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Liver: Hepatoblastoma or Hepatocellular Carcinoma				
Overall rate	9/49 (18%)	6/50 (12%)	47/50 (94%)	50/50 (100%)
Adjusted rate	18.7%	12.9%	97.9%	100.0%
Terminal rate	6/45 (13%)	4/44 (9%)	14/15 (93%)	0/0
First incidence (days)	586	453	540	401
Life table test	P<0.001	P=0.310N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.184N	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.274N	P<0.001	P<0.001
Liver: Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma				
Overall rate	23/49 (47%)	19/50 (38%)	50/50 (100%)	50/50 (100%)
Adjusted rate	47.9%	39.5%	100.0%	100.0%
Terminal rate	20/45 (44%)	15/44 (34%)	15/15 (100%)	0/0
First incidence (days)	586	453	486	401
Life table test	P<0.001	P=0.315N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.205N	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.243N	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	11/50 (22%)	17/50 (34%)	5/50 (10%)	1/50 (2%)
Adjusted rate	23.9%	36.1%	28.1%	25.0%
Terminal rate	10/45 (22%)	14/44 (32%)	4/15 (27%)	0/0
First incidence (days)	627	527	486	625
Life table test	P=0.501	P=0.128	P=0.493	P=0.127
Logistic regression test	P=0.001N	P=0.133	P=0.126N	P=0.570N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P=0.133	P=0.086N	P=0.002N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	13/50 (26%)	18/50 (36%)	5/50 (10%)	1/50 (2%)
Adjusted rate	28.2%	38.2%	28.1%	25.0%
Terminal rate	12/45 (27%)	15/44 (34%)	4/15 (27%)	0/0
First incidence (days)	627	527	486	625
Life table test	P=0.581	P=0.184	P=0.612	P=0.127
Logistic regression test	P<0.001N	P=0.195	P=0.063N	P=0.559N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P=0.194	P=0.033N	P<0.001N
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.2%	10.7%	2.6%	0.0%
Terminal rate	1/45 (2%)	3/44 (7%)	0/15 (0%)	0/0
First incidence (days)	729 (T)	508	642	–
Life table test	P=0.407N	P=0.104	P=0.620	–
Logistic regression test	P=0.016N	P=0.090	P=0.758N	–
Cochran-Armitage test	P=0.046N			
Fisher exact test		P=0.102	P=0.753N	P=0.500N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
All Organs: Malignant Lymphoma or Histiocytic Sarcoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.7%	4.5%	2.8%	2.9%
Terminal rate	3/45 (7%)	2/44 (5%)	0/15 (0%)	0/0
First incidence (days)	729 (T)	729 (T)	646	542
Life table test	P=0.246	P=0.510N	P=0.637N	P=0.433
Logistic regression test	P=0.347N	P=0.510N	P=0.352N	P=0.778N
Cochran-Armitage test	P=0.207N			
Fisher exact test		P=0.500N	P=0.309N	P=0.309N
All Organs: Benign Neoplasms				
Overall rate	32/50 (64%)	31/50 (62%)	37/50 (74%)	32/50 (66%)
Adjusted rate	68.1%	63.3%	94.0%	100.0%
Terminal rate	30/45 (67%)	26/44 (59%)	13/15 (87%)	0/0
First incidence (days)	627	453	486	401
Life table test	P<0.001	P=0.549N	P<0.001	P<0.001
Logistic regression test	P=0.135	P=0.576N	P=0.077	P=0.170
Cochran-Armitage test	P=0.092			
Fisher exact test		P=0.500N	P=0.101	P=0.339
All Organs: Malignant Neoplasms				
Overall rate	17/50 (34%)	18/50 (36%)	47/50 (94%)	50/50 (100%)
Adjusted rate	34.7%	36.7%	97.9%	100.0%
Terminal rate	13/45 (29%)	13/44 (30%)	14/15 (93%)	0/0
First incidence (days)	586	453	540	401
Life table test	P<0.001	P=0.475	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.493	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.500	P<0.001	P<0.001
All Organs: Benign and Malignant Neoplasms				
Overall rate	37/50 (74%)	35/50 (70%)	50/50 (100%)	50/50 (100%)
Adjusted rate	75.5%	70.0%	100.0%	100.0%
Terminal rate	33/45 (73%)	29/44 (66%)	15/15 (100%)	0/0
First incidence (days)	586	453	486	401
Life table test	P<0.001	P=0.481N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.409N	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.412N	P<0.001	P<0.001

(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 control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 C4
Historical Incidence of Liver Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls			
	Hepatoblastoma	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma
Historical Incidence at Battelle Columbus				
2,4-Dichlorophenol	0/50	4/50	7/50	10/50
4,4-Thiobis(6-*t*-butyl-*m*-cresol)	0/50	17/50	11/50	25/50
Diphenylhydantoin	0/50	19/50	13/50	29/50
Dowicide EC-7 pentachlorophenol	0/35	5/35	1/35	6/35
Ethylenethiourea	0/49	11/49	13/49	20/49
Firemaster FF-1 polybrominated biphenyl	0/50	9/50	8/50	16/50
Manganese (II) sulfate monohydrate	0/50	30/50	9/50	34/50
Technical grade pentachlorophenol	0/32	5/32	2/32	7/32
Triamterene	0/50	17/50	5/50	20/50
Triamterene	0/50	21/50	9/50	25/50
Tricresyl phosphate	0/52	18/52	15/52	28/52
Overall Historical Incidence				
Total	0/1,366	312/1,366 (22.8%)	223/1,366 (16.3%)	485/1,366 (35.5%)
Standard deviation		13.8%	7.2%	14.3%
Range		4%-60%	3%-29%	10%-68%

[a] Data as of 20 August 1992

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	2	4	22	30
Natural deaths	3	2	13	20
Survivors				
Terminal sacrifice	45	44	15	
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Basophilic focus				1 (10%)
Clear cell focus				1 (10%)
Eosinophilic focus		1 (10%)	9 (90%)	2 (20%)
Vacuolization cytoplasmic	7 (70%)	6 (60%)	1 (10%)	1 (10%)
Hepatocyte, centrilobular, hypertrophy		10 (100%)	10 (100%)	10 (100%)
Cardiovascular System				
None				
Endocrine System				
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, cyst	1 (10%)			
Thyroid gland	(10)	(10)	(10)	(10)
Inflammation, chronic active, focal				1 (10%)
Follicular cell, hyperplasia			5 (50%)	7 (70%)
General Body System				
None				
Genital System				
Preputial gland	(10)	(10)	(10)	(10)
Inflammation, chronic	1 (10%)			
Duct, ectasia	5 (50%)	2 (20%)	1 (10%)	
Hematopoietic System				
None				
Integumentary System				
None				

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
15-Month Interim Evaluation (continued)				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar epithelium, hyperplasia, focal	1 (10%)			1 (10%)
Special Senses System				
None				
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Nephropathy, chronic	10 (100%)	10 (100%)	5 (50%)	7 (70%)
Urinary bladder	(10)	(10)	(10)	(10)
Dilatation			1 (10%)	
2-Year Study				
Alimentary System				
Intestine small, jejunum	(49)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid	2 (4%)	1 (2%)		
Liver	(49)	(50)	(50)	(50)
Angiectasis			2 (4%)	
Basophilic focus	2 (4%)	1 (2%)		
Clear cell focus	9 (18%)	5 (10%)	1 (2%)	
Clear cell focus, multifocal	4 (8%)	1 (2%)		
Eosinophilic focus	18 (37%)	12 (24%)	8 (16%)	8 (16%)
Infarct				1 (2%)
Mixed cell focus	2 (4%)	1 (2%)	1 (2%)	
Necrosis	2 (4%)			
Necrosis, multifocal	1 (2%)			
Thrombosis			1 (2%)	5 (10%)
Vacuolization cytoplasmic	2 (4%)	2 (4%)		
Artery, angiectasis				1 (2%)
Centrilobular, hypertrophy		2 (4%)	26 (52%)	43 (86%)
Mesentery	(2)	(2)	(2)	(2)
Congestion				1 (50%)
Fat, inflammation, chronic	1 (50%)	1 (50%)		
Pancreas	(49)	(50)	(50)	(50)
Acinus, atrophy				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, focal, squamous	2 (4%)	4 (8%)	1 (2%)	
Hyperplasia, multifocal, squamous			1 (2%)	

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam
(continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Stomach, glandular	(50)	(50)	(50)	(50)
Necrosis, multifocal				2 (4%)
Tooth	(1)			
Gingiva, hyperplasia, focal, squamous	1 (100%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Angiectasis, focal	1 (2%)			
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(50)
Hypertrophy	13 (27%)	7 (14%)	1 (2%)	2 (4%)
Vacuolization cytoplasmic			1 (2%)	
Capsule, hyperplasia	3 (6%)	2 (4%)	1 (2%)	
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)			
Pituitary gland	(48)	(50)	(42)	(37)
Pars distalis, cyst	3 (6%)	3 (6%)	3 (7%)	
Pars distalis, vacuolization cytoplasmic				3 (8%)
Thyroid gland	(49)	(50)	(50)	(50)
Follicle, dilatation, multiple			1 (2%)	
Follicular cell, hyperplasia	4 (8%)	22 (44%)	49 (98%)	47 (94%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			
Infiltration cellular, lymphocyte	2 (4%)	14 (28%)	33 (66%)	21 (42%)
Inflammation, chronic			1 (2%)	
Spermatocele	2 (4%)			
Preputial gland	(50)	(50)	(50)	(49)
Ectasia	2 (4%)			
Infiltration cellular, lymphocyte	1 (2%)			
Inflammation, chronic	6 (12%)	7 (14%)	8 (16%)	5 (10%)
Duct, ectasia	35 (70%)	29 (58%)	9 (18%)	7 (14%)
Duct, hemorrhage			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Atrophy	1 (2%)		25 (50%)	38 (76%)
Hematopoietic System				
Lymph node	(2)	(1)	(1)	(2)
Inguinal, hyperplasia, lymphoid	1 (50%)			1 (50%)
Lumbar, hyperplasia, lymphoid				1 (50%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node, mandibular	(44)	(45)	(42)	(42)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid				1 (2%)
Lymph node, mesenteric	(48)	(49)	(45)	(41)
Angiectasis			1 (2%)	1 (2%)
Hematopoietic cell proliferation		1 (2%)	1 (2%)	
Hemorrhage				1 (2%)
Hyperplasia, lymphoid		1 (2%)		
Spleen	(49)	(50)	(50)	(50)
Atrophy				1 (2%)
Hematopoietic cell proliferation	3 (6%)	2 (4%)	6 (12%)	7 (14%)
Hyperplasia, lymphoid	1 (2%)			
Thymus	(43)	(43)	(37)	(31)
Atrophy	39 (91%)	40 (93%)	35 (95%)	24 (77%)
Cyst				1 (3%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Edema, subacute			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cartilage, vertebra, fracture		1 (2%)		
Joint, cartilage, fracture		1 (2%)		
Vertebra, hyperostosis				1 (2%)
Nervous System				
Peripheral nerve		(1)	(2)	
Sciatic, axon, degeneration		1 (100%)	2 (100%)	
Spinal cord		(1)	(2)	
Nerve, degeneration		1 (100%)	2 (100%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage, focal				1 (2%)
Infiltration cellular, multifocal, histiocyte		1 (2%)		
Alveolar epithelium, hyperplasia	4 (8%)	2 (4%)	1 (2%)	
Special Senses System				
Eye	(2)	(2)		
Bilateral, inflammation, chronic	1 (50%)			

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam
(continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydronephrosis	1 (2%)			
Nephropathy, chronic	43 (86%)	40 (80%)	3 (6%)	18 (36%)
Cortex, cyst	2 (4%)	1 (2%)		
Urinary bladder	(49)	(50)	(50)	(49)
Calculus gross observation				1 (2%)
Inflammation, chronic active				1 (2%)

[a] Number of animals examined microscopically at site and number of animals with lesion

APPENDIX D
SUMMARY OF LESIONS IN FEMALE B6C3F$_1$ MICE IN THE 2-YEAR FEED STUDY OF OXAZEPAM

TABLE D1
Summary of the Incidence of Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	8	7	22	15
Natural deaths	3	2	26	35
Survivors				
Terminal sacrifice	39	41	2	
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatoblastoma				2 (20%)
Hepatocellular carcinoma	1 (10%)		2 (20%)	3 (30%)
Hepatocellular carcinoma, multiple				7 (70%)
Hepatocellular adenoma	1 (10%)	1 (10%)		
Hepatocellular adenoma, multiple			9 (90%)	10 (100%)
Histiocytic sarcoma			1 (10%)	
Cardiovascular System				
None				
Endocrine System				
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, adenoma	1 (10%)	1 (10%)		
General Body System				
None				
Genital System				
Uterus	(10)	(10)	(10)	(10)
Cervix, histiocytic sarcoma			1 (10%)	
Hematopoietic System				
None				
Integumentary System				
None				
Musculoskeletal System				
None				

TABLE D1
Summary of the Incidence of Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
15-Month Interim Evaluation (continued)				
Nervous System				
None				
Respiratory System				
None				
Special Senses System				
None				
Urinary System				
None				
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
2-Year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatoblastoma		1 (2%)	8 (16%)	7 (14%)
Hepatoblastoma, multiple				1 (2%)
Hepatocellular carcinoma	9 (18%)	4 (8%)	7 (14%)	1 (2%)
Hepatocellular carcinoma, multiple		1 (2%)	42 (84%)	43 (86%)
Hepatocellular adenoma	12 (24%)	16 (32%)	6 (12%)	6 (12%)
Hepatocellular adenoma, multiple	13 (26%)	19 (38%)	29 (58%)	30 (60%)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Mesentery	(3)	(3)		
Histiocytic sarcoma		1 (33%)		
Teratoma malignant, metastatic, ovary	1 (33%)			
Fat, lipoma	1 (33%)			
Pancreas	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		1 (2%)	
Cardiovascular System				
None				

TABLE D1
Summary of the Incidence of Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Capsule, adenoma		1 (2%)		
Capsule, histiocytic sarcoma		1 (2%)		
Pituitary gland	(50)	(49)	(43)	(49)
Pars distalis, adenoma	2 (4%)	8 (16%)		
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma		4 (8%)	5 (10%)	6 (12%)
General Body System				
None				
Genital System				
Ovary	(50)	(50)	(48)	(50)
Cystadenoma	1 (2%)	3 (6%)	1 (2%)	
Granulosa cell tumor malignant	1 (2%)			
Granulosa cell tumor benign		1 (2%)		
Histiocytic sarcoma	1 (2%)	1 (2%)		
Luteoma	2 (4%)			
Teratoma malignant	1 (2%)	1 (2%)		
Follicle, adenoma	1 (2%)		2 (4%)	
Uterus	(50)	(50)	(50)	(49)
Histiocytic sarcoma	1 (2%)			
Leiomyosarcoma		1 (2%)		
Polyp stromal	1 (2%)		1 (2%)	
Cervix, histiocytic sarcoma	1 (2%)			
Endometrium, carcinoma		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(3)	(2)	(1)	
Pancreatic, histiocytic sarcoma		1 (50%)		
Renal, teratoma malignant, metastatic, ovary	1 (33%)			
Thoracic, histiocytic sarcoma		1 (50%)		
Lymph node, bronchial	(1)			
Lymph node, mandibular	(48)	(47)	(48)	(44)
Fibrosarcoma, metastatic, skin	1 (2%)			
Histiocytic sarcoma	1 (2%)			
Lymph node, mesenteric	(48)	(46)	(38)	(37)
Histiocytic sarcoma		1 (2%)		
Teratoma malignant, metastatic, ovary	1 (2%)			
Lymph node, mediastinal	(1)	(2)	(3)	
Histiocytic sarcoma		1 (50%)		
Spleen	(50)	(50)	(50)	(49)
Histiocytic sarcoma		1 (2%)		
Thymus	(50)	(49)	(34)	(34)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Thymoma NOS	1 (2%)	1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, fibrosarcoma, multiple	1 (2%)			
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Subcutaneous tissue, melanoma NOS			1 (2%)	
Musculoskeletal System				
Skeletal muscle	(50)	(50)	(50)	(50)
Diaphragm, teratoma malignant, metastatic, ovary	1 (2%)			
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	1 (2%)		1 (2%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)	3 (6%)	1 (2%)	
Fibrosarcoma, metastatic, skin	1 (2%)			
Hepatoblastoma, metastatic, liver			2 (4%)	2 (4%)
Hepatocellular carcinoma, metastatic, liver	3 (6%)	2 (4%)	8 (16%)	10 (20%)
Histiocytic sarcoma		1 (2%)		
Teratoma malignant, metastatic, ovary	1 (2%)			
Trachea	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Special Senses System				
Ear	(1)	(2)	(1)	
External ear, fibrosarcoma			1 (100%)	
Harderian gland	(3)	(3)	(1)	(1)
Adenoma	1 (33%)	3 (100%)	1 (100%)	1 (100%)
Carcinoma	1 (33%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)		
Teratoma malignant, metastatic, ovary	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Systemic Lesions				
Multiple organs	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	2 (4%)		
Lymphoma malignant			1 (2%)	
Lymphoma malignant lymphocytic	2 (4%)	2 (4%)	1 (2%)	
Lymphoma malignant mixed	1 (2%)	1 (2%)	2 (4%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]				
15-Month interim evaluation	3	2	9	10
2-Year study	37	43	50	47
Total primary neoplasms				
15-Month interim evaluation	3	2	12	22
2-Year study	60	75	110	96
Total animals with benign neoplasms				
15-Month interim evaluation	2	2	9	10
2-Year study	29	40	38	38
Total benign neoplasms				
15-Month interim evaluation	2	2	9	10
2-Year study	39	57	46	44
Total animals with malignant neoplasms				
15-Month interim evaluation	1		3	10
2-Year study	18	15	49	44
Total malignant neoplasms				
15-Month interim evaluation	1		3	12
2-Year study	20	17	63	52
Total animals with metastatic neoplasms				
2-Year study	6	2	8	12
Total metastatic neoplasms				
2-Year study	12	2	10	12
Total animals with uncertain neoplasms - benign or malignant				
2-Year study	1	1	1	
Total uncertain neoplasms				
2-Year study	1	1	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 D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm

Number of Days on Study	3 5 5 5 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 5 1 7 9 2 2 3 3 5 5 7 3 3 3 3 3 3 3 3 3 3 3 3 3 3 7 6 6 8 2 4 0 8 2 9 0 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Carcass ID Number	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 7 8 4 8 6 5 8 9 7 4 6 4 4 4 5 5 5 5 6 6 7 7 7 7 7 2 6 5 2 7 9 8 7 0 1 1 3 8 9 1 2 3 7 5 6 1 3 6 7 9
Alimentary System	
Esophagus	+ +
Gallbladder	+ +
Intestine large, colon	+ +
Intestine large, rectum	+ +
Intestine large, cecum	+ +
Intestine small, duodenum	+ +
Intestine small, jejunum	+ +
Intestine small, ileum	+ +
Liver	+ +
Hepatocellular carcinoma	X X X X
Hepatocellular adenoma	X X X X X X X
Hepatocellular adenoma, multiple	X X X X X
Histiocytic sarcoma	X
Mesentery	+
Teratoma malignant, metastatic, ovary	X
Fat, lipoma	
Pancreas	+ +
Salivary glands	+ +
Fibrosarcoma, metastatic, skin	X
Stomach, forestomach	+ +
Squamous cell papilloma	X
Stomach, glandular	+ +
Cardiovascular System	
Blood vessel	+ +
Heart	+ +
Endocrine System	
Adrenal cortex	+ +
Adrenal medulla	+ +
Islets, pancreatic	+ +
Parathyroid gland	+ + + + + + + + + M + M + + + + + + + + + + + + +
Pituitary gland	+ +
Pars distalis, adenoma	X
Thyroid gland	+ +
General Body System	
None	
Genital System	
Clitoral gland	+ + M + + + + + + M + + + + + + + + + + + + + + +
Ovary	+ +
Cystadenoma	
Granulosa cell tumor malignant	X
Histiocytic sarcoma	X
Luteoma	X
Teratoma malignant	X
Follicle, adenoma	

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 8 1	2 8 4	2 8 7	2 8 9	2 9 2	2 9 8	2 4 2	2 4 4	2 4 7	2 5 4	2 5 5	2 5 6	2 5 8	2 6 3	2 6 4	2 7 5	2 7 8	2 8 3	2 8 5	2 9 0	2 9 1	2 9 3	2 9 5	2 9 6	2 9 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma			X			X							X	X					X		X					9
Hepatocellular adenoma				X				X	X		X							X						X		12
Hepatocellular adenoma, multiple	X		X		X	X										X	X					X	X			13
Histiocytic sarcoma																										1
Mesentery																		+	+							3
Teratoma malignant, metastatic, ovary																										1
Fat, lipoma																			X							1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X																								2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma																			X							1
Granulosa cell tumor malignant																										1
Histiocytic sarcoma																										1
Luteoma																								X		2
Teratoma malignant																										1
Follicle, adenoma		X																								1

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	3 5 5 5 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 5 1 7 9 2 2 3 3 5 5 7 3 3 3 3 3 3 3 3 3 3 3 3 3 3 7 6 6 8 2 4 0 8 2 9 0 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Carcass ID Number	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 7 8 4 8 6 5 8 9 7 4 6 4 4 4 5 5 5 5 6 6 7 7 7 7 7 2 6 5 2 7 9 8 7 0 1 1 3 8 9 1 2 3 7 5 6 1 3 6 7 9
Genital System (continued)	
Uterus	+ +
Histiocytic sarcoma	X
Polyp stromal	
Cervix, histiocytic sarcoma	X
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ + +
Renal, teratoma malignant, metastatic, ovary	X
Lymph node, bronchial	+
Lymph node, mandibular	+ +
Fibrosarcoma, metastatic, skin	X
Histiocytic sarcoma	X
Lymph node, mesenteric	+ M +
Teratoma malignant, metastatic, ovary	X
Lymph node, mediastinal	+
Spleen	+ +
Thymus	+ +
Histiocytic sarcoma	X
Thymoma NOS	
Integumentary System	
Mammary gland	+ +
Skin	+ +
Subcutaneous tissue, fibrosarcoma	X
Subcutaneous tissue, fibrosarcoma, multiple	X
Subcutaneous tissue, hemangiosarcoma	X
Musculoskeletal System	
Bone	+ +
Skeletal muscle	+ +
Diaphragm, teratoma malignant, metastatic, ovary	X
Nervous System	
Brain	+ +
Peripheral nerve	+ +
Spinal cord	+ +

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 8 1	2 8 4	2 8 7	2 8 9	2 9 2	2 9 8	2 4 2	2 4 4	2 4 7	2 5 4	2 5 5	2 5 6	2 5 8	2 6 3	2 6 4	2 7 5	2 7 8	2 8 3	2 8 5	2 9 0	2 9 1	2 9 3	2 9 5	2 9 6	2 9 9	Total Tissues/ Tumors
Genital System (continued)																										
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Polyp stromal																	X									1
Cervix, histiocytic sarcoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										3
Renal, teratoma malignant, metastatic, ovary																										1
Lymph node, bronchial																										1
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Fibrosarcoma, metastatic, skin																										1
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Teratoma malignant, metastatic, ovary																										1
Lymph node, mediastinal																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Thymoma NOS																	X									1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, fibrosarcoma, multiple																										1
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Diaphragm, teratoma malignant, metastatic, ovary																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																										2

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	3 5 5 5 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 5 1 7 9 2 2 3 3 5 5 7 3 3 3 3 3 3 3 3 3 3 3 3 3 3 7 6 6 8 2 4 0 8 2 9 0 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Carcass ID Number	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 7 8 4 8 6 5 8 9 7 4 6 4 4 4 5 5 5 5 6 6 7 7 7 7 7 2 6 5 2 7 9 8 7 0 1 1 3 8 9 1 2 3 7 5 6 1 3 6 7 9
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X
Alveolar/bronchiolar adenoma, multiple	X
Alveolar/bronchiolar carcinoma	X
Fibrosarcoma, metastatic, skin	X
Hepatocellular carcinoma, metastatic, liver	X
Teratoma malignant, metastatic, ovary	X
Nose	+ +
Trachea	+ +
Special Senses System	
Ear	+
Eye	+ +
Harderian gland	+ + +
Adenoma	X
Carcinoma	X
Urinary System	
Kidney	+ +
Teratoma malignant, metastatic, ovary	X
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Histiocytic sarcoma	X
Lymphoma malignant lymphocytic	X X
Lymphoma malignant mixed	

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 0 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 8 1	2 8 4	2 8 7	2 8 9	2 9 2	2 9 8	2 4 2	2 4 4	2 4 7	2 5 4	2 5 5	2 5 6	2 5 8	2 6 3	2 6 4	2 7 5	2 7 8	2 8 3	2 8 5	2 9 0	2 9 1	2 9 3	2 9 5	2 9 6	2 9 9	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X																					X		3
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma																										1
Fibrosarcoma, metastatic, skin																										1
Hepatocellular carcinoma, metastatic, liver						X							X													3
Teratoma malignant, metastatic, ovary																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye																										2
Harderian gland																										3
Adenoma																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Teratoma malignant, metastatic, ovary																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant lymphocytic																										2
Lymphoma malignant mixed																								X		1

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm

Number of Days on Study	3 9 1	4 7 1	4 9 0	4 9 2	6 4 9	6 6 2	7 0 1	7 1 4	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 4 4	3 5 8	3 4 5	3 2 0	3 3 0	3 3 8	3 3 6	3 1 7	3 1 9	3 0 4	3 0 5	3 0 7	3 1 0	3 1 3	3 1 5	3 1 8	3 2 1	3 2 4	3 2 8	3 2 9	3 3 4	3 4 0	4 4 1	4 4 2	4 4 6
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																	
Gallbladder	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma								X																
Hepatocellular carcinoma														X										
Hepatocellular carcinoma, multiple								X																
Hepatocellular adenoma							X	X		X								X	X	X		X		X
Hepatocellular adenoma, multiple		X									X	X			X		X				X		X	
Histiocytic sarcoma																							X	
Mesentery					+		+		+															
Histiocytic sarcoma							X																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma														X										
Capsule, histiocytic sarcoma							X																	
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X			X											X
Pars intermedia, adenoma																X								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																	X		X					
General Body System																								
Tissue NOS																								+

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 4 8	3 5 0	3 5 2	3 5 3	3 5 7	3 0 1	3 0 2	3 0 3	3 1 4	3 1 6	3 2 2	3 2 3	3 2 7	3 3 2	3 3 3	3 3 5	3 3 7	3 4 3	3 4 7	3 4 9	3 5 1	3 5 5	3 5 6	3 5 9	3 6 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma																										1
Hepatocellular carcinoma										X			X						X							4
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma				X		X				X			X		X	X								X	X	16
Hepatocellular adenoma, multiple	X		X		X		X		X			X	X		X				X		X	X	X			19
Histiocytic sarcoma																										1
Mesentery																										3
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma																										1
Capsule, histiocytic sarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma					X										X		X				X	X				8
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma			X																				X			4
General Body System																										
Tissue NOS																										1

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	3 9 1	4 7 1	4 9 0	4 9 2	6 4 9	6 6 2	7 0 1	7 1 4	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 4 4	3 5 8	3 4 5	3 2 0	3 3 0	3 3 8	3 3 6	3 1 7	3 1 9	3 0 4	3 0 5	3 0 7	3 1 0	3 1 3	3 1 5	3 1 8	3 2 1	3 2 4	3 2 8	3 2 9	3 3 4	3 4 0	3 4 1	3 4 2	3 4 6
Genital System																									
Clitoral gland	M	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma							X																		
Granulosa cell tumor benign																		X							
Histiocytic sarcoma							X																		
Teratoma malignant												X													
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																							X		
Endometrium, carcinoma													X												
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+																	+		
Pancreatic, histiocytic sarcoma																							X		
Thoracic, histiocytic sarcoma																							X		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																		
Lymph node, mediastinal						+	+																		
Histiocytic sarcoma							X																		
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																		
Thymoma NOS																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve					+				+														+		
Spinal cord					+				+														+		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									X
Alveolar/bronchiolar carcinoma																			X						
Hepatocellular carcinoma, metastatic, liver								X						X											
Histiocytic sarcoma							X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																		

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 4 8	3 5 0	3 5 2	3 5 3	3 5 7	3 6 1	3 6 2	3 6 3	3 6 4	3 6 6	3 6 2	3 6 3	3 6 7	3 7 2	3 7 3	3 7 5	3 7 7	3 7 3	3 7 7	3 7 9	3 8 1	3 8 5	3 8 6	3 8 9	3 9 0	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma						X											X									3
Granulosa cell tumor benign																										1
Histiocytic sarcoma																										1
Teratoma malignant																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Endometrium, carcinoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										2
Pancreatic, histiocytic sarcoma																										1
Thoracic, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	M		46
Histiocytic sarcoma																										1
Lymph node, mediastinal																										2
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Thymoma NOS																				X						1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										3
Spinal cord																										3
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma							X																X			3
Hepatocellular carcinoma, metastatic, liver																										2
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	3 9 1	4 7 1	4 9 0	4 9 2	6 4 9	6 6 2	7 0 1	7 1 4	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 4 4	3 5 8	3 4 5	3 2 0	3 3 0	3 3 8	3 3 6	3 1 7	3 1 9	3 0 4	3 0 5	3 0 7	3 1 0	3 1 3	3 1 5	3 1 8	3 2 1	3 2 4	3 2 8	3 2 9	3 3 4	3 4 0	3 4 1	3 4 2	3 4 6
Special Senses System																									
Ear	+		+																						
Eye																									
Harderian gland									+									+							
Adenoma									X									X							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																X		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																X		
Lymphoma malignant lymphocytic						X																			
Lymphoma malignant mixed																								X	

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 125 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 4 8	3 5 0	3 5 2	3 5 3	3 5 7	3 0 1	3 0 2	3 0 3	3 1 4	3 1 6	3 2 2	3 2 3	3 2 7	3 3 2	3 3 3	3 3 5	3 3 7	3 4 3	3 4 7	3 4 9	3 5 1	3 5 5	3 5 6	3 5 9	3 6 0	Total Tissues/ Tumors
Special Senses System																										
Ear																										2
Eye											+															1
Harderian gland											+															3
Adenoma											X															3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant lymphocytic											X															2
Lymphoma malignant mixed																										1

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm

Number of Days on Study	3 9 3	3 9 6	4 7 0	5 7 5	5 9 6	6 1 4	6 1 5	6 2 2	6 3 0	6 3 4	6 3 5	6 3 6	6 3 8	6 4 2	6 4 4	6 4 5	6 4 8	6 6 3	6 6 4	6 6 5	6 6 6	6 6 7	6 7 7	6 8 9	6 9 1
Carcass ID Number	4 0 1	3 7 1	4 0 7	3 9 4	3 6 9	3 7 6	3 7 5	4 1 3	3 6 7	4 1 6	4 0 0	3 7 4	3 8 1	3 9 7	3 8 4	4 1 1	4 1 4	3 6 6	4 0 3	3 6 5	3 8 9	4 1 5	3 7 7	4 7 8	3 9 8
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+	M	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma						X			X	X				X		X		X							
Hepatocellular carcinoma	X	X						X			X					X						X			
Hepatocellular carcinoma, multiple			X	X	X	X	X		X	X		X	X	X	X		X	X	X	X	X		X	X	X
Hepatocellular adenoma				X	X						X			X											
Hepatocellular adenoma, multiple	X		X				X	X		X		X				X	X			X		X	X	X	X
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	M	M	+	M	M	+	+	+	+	+	+	+	M	+	+
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	M	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																		X		X					
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	I	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Cystadenoma																									
Follicle, adenoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal															X										

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	6 9 2	6 9 9	7 0 0	7 0 1	7 0 2	7 0 6	7 0 6	7 1 0	7 1 1	7 1 1	7 1 2	7 1 2	7 1 2	7 1 2	7 1 2	7 1 9	7 1 9	7 1 9	7 1 9	7 1 9	7 1 9	7 1 9	7 1 9	7 3 5	7 3 5	
Carcass ID Number	3 9 3	4 0 2	4 1 9	3 9 0	3 7 0	3 7 9	4 2 0	4 1 0	3 9 6	4 0 6	3 6 1	3 7 2	3 8 5	3 9 1	4 0 8	3 6 2	3 6 3	3 6 4	3 8 2	3 8 3	3 8 6	3 9 2	4 0 4	4 0 5	4 1 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	M	+	M	+	+	+	+	+	41
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma																X				X						8
Hepatocellular carcinoma																			X							7
Hepatocellular carcinoma, multiple	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	42
Hepatocellular adenoma																X	X									6
Hepatocellular adenoma, multiple						X	X	X		X	X	X	X	X	X			X	X	X	X	X	X		X	29
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																	X									1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	M	+	41
Pituitary gland	I	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	43
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																	X	X		X						5
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Ovary	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cystadenoma																								X		1
Follicle, adenoma																	X		X							2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal																										1

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F1 Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm (continued)

	3	3	4	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Number of Days on Study	9	9	7	7	9	1	1	2	3	3	3	3	3	4	4	4	4	6	6	6	6	6	7	8	9
	3	6	0	5	6	4	5	2	0	4	5	6	8	2	4	5	8	3	4	5	6	7	7	9	1
	4	3	4	3	3	3	3	4	3	4	4	3	3	3	3	4	4	3	4	3	3	3	4	3	3
Carcass ID Number	0	7	0	9	6	7	7	1	6	1	0	7	8	9	8	1	1	6	0	6	8	9	1	7	9
	1	1	7	4	9	6	5	3	7	6	0	4	1	7	4	1	4	6	3	5	9	5	7	8	8
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+																								
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	M	+
Lymph node, mediastinal	+							+	+																
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	M	M	M	M	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, melanoma NOS																				X					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																+	+								
Spinal cord																	+								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																X									
Hepatoblastoma, metastatic, liver						X																			
Hepatocellular carcinoma, metastatic, liver						X									X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear								+																	
External ear, fibrosarcoma								X																	
Harderian gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant								X																	
Lymphoma malignant lymphocytic								X																	
Lymphoma malignant mixed	X																								

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 2,500 ppm (continued)

Number of Days on Study	6 9 2	6 9 9	7 0 0	7 0 1	7 0 2	7 0 6	7 0 6	7 1 0	7 1 1	7 1 1	7 1 2	7 1 2	7 1 2	7 1 2	7 1 2	7 1 9	7 1 9	7 1 9	7 1 9	7 1 9	7 1 9	7 1 9	7 1 9	7 3 5	7 3 5	
Carcass ID Number	3 9 3	4 0 2	4 1 9	3 9 0	3 7 0	3 7 9	4 2 0	4 1 0	3 9 6	4 0 6	3 6 1	3 7 2	3 8 5	3 9 1	4 0 8	3 6 2	3 6 3	3 6 4	3 8 2	3 8 3	3 8 6	3 9 2	4 0 4	4 0 5	4 0 5	4 1 5	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	M	+	+	+	M	M	M	+	M	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	38
Lymph node, mediastinal																										3
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	M	+	+	+	+	+	M	+	+	+	M	+	M	+	+	+	+	+	M	M	+	+	M	M	+	34
Integumentary System																										
Mammary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	47
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, melanoma NOS																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord										+																2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																										1
Hepatoblastoma, metastatic, liver																X										2
Hepatocellular carcinoma, metastatic, liver		X		X		X										X			X					X		8
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
External ear, fibrosarcoma																										1
Harderian gland								+																		1
Adenoma								X																		1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																										1
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																				X						2

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 5,000 ppm

Number of Days on Study	0 0 0 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 0 0 0 0 1 1 1 1 5 5 5 7 7 7 7 9 9 9 0 2 3 3 4 4 5 6 7 7 3 0 2 4 5 0 4 7 0 1 8 9 1 4 9 9 4 6 3 5 1 7 4
Carcass ID Number	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 7 5 6 4 7 5 7 2 3 6 4 3 5 7 5 6 7 7 7 2 5 4 5 6 4 0 3 0 7 3 7 6 3 2 4 4 8 2 1 0 8 8 5 7 1 4 3 6 1 9
Alimentary System	
Esophagus	+ +
Gallbladder	+ + + + + M + + + + + + + + + + + + + + + + M + M
Intestine large, colon	+ +
Intestine large, rectum	+ +
Intestine large, cecum	+ +
Intestine small, duodenum	+ +
Intestine small, jejunum	+ +
Intestine small, ileum	+ +
Liver	+ +
Hepatoblastoma	X X
Hepatoblastoma, multiple	
Hepatocellular carcinoma	
Hepatocellular carcinoma, multiple	X X X X X X X X X X X X X X X X X X X X X
Hepatocellular adenoma	X X
Hepatocellular adenoma, multiple	X X X X X X X X X X X X X X X X X X X X X
Pancreas	+ +
Salivary glands	+ +
Stomach, forestomach	+ +
Stomach, glandular	+ +
Cardiovascular System	
Blood vessel	M +
Heart	+ +
Endocrine System	
Adrenal cortex	+ +
Adrenal medulla	+ +
Islets, pancreatic	+ +
Parathyroid gland	+ + + + M M + + + + M + + + + + M M + + + + + M M
Pituitary gland	+ +
Thyroid gland	+ +
Follicular cell, adenoma	X X
General Body System	
None	
Genital System	
Clitoral gland	+ + + M + M + + + + + + M M + + + + + + + + + + +
Ovary	+ +
Uterus	+ + + + + M + + + + + + + + + + + + + + + + + + +
Hematopoietic System	
Bone marrow	+ +
Lymph node, mandibular	+ + M M + + + + + + + + + + + + M + + + + + + M + +
Lymph node, mesenteric	+ M + M M M + + + + + + + + + + + + M + M + + + + +
Spleen	+ + + A +
Thymus	+ + + + + + + + + + + + + M + + + + M M M + + + +

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	5 6 2	5 6 3	5 6 6	5 6 7	5 6 8	5 6 9	5 6 9	5 7 0	5 7 0	5 7 7	5 8 2	5 8 3	5 8 9	5 9 4	5 9 5	5 9 6	5 9 6	6 0 0	6 1 2	6 1 3	6 1 4	6 1 4	6 1 7	6 1 7	6 1 7	
Carcass ID Number	4 6 7	4 6 2	4 6 4	4 4 1	4 4 0	4 3 4	4 7 4	4 5 9	4 8 0	4 2 5	4 6 9	4 6 3	4 6 5	4 5 5	4 3 0	4 3 5	4 3 9	4 5 8	4 4 6	4 4 2	4 2 9	4 3 7	4 2 2	4 3 3	4 5 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	M	M	M	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	M	M	+	40
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma			X				X						X					X						X		7
Hepatoblastoma, multiple														X												1
Hepatocellular carcinoma							X																			1
Hepatocellular carcinoma, multiple	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	43
Hepatocellular adenoma								X											X	X			X			6
Hepatocellular adenoma, multiple		X		X	X	X	X		X				X	X	X	X		X						X	X	30
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma						X											X			X	X					6
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Lymph node, mesenteric	M	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	M	+	+	M	+	M	37
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	M	M	M	+	M	M	M	+	+	+	+	M	+	+	+	M	+	M	M	+	M	M	+	34

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	0	0	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5
	0	0	0	0	1	1	1	1	5	5	5	7	7	7	7	9	9	9	0	2	3	3	4	4	5
	6	7	7	3	0	2	4	5	0	4	7	0	1	8	9	1	4	9	4	6	3	5	1	7	4
Carcass ID Number	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	7	5	6	4	7	5	7	2	3	6	4	3	5	7	5	6	7	7	7	2	5	4	5	6	4
	0	3	0	7	3	7	6	3	2	4	4	8	2	1	0	8	8	5	7	1	4	3	6	1	9
Integumentary System																									
Mammary gland	M	M	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																									
Spinal cord																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Hepatoblastoma, metastatic, liver													X												
Hepatocellular carcinoma, metastatic, liver					X										X								X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland															+										
Adenoma															X										
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam: 5,000 ppm (continued)

Number of Days on Study	5 6 2	5 6 3	5 6 6	5 6 7	5 6 8	5 6 9	5 6 9	5 7 0	5 7 0	5 7 7	5 8 2	5 8 3	5 8 9	5 9 4	5 9 5	5 9 6	5 9 6	6 0 0	6 1 2	6 1 3	6 1 4	6 1 4	6 1 7	6 1 7	6 1 7	
Carcass ID Number	4 6 7	4 6 2	4 4 4	4 4 1	4 4 0	4 3 4	4 7 4	4 5 9	4 8 0	4 2 5	4 6 9	4 6 3	4 6 5	4 5 5	4 3 0	4 3 5	4 3 9	4 5 8	4 4 6	4 4 2	4 2 9	4 3 7	4 2 2	4 3 3	4 5 1	Total Tissues/ Tumors
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	43
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve							+																			1
Spinal cord							+																			1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																	X									1
Hepatoblastoma, metastatic, liver																		X								2
Hepatocellular carcinoma, metastatic, liver	X			X	X		X								X					X	X					10
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland																										1
Adenoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D3
Statistical Analysis of Primary Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Harderian Gland: Adenoma				
Overall rate[a]	1/50 (2%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate[b]	2.0%	7.1%	5.6%	2.8%
Terminal rate[c]	0/39 (0%)	2/41 (5%)	0/2 (0%)	0/0
First incidence (days)	516	732	710	479
Life table test[d]	P=0.202	P=0.318	P=0.673	P=0.673
Logistic regression test[d]	P=0.300N	P=0.301	P=0.706N	P=0.619N
Cochran-Armitage test[d]	P=0.320N			
Fisher exact test[d]		P=0.309	P=0.753N	P=0.753N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	4.2%	7.1%	5.6%	2.8%
Terminal rate	0/39 (0%)	2/41 (5%)	0/2 (0%)	0/0
First incidence (days)	516	732	710	479
Life table test	P=0.304	P=0.512	P=0.599N	P=0.673
Logistic regression test	P=0.159N	P=0.495	P=0.407N	P=0.305N
Cochran-Armitage test	P=0.214N			
Fisher exact test		P=0.500	P=0.500N	P=0.500N
Liver: Hepatoblastoma				
Overall rate	0/50 (0%)	1/50 (2%)	8/50 (16%)	8/50 (16%)
Adjusted rate	0.0%	2.3%	31.7%	57.8%
Terminal rate	0/39 (0%)	0/41 (0%)	0/2 (0%)	0/0
First incidence (days)	–[e]	714	614	471
Life table test	P<0.001	P=0.519	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.502	P=0.007	P=0.003
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.500	P=0.003	P=0.003
Liver: Hepatocellular Adenoma				
Overall rate	25/50 (50%)	35/50 (70%)	35/50 (70%)	36/50 (72%)
Adjusted rate	59.3%	79.5%	96.7%	100.0%
Terminal rate	22/39 (56%)	32/41 (78%)	1/2 (50%)	0/0
First incidence (days)	598	471	393	403
Life table test	P<0.001	P=0.062	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.037	P=0.014	P=0.001
Cochran-Armitage test	P=0.064			
Fisher exact test		P=0.033	P=0.033	P=0.020
Liver: Hepatocellular Carcinoma				
Overall rate	9/50 (18%)	5/50 (10%)	49/50 (98%)	44/50 (88%)
Adjusted rate	21.6%	11.9%	100.0%	100.0%
Terminal rate	7/39 (18%)	4/41 (10%)	2/2 (100%)	0/0
First incidence (days)	598	714	393	410
Life table test	P<0.001	P=0.173N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.185N	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.194N	P<0.001	P<0.001

TABLE D3
Statistical Analysis of Primary Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Liver: Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma				
Overall rate	28/50 (56%)	36/50 (72%)	50/50 (100%)	47/50 (94%)
Adjusted rate	66.5%	81.8%	100.0%	100.0%
Terminal rate	25/39 (64%)	33/41 (80%)	2/2 (100%)	0/0
First incidence (days)	598	471	393	403
Life table test	P<0.001	P=0.126	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.084	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.072	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adjusted rate	10.3%	2.4%	0.0%	10.0%
Terminal rate	4/39 (10%)	1/41 (2%)	0/2 (0%)	0/0
First incidence (days)	735 (T)	735 (T)	–	596
Life table test	P=0.111	P=0.165N	P=0.769N	P=0.197
Logistic regression test	P=0.638N	P=0.165N	P=0.769N	P=0.665
Cochran-Armitage test	P=0.152N			
Fisher exact test		P=0.181N	P=0.059N	P=0.181N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.6%	7.3%	2.9%	0.0%
Terminal rate	1/39 (3%)	3/41 (7%)	0/2 (0%)	0/0
First incidence (days)	735 (T)	735 (T)	645	–
Life table test	P=0.390	P=0.323	P=0.502	–
Logistic regression test	P=0.578N	P=0.323	P=0.762	–
Cochran-Armitage test	P=0.116N			
Fisher exact test		P=0.309	P=0.753N	P=0.500N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted rate	12.8%	9.8%	2.9%	10.0%
Terminal rate	5/39 (13%)	4/41 (10%)	0/2 (0%)	0/0
First incidence (days)	735 (T)	735 (T)	645	596
Life table test	P=0.042	P=0.468N	P=0.615	P=0.197
Logistic regression test	P=0.458N	P=0.468N	P=0.247N	P=0.662
Cochran-Armitage test	P=0.038N			
Fisher exact test		P=0.500N	P=0.102N	P=0.102N
Ovary: Cystadenoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/48 (2%)	0/50 (0%)
Adjusted rate	2.6%	7.0%	50.0%	0.0%
Terminal rate	1/39 (3%)	2/41 (5%)	1/2 (50%)	0/0
First incidence (days)	735 (T)	701	735 (T)	–
Life table test	P=0.328	P=0.331	P=0.090	–
Logistic regression test	P=0.708	P=0.319	P=0.090	–
Cochran-Armitage test	P=0.118N			
Fisher exact test		P=0.309	P=0.742	P=0.500N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	2/50 (4%)	8/49 (16%)	0/43 (0%)	0/49 (0%)
Adjusted rate	5.1%	20.0%	0.0%	0.0%
Terminal rate	2/39 (5%)	8/40 (20%)	0/2 (0%)	0/0
First incidence (days)	735 (T)	735 (T)	–	–
Life table test	P=0.813N	P=0.051	P=0.910N	–
Logistic regression test	P=0.813N	P=0.051	P=0.910N	–
Cochran-Armitage test	P=0.005N			
Fisher exact test		P=0.043	P=0.286N	P=0.253N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	0.0%	9.8%	34.3%	46.1%
Terminal rate	0/39 (0%)	4/41 (10%)	0/2 (0%)	0/0
First incidence (days)	–	735 (T)	663	470
Life table test	P<0.001	P=0.070	P=0.001	P<0.001
Logistic regression test	P=0.007	P=0.070	P=0.019	P=0.017
Cochran-Armitage test	P=0.042			
Fisher exact test		P=0.059	P=0.028	P=0.013
All Organs (Malignant Lymphoma): Lymphocytic and Mixed				
Overall rate	3/50 (6%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	7.2%	7.0%	13.9%	0.0%
Terminal rate	2/39 (5%)	2/41 (5%)	0/2 (0%)	0/0
First incidence (days)	622	662	393	–
Life table test	P=0.309	P=0.636N	P=0.233	–
Logistic regression test	P=0.072N	P=0.662	P=0.563N	P=0.457N
Cochran-Armitage test	P=0.090N			
Fisher exact test		P=0.661N	P=0.661N	P=0.121N
All Organs: Malignant Lymphoma or Histiocytic Sarcoma				
Overall rate	4/50 (8%)	5/50 (10%)	3/50 (6%)	0/50 (0%)
Adjusted rate	9.3%	11.4%	13.9%	0.0%
Terminal rate	2/39 (5%)	3/41 (7%)	0/2 (0%)	0/0
First incidence (days)	622	662	393	–
Life table test	P=0.475	P=0.536	P=0.415	–
Logistic regression test	P=0.017N	P=0.497	P=0.378N	P=0.134N
Cochran-Armitage test	P=0.025N			
Fisher exact test		P=0.500	P=0.500N	P=0.059N
All Organs: Benign Neoplasms				
Overall rate	29/50 (58%)	40/50 (80%)	38/50 (76%)	38/50 (76%)
Adjusted rate	67.2%	88.9%	100.0%	100.0%
Terminal rate	25/39 (64%)	36/41 (88%)	2/2 (100%)	0/0
First incidence (days)	516	471	393	403
Life table test	P<0.001	P=0.041	P<0.001	P<0.001
Logistic regression test	P=0.001	P=0.015	P=0.022	P=0.002
Cochran-Armitage test	P=0.170			
Fisher exact test		P=0.015	P=0.044	P=0.044

TABLE D3
Statistical Analysis of Primary Neoplasms in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
All Organs: Malignant Neoplasms				
Overall rate	18/50 (36%)	15/50 (30%)	49/50 (98%)	44/50 (88%)
Adjusted rate	38.4%	34.0%	100.0%	100.0%
Terminal rate	11/39 (28%)	12/41 (29%)	2/2 (100%)	0/0
First incidence (days)	357	662	393	410
Life table test	P<0.001	P=0.296N	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.344N	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.335N	P<0.001	P<0.001
All Organs: Benign and Malignant Neoplasms				
Overall rate	37/50 (74%)	43/50 (86%)	50/50 (100%)	47/50 (94%)
Adjusted rate	78.5%	93.5%	100.0%	100.0%
Terminal rate	29/39 (74%)	38/41 (93%)	2/2 (100%)	0/0
First incidence (days)	357	471	393	403
Life table test	P<0.001	P=0.266	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.107	P<0.001	P=0.048
Cochran-Armitage test	P=0.002			
Fisher exact test		P=0.105	P<0.001	P=0.006

(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, 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 control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 D4a
Historical Incidence of Liver Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls			
	Hepatoblastoma	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma
Historical Incidence at Battelle Columbus				
2,4-Dichlorophenol	0/50	0/50	2/50	2/50
4,4-Thiobis(6-*t*-butyl-*m*-cresol)[b]	0/51	17/51	4/51	20/51
Diphenylhydantoin[c]	0/48	5/48	0/48	5/48
Dowicide EC-7 pentachlorophenol	0/34	1/34	0/34	1/34
Ethylene thiourea[c]	0/50	2/50	2/50	4/50
Firemaster FF-1 polybrominated biphenyl[c]	0/50	4/50	1/50	5/50
Manganese (II) sulfate monohydrate	0/51	12/51	3/51	13/51
Technical grade pentachlorophenol	0/33	3/33	0/33	3/33
Triamterene[b]	0/50	10/50	4/50	13/50
Triamterene[b]	0/50	7/50	5/50	10/50
Tricresyl phosphate[b]	0/50	12/50	10/50	21/50
Overall Historical Incidence				
Total	1/1,363 (0.1%)	159/1,363 (11.7%)	80/1,363 (5.9%)	223/1,363 (16.4%)
Standard deviation	0.4%	8.3%	5.5%	10.7%
Range	0%-2%	0%-33%	0%-20%	3%-42%

[a] Data as of 20 August 1992
[b] Mice housed individually
[c] Mice housed individually after becoming pregnant

TABLE D4b
Historical Incidence of Thyroid Gland Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Follicular Cell Adenoma	Follicular Cell Carcinoma	Follicular Cell Adenoma or Carcinoma
Historical Incidence at Battelle Columbus			
2,4-Dichlorophenol	1/49	0/49	1/49
4,4-Thiobis(6-*t*-butyl-*m*-cresol)[b]	0/51	0/51	0/51
Diphenylhydantoin[c]	4/47	0/47	4/47
Dowicide EC-7 pentachlorophenol	3/34	0/34	3/34
Ethylenethiourea[c]	0/50	0/50	0/50
Firemaster FF-1 polybrominated biphenyl[c]	0/49	0/49	0/49
Manganese (II) sulfate monohydrate	2/50	0/50	2/50
Technical grade pentachlorophenol	0/33	0/33	0/33
Triamterene[b]	1/49	1/49	2/49
Triamterene[b]	0/50	0/50	0/50
Tricresyl phosphate[b]	1/49	0/49	1/49
Overall Historical Incidence			
Total	32/1,348 (2.4%)	2/1,348 (0.1%)	34/1,348 (2.5%)
Standard deviation	2.8%	0.5%	2.9%
Range	0%-9%	0%-2%	0%-9%

[a] Data as of 20 August 1992
[b] Mice housed individually
[c] Mice housed individually after becoming pregnant

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	8	7	22	15
Natural deaths	3	2	26	35
Survivors				
Terminal sacrifice	39	41	2	
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Eosinophilic focus	1 (10%)		8 (80%)	7 (70%)
Infarct				1 (10%)
Hepatocyte, centrilobular, hypertrophy			10 (100%)	10 (100%)
Pancreas	(10)	(10)	(10)	(10)
Ectopic liver			1 (10%)	
Infarct, focal				1 (10%)
Acinus, atrophy	1 (10%)	1 (10%)		
Stomach, glandular	(10)	(10)	(10)	(10)
Cyst epithelial inclusion		1 (10%)		
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hyperplasia				1 (10%)
Capsule, accessory adrenal cortical nodule		1 (10%)	1 (10%)	
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, hyperplasia		1 (10%)	2 (20%)	2 (20%)
Thyroid gland	(10)	(10)	(10)	(10)
Inflammation, chronic active, focal		1 (10%)		
Follicular cell, hyperplasia			10 (100%)	10 (100%)
General Body System				
None				
Genital System				
Ovary	(10)	(10)	(10)	(10)
Follicle, cyst	2 (20%)	2 (20%)	1 (10%)	
Uterus	(10)	(10)	(10)	(10)
Endometrium, hyperplasia, cystic, glandular	10 (100%)	10 (100%)	10 (100%)	8 (80%)

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam (continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
15-Month Interim Evaluation (continued)				
Hematopoietic System				
Spleen	(10)	(10)	(10)	(10)
Developmental malformation			1 (10%)	
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar epithelium, hyperplasia, focal				1 (10%)
Arteriole, infiltration cellular, lymphocyte			1 (10%)	
Special Senses System				
None				
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Nephropathy, chronic	3 (30%)	3 (30%)	1 (10%)	2 (20%)
2-Year Study				
Alimentary System				
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation, granulomatous				1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid	1 (2%)		1 (2%)	
Liver	(50)	(50)	(50)	(50)
Basophilic focus	4 (8%)			
Clear cell focus	2 (4%)	3 (6%)		
Congestion				1 (2%)
Developmental malformation		1 (2%)		
Eosinophilic focus	9 (18%)	19 (38%)	2 (4%)	5 (10%)
Hematopoietic cell proliferation, focal				1 (2%)
Hemorrhage			1 (2%)	1 (2%)
Infiltration cellular, lymphocyte			1 (2%)	
Mixed cell focus				1 (2%)
Necrosis, multifocal	2 (4%)			1 (2%)
Thrombosis				2 (4%)

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam
(continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Liver (continued)	(50)	(50)	(50)	(50)
Vacuolization cytoplasmic	1 (2%)			
Bile duct, hyperplasia, focal		1 (2%)		
Centrilobular, hypertrophy		2 (4%)	11 (22%)	29 (58%)
Mesentery	(3)	(3)		
Artery, inflammation, chronic		1 (33%)		
Fat, inflammation, chronic		1 (33%)		
Pancreas	(50)	(50)	(50)	(50)
Inflammation, granulomatous				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Dysplasia	1 (2%)			
Hyperplasia, diffuse, squamous			1 (2%)	
Hyperplasia, focal, squamous	1 (2%)	1 (2%)		
Hyperplasia, multifocal, squamous			1 (2%)	
Ulcer	2 (4%)	1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Congestion, multifocal				1 (2%)
Blood vessel	(50)	(50)	(50)	(49)
Aorta, mineralization	1 (2%)			
Artery, inflammation, chronic		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Mineralization	1 (2%)			
Atrium, inflammation, chronic		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Hyperplasia	1 (2%)			
Hypertrophy	2 (4%)	1 (2%)		2 (4%)
Vacuolization cytoplasmic	2 (4%)			
Pituitary gland	(50)	(49)	(43)	(49)
Pars distalis, angiectasis	2 (4%)	2 (4%)		
Pars distalis, cyst	2 (4%)		1 (2%)	
Pars distalis, hyperplasia	6 (12%)	8 (16%)	4 (9%)	1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte				1 (2%)
Follicular cell, hyperplasia	16 (32%)	34 (68%)	49 (98%)	44 (88%)
General Body System				
None				
Genital System				
Clitoral gland	(48)	(47)	(47)	(46)
Duct, ectasia		1 (2%)		1 (2%)

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam
(continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Genital System (continued)				
Ovary	(50)	(50)	(48)	(50)
Atrophy	39 (78%)	39 (78%)	44 (92%)	38 (76%)
Cyst	2 (4%)			
Mineralization			1 (2%)	
Bilateral, follicle, cyst		1 (2%)		
Follicle, cyst	13 (26%)	14 (28%)	3 (6%)	5 (10%)
Periovarian tissue, cyst		1 (2%)	1 (2%)	
Uterus	(50)	(50)	(50)	(49)
Endometrium, hyperplasia, cystic	46 (92%)	50 (100%)	19 (38%)	14 (29%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Myelofibrosis			1 (2%)	
Calvarium, myelofibrosis		1 (2%)		
Myeloid cell, erythroid cell, atrophy		1 (2%)		
Lymph node	(3)	(2)	(1)	
Bronchial, hyperplasia, lymphoid	1 (33%)			
Lymph node, mandibular	(48)	(47)	(48)	(44)
Atrophy			1 (2%)	
Hyperplasia, plasma cell	1 (2%)			
Lymph node, mesenteric	(48)	(46)	(38)	(37)
Atrophy			1 (3%)	
Hematopoietic cell proliferation		1 (2%)		
Hemorrhage				1 (3%)
Hyperplasia, lymphoid	1 (2%)			
Lymph node, mediastinal	(1)	(2)	(3)	
Angiectasis			1 (33%)	
Spleen	(50)	(50)	(50)	(49)
Hematopoietic cell proliferation	13 (26%)	4 (8%)	13 (26%)	4 (8%)
Hyperplasia, focal, reticulum cell	1 (2%)			
Hyperplasia, lymphoid	2 (4%)	1 (2%)		
Capsule, mineralization	1 (2%)			
Lymphoid follicle, atrophy	2 (4%)			
Lymphoid follicle, degeneration		1 (2%)		
Red pulp, atrophy	2 (4%)	1 (2%)		
Thymus	(50)	(49)	(34)	(34)
Atrophy	41 (82%)	44 (90%)	30 (88%)	22 (65%)
Hyperplasia, focal, lymphoid	1 (2%)			
Integumentary System				
Mammary gland	(50)	(49)	(47)	(43)
Hyperplasia, cystic	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Ulcer, multifocal		1 (2%)		
Dermis, sebaceous gland, hyperplasia		1 (2%)		

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam
(continued)

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Femur, hyperostosis, focal	1 (2%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression		1 (2%)		
Peripheral nerve	(2)	(3)	(2)	(1)
Sciatic, axon, degeneration	2 (100%)	3 (100%)	1 (50%)	1 (100%)
Spinal cord	(2)	(3)	(2)	(1)
Degeneration	1 (50%)	1 (33%)	1 (50%)	
Nerve, degeneration	1 (50%)	3 (100%)	1 (50%)	1 (100%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Infiltration cellular, lymphocyte			1 (2%)	
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Special Senses System				
Eye	(2)	(1)		
Phthisis bulbi	1 (50%)	1 (100%)		
Harderian gland	(3)	(3)	(1)	(1)
Cyst	1 (33%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Inflammation, subacute				1 (2%)
Nephropathy, chronic	14 (28%)	12 (24%)	9 (18%)	3 (6%)
Artery, pelvis, inflammation, chronic		1 (2%)		
Renal tubule, necrosis, acute, diffuse		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation, chronic				1 (2%)
Ulcer				1 (2%)

[a] Number of animals examined microscopically at site and number of animals with lesion

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1992). Oxazepam was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). Oxazepam was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA102, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. 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 oxazepam. The high dose was limited by solubility and toxicity. All assays 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 is not dose related, not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. No minimum percentage or fold increase is required for a chemical to be judged positive or weakly positive.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). Oxazepam was sent to the laboratory as a coded aliquot by Radian Corporation. Oxazepam 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 oxazepam. The high dose was limited by toxicity. A single flask per dose was used, and tests yielding positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with oxazepam 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 oxazepam 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 oxazepam, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no oxazepam and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. 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. Because significant chemical-induced cell cycle delay was seen at the 50 μg/mL dose without S9, incubation time was lengthened to ensure a sufficient number of scorable (second-division metaphase) cells.

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 ($P \leq 0.05$) 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 oxazepam 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 oxazepam and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

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. Two 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 ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) 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).

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay can be found in MacGregor *et al.* (1990). Peripheral blood samples were obtained from 10 male and 10 female $B6C3F_1$ mice from each dose group at the end of the 14-week toxicity study. Smears were immediately prepared and fixed in absolute methanol, stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983), and coded. Slides were scanned to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) per animal. The criteria of Schmidt (1976) were used to define micronuclei, with the additional requirement that the micronuclei exhibit the characteristic fluorescent emissions of DNA (blue with 360 nm and orange with 510 nm UV illumination); the minimum size limit was approximately one-twentieth the diameter of the NCE cell. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package (ILS, 1990), which employed a one-tailed trend test across dose groups and a *t*-test for pairwise comparisons of each dose group to the concurrent control.

RESULTS

Oxazepam (3 to 3,333 μg/plate) did not induce mutations in *Salmonella typhimurium* strains TA102, TA100, TA1535, TA97, or TA98 when tested in a preincubation protocol with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table E1). In cytogenetic tests with Chinese hamster ovary cells, oxazepam did not induce sister chromatid exchanges (Table E2) or chromosomal aberrations (Table E3), with or without S9. Cell cycle delay was noted at the 50 μg/mL dose in the SCE test without S9; harvest time was extended to allow accumulation of sufficient second-division metaphase cells for analysis. Peripheral blood samples obtained from $B6C3F_1$ mice in the 14-week toxicity study were analyzed for frequency of micronucleated NCEs; no increase in micronucleated NCEs was observed in any of the dose groups (Table E4).

TABLE E1
Mutagenicity of Oxazepam in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 10%	+ rat S9 30%
TA102						
	0	131 ± 2.6	213 ± 5.7	341 ± 23.3	197 ± 11.5	443 ± 24.8
	3	136 ± 5.7				
	10	128 ± 10.7				
	33	139 ± 1.8	223 ± 15.0	295 ± 8.1	216 ± 9.5	403 ± 17.1
	100	130 ± 8.3	196 ± 10.0	340 ± 24.6	208 ± 6.4	436 ± 38.4
	333	43 ± 3.9[c]	170 ± 2.7	358 ± 25.0	204 ± 14.3	413 ± 22.0
	1,000	155 ± 12.4	318 ± 1.3	166 ± 28.6	375 ± 19.9	
	1,666		103 ± 21.5[c]		102[c]	
	3,333		172 ± 1.2[c]		133 ± 17.3[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control[d]		676 ± 9.5	879 ± 17.9	2,127 ± 104.3	1,291 ± 49.3	1,104 ± 125.0
TA100						
	0	159 ± 8.0	146 ± 1.9	145 ± 8.1	161 ± 10.0	149 ± 2.5
	3	118 ± 4.6				
	10	128 ± 5.5	144 ± 2.3		160 ± 9.0	
	33	137 ± 14.0	159 ± 17.2	145 ± 9.1	157 ± 8.9	137 ± 7.4
	100	139 ± 8.9	137 ± 15.4	146 ± 2.1	156 ± 8.9	127 ± 1.2
	333	115 ± 9.7	141 ± 2.9	113 ± 8.7	133 ± 7.0	134 ± 4.8
	1,000	102 ± 5.8		93 ± 2.8	126 ± 2.5	134 ± 4.1
	1,666			100 ± 14.9	135 ± 13.2	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		316 ± 3.5	445 ± 11.7	462 ± 31.2	599 ± 23.1	244 ± 6.7
TA1535						
	0	20 ± 1.5	8 ± 0.9	12 ± 2.9	12 ± 1.7	14 ± 2.1
	3	20 ± 2.3				
	10	19 ± 1.5				
	33	19 ± 2.9	8 ± 3.0	10 ± 2.0	10 ± 2.0	14 ± 0.3
	100	15 ± 4.2	11 ± 2.0	11 ± 3.4	11 ± 1.5	15 ± 1.5
	333	18 ± 2.0	8 ± 1.0	9 ± 2.5	11 ± 0.6	15 ± 1.9
	1,000		9 ± 1.3	8 ± 0.3	9 ± 0.7	12 ± 2.7
	1,666		8 ± 1.2[e]		7 ± 1.5[e]	
	3,333			5 ± 0.7[e]		9 ± 0.6[e]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		332 ± 10.2	180 ± 18.4	340 ± 22.8	214 ± 27.7	206 ± 4.7

TABLE E1
Mutagenicity of Oxazepam in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA97						
	0	184 ± 6.1	179 ± 3.0	164 ± 8.3	206 ± 1.5	209 ± 6.1
	3	193 ± 7.4				
	10	182 ± 2.6	163 ± 8.2		204 ± 4.2	
	33	180 ± 10.5	163 ± 5.0	183 ± 6.9	203 ± 0.9	191 ± 9.1
	100	160 ± 7.3	167 ± 9.0	187 ± 12.4	200 ± 3.1	150 ± 6.2
	333	148 ± 12.7	175 ± 11.0	174 ± 14.0	198 ± 3.0	162 ± 17.4
	1,000		172 ± 17.3	168 ± 9.1	168 ± 5.8	184 ± 16.8
	3,333			107 ± 5.7[c]		139 ± 11.2[c]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		403 ± 11.1	463 ± 15.8	368 ± 24.9	449 ± 10.1	454 ± 25.6
TA98						
	0	18 ± 2.3	28 ± 2.0	19 ± 0.9	19 ± 1.5	23 ± 1.2
	3	15 ± 2.2				
	10	16 ± 2.0				
	33	19 ± 2.3	18 ± 0.6	27 ± 4.3	24 ± 3.0	28 ± 2.2
	100	20 ± 0.7	21 ± 2.1	27 ± 4.1	22 ± 3.7	33 ± 2.6
	333	17 ± 1.7	20 ± 0.3	23 ± 3.3	23 ± 4.6	31 ± 4.7
	1,000		20 ± 2.3	34 ± 2.3	14 ± 0.7	23 ± 1.7
	1,666		17 ± 0.6[e]	27 ± 1.5	22 ± 3.5[e]	21 ± 2.3
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		423 ± 27.7	523 ± 10.6	495 ± 21.7	202 ± 10.9	104 ± 5.9

[a] Study performed at SRI, International. A detailed description of the protocol is presented in Zeiger *et al.* (1992).
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] 2-aminoanthracene was used on all strains in the presence of S9; in the absence of S9, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, 9-aminoacridine was tested on TA97, and mitomycin-C was tested on TA102.
[e] Precipitate on plate

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Oxazepam[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[b]
-S9								
Summary: Negative								
Dimethylsulfoxide		50	1,049	424	0.40	8.5	26.0	
		50	1,045	393	0.37	7.9	31.0[c]	
Mitomycin-C	0.001	50	1,048	579	0.55	11.6	26.0	46.91
	0.004	10	210	223	1.06	22.3	26.0	182.37
Oxazepam	5	50	1,048	448	0.42	9.0	26.0	13.67
	17	50	1,048	428	0.40	8.6	26.0	8.59
	50	50	1,048	449	0.42	9.0	31.0[c]	13.92
								P=0.061[d]
+S9								
Summary: Negative								
Dimethylsulfoxide		50	1,046	402	0.38	8.0	26.0	
Cyclophosphamide	0.125	50	1,043	579	0.55	11.6	26.0	44.44
	0.500	10	209	205	0.98	20.5	26.0	155.22
Oxazepam	5	50	1,048	452	0.43	9.0	26.0	12.22
	17	50	1,050	460	0.43	9.2	26.0	13.99
	50	50	1,048	445	0.42	8.9	26.0	10.48
								P=0.075

[a] Study performed at Sitek Research Laboratories. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the protocol is presented in Galloway *et al.* (1987).
[b] SCEs/chromosome of culture exposed to oxazepam relative to those of culture exposed to solvent
[c] Because oxazepam induced a delay in the cell division cycle, harvest time was extended to maximize the number of second-division metaphase cells available for analysis.
[d] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Oxazepam[a]

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Harvest time: 14.0 hours Summary: Negative					Harvest time: 12.0 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	200	5	0.03	1.0		200	1	0.01	0.5
Mitomycin-C					Cyclophosphamide				
0.4	25	9	0.36	36.0	20	25	12	0.48	32.0
Oxazepam					Oxazepam				
25	200	1	0.01	0.5	43	200	0	0.00	0.0
54	200	3	0.02	1.0	93	200	3	0.02	1.5
116	200	0	0.00	0.0	200	200	2	0.01	1.0
				P=0.842[b]					P=0.135

[a] Study performed at Sitek Research Laboratories. Abs = aberrations. A detailed description of the protocol is 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 E4
Frequency of Micronuclei in B6C3F$_1$ Mouse Peripheral Blood Erythrocytes Following Administration of Oxazepam in Feed for 14 Weeks[a]

	Dose (ppm)	Percent Micronucleated NCE Cells
Male	0	0.082 ± 0.008
	625	0.081 ± 0.009
	1,250	0.078 ± 0.007
	2,500	0.085 ± 0.010
	5,000	0.074 ± 0.008
	10,000	0.069 ± 0.007
		P=0.899[b]
Female	0	0.042 ± 0.006
	625	0.039 ± 0.005
	1,250	0.034 ± 0.007
	2,500	0.031 ± 0.005
	5,000	0.042 ± 0.005
	10,000	0.043 ± 0.007
		P=0.194

[a] NCE = normochromatic erythrocyte. A minimum of 10,000 NCEs scored per animal, 10 animals per dose group; data presented as mean ± standard error. A detailed description of the protocol is presented in MacGregor *et al.* (1990).
[b] One-tailed trend test

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

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	10	10	10	9
Necropsy body wt	35.8 ± 0.8	38.5 ± 0.9	35.2 ± 0.7	38.2 ± 1.1	35.5 ± 1.0	35.4 ± 0.7
Heart						
Absolute	0.179 ± 0.009	0.174 ± 0.007	0.144 ± 0.004**	0.159 ± 0.006	0.162 ± 0.008	0.141 ± 0.005**
Relative	5.00 ± 0.24	4.53 ± 0.23	4.10 ± 0.12**	4.19 ± 0.20*	4.59 ± 0.24	3.98 ± 0.10**
R. Kidney						
Absolute	0.292 ± 0.010	0.294 ± 0.006	0.280 ± 0.012	0.280 ± 0.012	0.270 ± 0.008	0.269 ± 0.012
Relative	8.18 ± 0.23	7.66 ± 0.21	7.94 ± 0.27	7.35 ± 0.28	7.62 ± 0.26	7.59 ± 0.25
Liver						
Absolute	1.808 ± 0.080	2.354 ± 0.074**	2.275 ± 0.112*	2.761 ± 0.134**	3.035 ± 0.115**	3.528 ± 0.137**
Relative	50.43 ± 1.35	61.23 ± 1.88	64.60 ± 2.75**	72.61 ± 3.92**	85.52 ± 2.61**	99.98 ± 4.27**
Lungs						
Absolute	0.251 ± 0.009	0.293 ± 0.020	0.266 ± 0.012	0.283 ± 0.011	0.290 ± 0.014	0.245 ± 0.008
Relative	7.04 ± 0.26	7.63 ± 0.56	7.59 ± 0.37	7.43 ± 0.26	8.14 ± 0.32	6.95 ± 0.32
R. Testis						
Absolute	0.097 ± 0.003	0.099 ± 0.004	0.095 ± 0.004	0.100 ± 0.004	0.093 ± 0.002	0.096 ± 0.004
Relative	2.71 ± 0.10	2.57 ± 0.10	2.71 ± 0.14	2.65 ± 0.12	2.64 ± 0.07	2.72 ± 0.10
Thymus						
Absolute	0.037 ± 0.003	0.045 ± 0.002	0.045 ± 0.003	0.049 ± 0.004*	0.048 ± 0.003	0.043 ± 0.003
Relative	1.03 ± 0.08	1.16 ± 0.05	1.28 ± 0.08	1.26 ± 0.08	1.34 ± 0.06*	1.23 ± 0.08
Female						
n	10	10	10	10	10	10
Necropsy body wt	29.8 ± 0.6	32.0 ± 0.7	31.3 ± 0.5	32.2 ± 0.8*	31.0 ± 0.6	30.7 ± 0.4
Heart						
Absolute	0.135 ± 0.005	0.137 ± 0.004	0.143 ± 0.008	0.142 ± 0.004	0.136 ± 0.003	0.140 ± 0.005
Relative	4.54 ± 0.17	4.31 ± 0.13	4.59 ± 0.29	4.43 ± 0.17	4.41 ± 0.15	4.56 ± 0.12
R. Kidney						
Absolute	0.204 ± 0.007	0.223 ± 0.006	0.229 ± 0.007*	0.229 ± 0.006*	0.240 ± 0.005**	0.233 ± 0.007**
Relative	6.90 ± 0.31	6.97 ± 0.13	7.30 ± 0.13	7.14 ± 0.20	7.74 ± 0.19*	7.60 ± 0.19
Liver						
Absolute	1.405 ± 0.028	1.731 ± 0.049**	1.910 ± 0.052**	2.328 ± 0.109**	2.610 ± 0.064**	3.084 ± 0.070**
Relative	47.32 ± 1.14	54.28 ± 1.47	61.16 ± 2.12**	72.42 ± 2.77**	84.16 ± 1.93**	100.63 ± 1.95**
Lungs						
Absolute	0.268 ± 0.015	0.256 ± 0.008	0.257 ± 0.008	0.273 ± 0.013	0.260 ± 0.010	0.243 ± 0.010
Relative	9.04 ± 0.57	8.03 ± 0.25	8.22 ± 0.27	8.59 ± 0.55	8.39 ± 0.34	7.92 ± 0.28
Thymus						
Absolute	0.050 ± 0.004	0.049 ± 0.004	0.049 ± 0.002	0.052 ± 0.002	0.050 ± 0.003	0.047 ± 0.003
Relative	1.68 ± 0.11	1.52 ± 0.10	1.57 ± 0.06	1.62 ± 0.07	1.63 ± 0.10	1.52 ± 0.11

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤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)

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	9	10	10	10	10
Necropsy body wt	34.6 ± 0.7	36.3 ± 0.7	35.0 ± 0.6	35.0 ± 0.5	34.0 ± 0.6	33.7 ± 0.6
Heart						
Absolute	0.170 ± 0.009	0.162 ± 0.003	0.172 ± 0.007	0.151 ± 0.004	0.163 ± 0.005	0.165 ± 0.003
Relative	4.90 ± 0.21	4.49 ± 0.13	4.90 ± 0.17	4.33 ± 0.12*	4.79 ± 0.14	4.90 ± 0.10
R. Kidney						
Absolute	0.280 ± 0.008	0.285 ± 0.007	0.296 ± 0.007	0.249 ± 0.005*	0.259 ± 0.007	0.266 ± 0.008
Relative	8.08 ± 0.13	7.86 ± 0.17	8.45 ± 0.14	7.12 ± 0.10**	7.62 ± 0.16	7.89 ± 0.15
Liver						
Absolute	1.634 ± 0.052	1.919 ± 0.044**	2.150 ± 0.037**	2.186 ± 0.038**	2.545 ± 0.065**	2.966 ± 0.057**
Relative	47.24 ± 0.96	52.91 ± 0.67**	61.42 ± 0.47**	62.50 ± 0.94**	74.83 ± 1.09**	88.09 ± 1.19**
Lungs						
Absolute	0.227 ± 0.009	0.229 ± 0.009	0.242 ± 0.010	0.272 ± 0.013*	0.222 ± 0.009	0.271 ± 0.020
Relative	6.55 ± 0.18	6.34 ± 0.30	6.94 ± 0.31	7.75 ± 0.31	6.54 ± 0.30	8.04 ± 0.57*
R. Testis						
Absolute	0.124 ± 0.003	0.127 ± 0.003	0.126 ± 0.002	0.129 ± 0.002	0.125 ± 0.002	0.129 ± 0.003
Relative	3.60 ± 0.08	3.50 ± 0.09	3.59 ± 0.07	3.69 ± 0.07	3.69 ± 0.07	3.85 ± 0.10
Thymus						
Absolute	0.040 ± 0.002	0.054 ± 0.002*	0.052 ± 0.003*	0.057 ± 0.004**	0.050 ± 0.002	0.057 ± 0.005**
Relative	1.16 ± 0.07	1.50 ± 0.06	1.49 ± 0.08	1.64 ± 0.12**	1.47 ± 0.05	1.71 ± 0.15**
Female						
n	10	10	9	10	10	9
Necropsy body wt	29.9 ± 0.6	31.5 ± 0.8	30.4 ± 0.6	30.1 ± 0.5	30.9 ± 0.6	30.3 ± 0.4
Heart						
Absolute	0.135 ± 0.003	0.145 ± 0.003	0.155 ± 0.005**	0.140 ± 0.005	0.144 ± 0.004	0.146 ± 0.003
Relative	4.55 ± 0.14	4.62 ± 0.14	5.11 ± 0.22*	4.67 ± 0.13	4.67 ± 0.09	4.81 ± 0.09
R. Kidney						
Absolute	0.202 ± 0.006	0.241 ± 0.005**	0.247 ± 0.004**	0.222 ± 0.006*	0.228 ± 0.005**	0.232 ± 0.002**
Relative	6.77 ± 0.13	7.67 ± 0.16**	8.15 ± 0.18**	7.38 ± 0.15*	7.39 ± 0.13*	7.68 ± 0.08**
Liver						
Absolute	1.387 ± 0.021	1.743 ± 0.031**	1.871 ± 0.046**	1.898 ± 0.044**	2.391 ± 0.072**	2.837 ± 0.061**
Relative	46.59 ± 1.01	55.44 ± 1.02**	61.54 ± 0.76**	63.20 ± 1.19**	77.35 ± 1.16**	93.64 ± 1.03**
Lungs						
Absolute	0.235 ± 0.010	0.258 ± 0.009	0.240 ± 0.011	0.230 ± 0.010	0.245 ± 0.015	0.240 ± 0.010
Relative	7.91 ± 0.35	8.21 ± 0.33	7.90 ± 0.34	7.66 ± 0.29	7.94 ± 0.43	7.91 ± 0.27
Thymus						
Absolute	0.056 ± 0.002	0.059 ± 0.002	0.058 ± 0.003	0.064 ± 0.004	0.064 ± 0.004	0.056 ± 0.001
Relative	1.86 ± 0.07	1.88 ± 0.05	1.90 ± 0.09	2.13 ± 0.13	2.07 ± 0.12	1.84 ± 0.03

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤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)

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice at the 15-Month Interim Evaluation in the 2-Year Feed Study of Oxazepam[a]

	0 ppm	125 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10
Male				
Necropsy body wt	48.9 ± 1.2	50.0 ± 1.2	40.1 ± 0.6**	35.5 ± 0.7**
R. Kidney				
Absolute	0.388 ± 0.007	0.335 ± 0.010**	0.279 ± 0.005**	0.257 ± 0.007**
Relative	7.96 ± 0.17	6.71 ± 0.13**	6.95 ± 0.13**	7.23 ± 0.10**
Liver				
Absolute	2.264 ± 0.120	2.561 ± 0.196	3.450 ± 0.157*	7.162 ± 0.605**
Relative	46.02 ± 1.35	51.30 ± 3.93	86.39 ± 4.85**	201.34 ± 16.73**
Female				
Necropsy body wt	53.3 ± 2.0	47.3 ± 1.9**	40.2 ± 1.3**	35.8 ± 0.6**
R. Kidney				
Absolute	0.258 ± 0.008	0.248 ± 0.006	0.239 ± 0.007	0.242 ± 0.006
Relative	4.87 ± 0.14	5.29 ± 0.15*	5.95 ± 0.10**	6.75 ± 0.12**
Liver				
Absolute	2.008 ± 0.079	1.874 ± 0.044	3.262 ± 0.312*	6.980 ± 0.729**
Relative	37.76 ± 0.94	39.95 ± 0.99	80.14 ± 5.22**	195.30 ± 20.16**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or 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).

APPENDIX G
NEUROBEHAVIORAL STUDIES

NEUROBEHAVIORAL STUDIES

INTRODUCTION

Although the acute and short-term neurobehavioral effects of benzodiazepines have been fairly extensively investigated in animals, few studies of repeated measures have been performed throughout the course of a prolonged chronic treatment regimen. Therefore, a battery of neurobehavioral assays including forelimb and hindlimb grip strength, thermal sensitivity, motor activity, and startle response were performed on groups of Swiss-Webster and B6C3F$_1$ mice during weeks 2 and 12 of the 14-week studies; before study initiation and during months 6 and 12 of the 57-week study in Swiss-Webster mice; and before study initiation and during months 6, 12, 18, and 24 in the 2-year study in B6C3F$_1$ mice.

METHODS

In the 14-week studies, 10 male and 10 female mice of each strain and exposure group (0, 625, 1,250, 2,500, 5,000, and 10,000 ppm) were tested. Ten male and 10 female mice of each strain and exposure group [0, 125 (B6C3F$_1$ mice only), 2,500, and 5,000 ppm] were also tested in the chronic studies. Thirty animals per day underwent neurobehavioral testing; each testing period occurred over 4 consecutive days. To reduce extraneous variation, animals designated for neurobehavioral testing were placed on a separate cage rack in the study room and were undisturbed for at least 12 hours prior to testing. This rack of animals was moved to the behavior laboratory for acclimation at least 1 hour before testing began. For the most part, testing was limited to the hours between 8:30 a.m. and 1 p.m.

Grip strength. Forelimb and hindlimb grip strength were measured using a device similar to that described by Meyer *et al.* (1979), and data were entered directly into a Xybion® electronic data collection system. The animal was allowed to grip a triangular ring with its forepaws and was pulled back along a platform until its grip was broken. As the backward motion continued, the animal's hindpaws reached a T-shaped hindlimb grip bar, which it was allowed to grasp and then was forced to release by continued pulling. Chatillon push-pull strain gauges (Kew Gardens, NY) were used to record the maximum strain required to break the forelimb and hindlimb grip. Each animal was given five trials with less than 1 minute between trials so that a measure of degree of habituation or fatigue could be observed. The body weight of each animal was recorded.

Thermal sensitivity (analgesia test). A model 550 Analgesia Meter (Omnitech Electronics Inc., Columbus, OH) was used for this test and data were entered directly into Xybion®. The device consisted of a square acrylic plate arena with a clear acrylic plate cover mounted on a heat source to ensure maximum experimenter visibility. The animal was placed on the heat source and the arena covered. The dependent variable was rodent reaction time to the heat stimulus (55° C). The response monitored was characteristically a vigorous licking of the hind paws. Latency was measured manually using a built-in timer. Measurement began when the animal touched the plate and ended at the onset of the pain-sensing response. Animals failing to make the response in 30 seconds were removed and assigned an arbitrary maximum score of 30 seconds.

Motor activity. Motor activity was measured using a photocell movement detection procedure under stress-free conditions. Chambers were sound insulated and darkened, and each contained an individual acrylic plate test cage. A ventilation fan with baffled air intake and exhaust system was mounted in each cubicle, and a 4-inch speaker was used for delivery of 75 dB white noise. A U-shaped photocell/light source holder was placed under the test cages and photo beam detector units were inserted so that infrared photo beams 6 cm apart passed through the test cage just above the cage floor. Animal movement inside the cage interrupted these photo beams and was translated into activity counts by means of modular signal processing equipment (Coulburn Instruments, Lehigh Valley, PA). Motor activity was determined over

five continuous 3-minute periods, with the totals at the end of each period printed on a microprocessor-based, 10-channel printer. This gave an indication of habituation of activity.

Startle response. Startle response is measured using an SR-LAB Startle Response System (San Diego Instruments, San Diego, CA). It is composed of four isolation cabinets, a computer control unit and connection box, four startle chambers, and four test station control boxes. Response measurement takes place within a sound isolated cubicle (Coulburn Instruments Isolation Cubicle Model E10-20) equipped with a light, a ventilation fan, a small viewing port, and a thermometer. The computer unit controls the presentation of all stimuli in four chambers simultaneously. The startle chamber in which the test animal is enclosed is constructed of transparent acrylic. The sensitivity of each chamber may be individually and reproducibly adjusted. A calibration routine is performed prior to each testing session to detect significant sensitivity differences among the chambers. Each test station control box contains a complete sound generation system to produce high frequency noise stimuli up to 120 dB(A), an adjustable background noise level [70 dB(A)], and an AC relay for tactile (air-puff) stimuli.

In the 14-week studies, startle response was examined by measuring the interaction between prepulse inhibition and the habituation of the startle reflex over 80 repeated trials. An acclimation period of 3 minutes was followed by 10 startle trials with a tactile airpuff (15 to 20 psi, 20 msec duration per trial) as the main startle stimulus (block 1). This was followed by 60 prepulse trials in which an 80 to 90 dB(A) white noise prepulse preceded the tactile stimulus by 100 msec (block 2). The final 10 trials were identical to the first 10 trials (block 3). In the chronic studies, each block consisted of 20 trials, for a total of 60 repeated trials. Startle response for each trial was recorded 20 msec after the main startle stimulus was turned off in order to demonstrate a response in which there was no stimulus interference. The 20 msec wait time was added to all latencies. All trials were separated by an 8 second inter-trial interval. A background noise level of 70 dB(A) prevailed when the prepulse or main stimulus was off. Each session took approximately 15 minutes to complete. Inclusion of a prepulse and measurement of the startle response over repeated trials provided data for the initial reactivity, habituation, and prepulse inhibition of the startle response in the same test.

Multiple aspects of the startle response were studied simultaneously. Initial reactivity to an air-puff tactile stimulus is determined by values obtained for block 1 (mean of tactile trials 1-10 or 1-20). Startle amplitude can be modified by presenting very brief, low intensity stimuli (prepulses) shortly before an intense, startle eliciting stimulus. Prepulse inhibition occurs using prepulses at the threshold of audibility which, by themselves, do not elicit startle responses. The inhibitory effect of an auditory prepulse was determined by the responses of block 2 (mean of trials 11-70 or 21-40), and comparison with those of block 1. A general decrease in response amplitude over repeated stimulus presentations was also shown and was referred to as habituation. The extent of habituation was determined by comparison of block 3 (mean of trials 71-80 or 41-60) to block 1. The startle response-dependent variable that was statistically analyzed was the average amplitude across the entire response window of each trial.

Statistical methods. Neurobehavioral data were analyzed by analysis of variance and Dunnett's test (Dunnett, 1955).

RESULTS

Swiss-Webster Mice

14-Week Study

Grip strength. No significant differences in forelimb grip strength were observed in male or female mice during prestudy testing. One week of exposure to oxazepam led to a significant reduction in mean forelimb grip strength in male [$F(5,54) = 2.82$, $P \leq 0.025$] and female [$F(5,54) = 3.11$, $P \leq 0.015$] mice (the two numbers in parentheses represent the degrees of freedom for the F test). Results of an analysis of variance followed by Dunnett's test for comparison of individual means revealed that the 5,000 and

10,000 ppm groups were different from controls during week 2 (Table G1). For males in these groups, the deficits represented a 15% decrease compared to controls. Similar decreases were seen in 5,000 (12%) and 10,000 ppm (14%) females. Forelimb grip strength deficits of the same order of magnitude [$F(5,53) = 4.33$, $P \leq 0.002$] were still evident at week 12 in 625, 5,000, and 10,000 ppm males, but not in females. There were no consistent differences in habituation between exposed and control groups of either sex.

No significant differences in prestudy hindlimb grip strength were observed in male or female mice. Significant deficits in mean hindlimb grip strength were evident in males [$F(5,54) = 4.32$, $P \leq 0.002$] and females [$F(5,54) = 6.19$, $P \leq 0.0001$] during week 2 (Table G2). The 5,000 ppm females and 10,000 ppm males and females had significantly lower grip strength scores compared to those of the controls at 2 weeks. The extent of the hindlimb grip strength deficit was slightly greater than that of the forelimb grip strength. Decreases for those groups significantly different from control were between 20% and 24%. Hindlimb grip strength deficits had disappeared in female mice at week 12, but were still evident to a somewhat lesser degree (8%) in male mice [$F(5,53) = 2.37$, $P \leq 0.051$] compared to controls. Overall, the degree of habituation of hindlimb grip strength across trials was similar in exposed and control mice of each sex at weeks 2 and 12. Decreases in grip strength could not be related to decreases in body weight as all exposed groups had similar or greater body weights than those of control groups.

Thermal sensitivity. No evidence of chemical-related changes in analgesia or pain sensation were indicated in either sex at week 2. However, at week 12, significant decreases in paw lick latency were demonstrated in males [$F(5,53) = 4.13$, $P \leq 0.003$] and females [$F(5,53) = 3.10$, $P \leq 0.016$]. Paw lick latency was reduced in male mice in the 625, 5,000, and 10,000 ppm groups, the same groups with grip strength deficits. The 625, 1,250, 2,500, and 5,000 ppm groups of female mice also had decreased paw lick latency (Table G3).

Motor activity. Chemical-related increases in motor activity were evident in all groups of male and female mice at week 2 [male, $F(5,54) = 5.10$, $P \leq 0.0007$; female, $F(5,54) = 4.53$, $P \leq 0.0016$; Table G4]. Although there appeared to be no dose-related effects, exposed males (46%) showed an overall greater increase in motor activity compared to controls than did females (35%). Motor activity also increased from prestudy to week 2. The facilitatory effect of oxazepam on motor activity was no longer evident in male mice at week 12. Change from prestudy to week 12 was similar in all groups of male mice. In contrast, increased motor activity in female mice persisted to week 12 [$F(5,53) = 2.50$, $P \leq 0.0415$]. Females in the 1,250 and 10,000 ppm groups showed significantly greater activity counts than did controls at 12 weeks.

Startle response. Table G5 shows the startle response profiles of each exposure group. The mean initial reactivities (block 1) of male and female mice collectively were 59 ± 3 and 59 ± 4 amplitude units, respectively. Mean prestudy startle responses of males and females collectively during block 2 were 30 ± 1 and 34 ± 3 amplitude units, respectively. Mean startle responses for male and female mice collectively during block 3 were 51 ± 3 and 45 ± 3 amplitude units, respectively. Thus, a 13% habituation of the startle response was observed overall in male mice whereas a 24% habituation occurred in females at study initiation.

A group (dose) by block design was used to analyze startle response data. An analysis of variance on startle response data from males at week 2 (Table G6) indicated significant overall effects of group, $F(5,4782) = 56.35$, $P \leq 0.0001$; block, $F(2,4782) = 303.60$, $P \leq 0.0001$; and group $\times$ block, $F(10,4782) = 8.98$, $P \leq 0.0001$. The group by block interaction suggested differences between exposure groups in startle response across trial blocks. Separate analyses of each trial block provided additional evidence of chemical-related effects on startle behavior. Significant F-ratios were demonstrated for all trial blocks [block 1, $F(5,594) = 9.38$, $P \leq 0.0001$; block 2, $F(5,3594) = 48.27$, $P \leq 0.0001$; block 3, $F(5,594) = 11.02$, $P \leq 0.0001$]. Initial reactivity (block 1) of males to the startle eliciting stimulus was reduced for all exposed groups compared to that of the controls; this general tendency was also prevalent in blocks 2 and 3. This reduction was significant in the 625, 5,000, and 10,000 ppm groups during block 1 and all exposed groups

during block 2. For the most part, no consistent changes in the inhibitory effect of a prepulse were evident in males at week 2. The effect of the prepulse on control animals at week 2 was identical to that during prestudy. Compared to prestudy the 625 and 10,000 ppm groups showed decreases while the 1,250, 2,500, and 5,000 ppm groups demonstrated increases in prepulse inhibition of the startle response. During block 3, all exposed groups, except the 1,250 ppm group, had startle responses significantly less than those of controls. The 2,500 and 5,000 ppm groups also showed the highest percent habituation.

For female mice at week 2 (Table G6), significant effects were indicated for group, $F(5,4782) = 27.42$, $P \leq 0.0001$; block, $F(2,4782) = 390.98$, $P \leq 0.0001$; and group × block, $F(10,4782) = 5.05$, $P \leq 0.0001$. Separate analyses for each trial block indicated significant differences between groups [block 1, $F(5,594) = 2.39$, $P \leq 0.0367$; block 2, $F(5,3594) = 22.43$, $P \leq 0.0001$; block 3 $F(5,594) = 8.47$, $P \leq 0.0001$]. The average startle response for females at week 2 was reduced for all groups compared to their respective scores at study initiation. The overall chemical-related reduction of the startle response that was clearly demonstrated in males was not evident in females. The prepulse inhibition was similar among groups at week 2 and was similar to that exhibited during prestudy. Habituation was generally reduced from prestudy to 2 weeks for all female mice. Habituation seemed to be impaired to a greater extent in all exposed groups, except in the 10,000 ppm group, compared to controls.

Analysis of variance of startle behavior for male mice during week 12 (Table G7) revealed significant effects for group, $F(5,4702) = 45.67$, $P \leq 0.0001$; block, $F(2,4702) = 554.61$, $P \leq 0.0001$; and group × block, $F(10,4702) = 18.42$, $P \leq 0.0001$. Individual analyses indicated significant effects at each trial block [block 1, $F(5,584) = 12.48$, $P \leq 0.0001$; block 2, $F(5,3534) = 41.60$, $P \leq 0.0001$; block 3, $F(5,584) = 9.33$, $P \leq 0.0001$]. Compared to prestudy and week 2, initial startle responses were lower in all males at week 12. Repeated testing may have resulted in an overall dampening of the response. Nonetheless, exposure led to a reduction in startle behavior throughout the studies. During block 1, the 1,250, 2,500, 5,000, and 10,000 ppm groups had startle responses significantly lower than those of the controls. All exposed males had startle responses significantly lower than those of the controls during block 2. The prepulse inhibition was similar to that of the controls for all groups except the 2,500 ppm group, which showed decreased sensitivity to the effects of the auditory prepulse. During block 3, the 2,500, 5,000, and 10,000 ppm groups demonstrated decreased startle responses. The degree of habituation was similar to that reported for prestudy with the exception of the 2,500 ppm group, which showed no habituation.

Significant effects of group [$F(5,4702) = 45.67$, $P \leq 0.0001$], block [$F(2,4702) = 554.61$, $P \leq 0.0001$], and group × block [$F(10,4702) = 18.42$, $P \leq 0.0001$] were indicated by analysis of variance performed on week 12 data for females (Table G7). Separate analyses of each trial block suggested significant differences between exposure groups [block 1, $F(5,584) = 12.48$, $P \leq 0.0001$; block 2, $F(5,584) = 41.60$, $P \leq 0.0001$; block 3, $F(5,584) = 9.33$, $P \leq 0.0001$)]. All scores were lower at week 12 compared to prestudy or week 2. Initial startle reactivity of 1,250 ppm females was significantly less than that of the controls. While the percent prepulse inhibition was higher overall at week 12 than at week 2, again, no differences between exposed and control females were observed. Startle response of the 5,000 and 10,000 ppm groups was significantly less than that of controls during block 3. The 5,000 and 10,000 ppm groups demonstrated the greatest habituation of the startle response as well. The 625, 1,250, and 2,500 ppm groups showed little or no habituation compared to control mice.

57-Week Study

Grip strength. During the 6-month evaluation in the 57-week study of Swiss-Webster mice, forelimb grip strength was significantly decreased in 5,000 ppm male mice (Table G8). Hindlimb grip strength was not affected by oxazepam exposure (Table G9). At 12 months, neither forelimb nor hindlimb grip strength in either sex was altered by oxazepam exposure. Although for males, forelimb decreases were demonstrated in the 2,500 (18%) and 5,000 ppm (24%) groups, the reduction in the number of animals in these groups may have influenced the statistical analysis and resulting lack of difference between these groups and controls.

Thermal sensitivity. Results of the thermal sensitivity test revealed decreases in paw lick latency in the exposed mice at 6 months (Table G10). Paw lick latencies in the 2,500 and 5,000 ppm female groups were significantly different from those of the controls. Decreases in paw lick latencies were observed in males in the 2,500 (29%) and 5,000 ppm (48%) groups. However, this effect was significant only in the 5,000 ppm group compared to the control group. At 12 months, significant differences in paw lick latency scores were not evident, although the 5,000 ppm female group showed a 38% decrease compared to the control group.

Motor activity and startle response. Motor activity at 6 months was not affected by oxazepam exposure, but motor activity was reduced by 53% in 5,000 ppm females at 12 months (Table G11). Startle response was not altered by oxazepam at either 6 or 12 months (data not shown).

B6C3F$_1$ Mice

14-Week Study

Grip strength. One week of oxazepam exposure led to a significant reduction in mean forelimb grip strength in males [$F(5,54) = 5.51$, $P \leq 0.001$] but not in females. Results of an analysis of variance followed by Dunnett's test for comparison of individual means showed that all male exposure groups were significantly different from controls during week 2 (Table G12). No effect of oxazepam was observed during week 12 in male or female mice. For male mice there were no consistent differences between exposed and control groups in performance across trials. However, exposed females showed a slightly greater degree of habituation than controls. This might have been due, in part, to the lack of any difference in performance of the control animals, across five trials.

Significant decrements in mean hindlimb grip strength were evident in male [$F(5,54) = 12.45$, $P \leq 0.001$] and female [$F(5,54) = 3.93$, $P \leq 0.004$] mice. All exposed mice, except females receiving 625 ppm, had lower hindlimb grip strength scores compared to controls (Table G13). Males were slightly more sensitive to the chemical-related grip strength deficit than females. The mean decreases for exposed males and females were 20% and 13%, respectively. During week 12, all hindlimb grip strength deficits had disappeared in all mice. For most exposed groups, the degree of habituation of hindlimb grip strength performance was greater than controls during week 2, but not during week 12.

Body weights of exposed animals were similar to or greater than those of the controls. Decreases in grip strength were not considered related to body weights.

Thermal sensitivity. No evidence of chemical-related changes in analgesia or pain sensation were indicated at week 2. However, at week 12, significant decreases in paw lick latency were demonstrated in female mice [$F(5,54) = 3.06$, $P \leq 0.017$]. The 625, 1,250, and 10,000 ppm groups had latency scores that were lower than controls (Table G14).

Motor activity. Chemical-related increases in motor activity were evident in both male and female mice at weeks 2 and 12 [male, week 2: $F(5,54) = 13.37$, $P \leq 0.0001$; female, week 2: $F(5,54) = 6.31$, $P \leq 0.0001$; male, week 12: $F(5,54) = 3.98$, $P \leq 0.0038$; female, week 12: $F(5,54) = 6.16$, $P \leq 0.0001$]. Significant differences in motor activity between exposed and control groups of each sex were observed at each time period (Table G15). In exposed males, the changes in motor activity from prestudy to week 2 and from prestudy to week 12 were similar. Exposed females showed greater changes in motor activity from prestudy to week 12 than from prestudy to week 2.

Startle response. Examination of data from the prestudy period provided various indices of the startle response (Table G16). The mean initial reactivities of male and female mice collectively were 67 ± 1 and 61 ± 2 amplitude units, respectively. Mean startle responses collectively during block 2 were 52 ± 2 and 45 ± 1 amplitude units for males and females, respectively. Thus, the inclusion of an auditory prepulse

caused an inhibition of the startle response, overall, in male (22%) and female (26%) mice. Mean startle responses for male and female mice collectively during block 3 were 58 ± 2 and 59 ± 2 amplitude units, respectively. A 13% habituation of the startle response was observed overall in male mice, whereas only a 3% habituation occurred in females.

An analysis of variance on startle data from male mice at week 2 (Table G17) indicated significant overall effects of group, $F(5,4782) = 62.95$, $P \leq 0.0001$; block, $F(2,4782) = 331.42$, $P \leq 0.0001$; and group × block, $F(10,4782) = 4.41$, $P \leq 0.0001$. The group × block interaction suggested that there were differences between exposure groups in startle performance across trial blocks. Separate analyses for each trial block provided additional evidence for chemical-related effects on startle behavior. Significant F-ratios were demonstrated for all trial blocks [block 1, $F(5,594) = 9.20$, $P \leq 0.0001$; block 2, $F(5,3594) = 49.08$, $P \leq 0.0001$; block 3, $F(5,594) = 10.86$, $P \leq 0.0001$]. Initial reactivity (block 1) to the startle eliciting stimulus was significantly greater ($P \leq 0.05$) in the 625 and 1,250 ppm groups of males. During block 2, the 625, 2,500, 5,000, and 10,000 ppm groups had average startle responses that were significantly less than those of the controls while the 1,250 ppm group had responses greater than controls. In general, the inhibiting effects of a prepulse were greater for exposed mice than controls. Comparison of blocks 1 and 3 revealed no habituation of the startle response in male controls. For the most part, exposed groups showed a greater degree of habituation.

Analysis of variance of startle response behavior for female mice at week 2 (Table G17) revealed significant effects for group, $F(5,4782) = 57.45$, $P \leq 0.0001$; block, $F(2,4782) = 276.50$, $P \leq 0.0001$; and group × block, $F(10,4782) = 4.95$, $P \leq 0.0001$. Individual analyses indicated significant dose-related effects at each trial block [block 1, $F(5,594) = 6.64$, $P \leq 0.0001$; block 2, $F(5,3594) = 63.08$, $P \leq 0.0001$; block 3, $F(5,594) = 4.79$, $P \leq 0.0003$]. Compared to prestudy, little change in initial reactivity to the startle stimulus was observed at week 2 except in the 2,500 ppm group, which had scores greater than controls. This is different from the male 625 and 1,250 ppm groups in which startle amplitudes were significantly greater than those of the controls at week 2. During block 2, the 1,250, 5,000, and 10,000 ppm groups had average startle responses less than controls while the 2,500 ppm group had greater scores. Similar to males at week 2, prepulse inhibition was generally greater in exposed females than controls. This can be compared to an overall 26% prepulse inhibition for females at prestudy. During block 3, the 625 ppm group had startle scores greater than controls. Repeated trial presentation resulted in a greater degree of habituation of the startle response in the 1,250 and 2,500 ppm groups.

At week 12, significant effects were indicated in males for group, $F(5,4782) = 16.52$, $P \leq 0.0001$; block, $F(2,4782) = 772.01$, $P \leq 0.0001$; and group × block, $F(10,4782) = 9.30$, $P \leq 0.0001$ (Table G18). Separate analyses for each trial block indicated significant differences between groups [block 1, $F(5,594) = 2.41$, $P \leq 0.0354$; block 2, $F(5,3594) = 21.37$, $P \leq 0.0001$; block 3, $F(5,594) = 7.72$, $P \leq 0.0001$]. The average startle response for the 625, 2,500, and 10,000 ppm males was lower than their responses at prestudy or week 2. This may have been due to the fact that repeated testing caused an overall reduction in response, or alternatively, the increase in age may have made the animals less hyperactive. During block 2, the 625, 2,500, and 10,000 ppm males had startle scores significantly lower than controls. Similar to week 2, prepulse inhibition was, for the most part, greater in exposed males than in controls. Overall, the effects of an auditory prepulse on tactile startle response were greater at week 12 than at week 2. Startle responses of all but the 625 and 2,500 ppm males were greater than those of the controls during block 3. In general, startle response habituation was greater in controls than in exposed mice. Compared to the degree of habituation at week 2, exposed males showed little change, whereas control males showed greater habituation at week 12 (−34%) than at week 2 (+4%).

Significant effects of group [$F(5,4782) = 11.10$, $P \leq 0.0001$], block [$F(2,4782) = 612.92$, $P \leq 0.0001$], and group × block [$F(10,4782) = 7.85$, $P \leq 0.0001$] were indicated by analysis of variance performed on females at week 12 (Table G18). Separate analyses of each trial block suggested significant differences between exposure groups [block 1, $F(5,594) = 4.48$, $P \leq 0.0005$; block 2, $F(5,3594) = 13.13$, $P \leq 0.0001$; block 3,

$F(5,594) = 3.52$, $P \leq 0.0038$]. The initial startle response of the 10,000 ppm females was significantly less than that of the controls. Similar to male mice, the startle behavior of all female groups at week 12 was less, overall, than at prestudy or week 2. Startle responses for the 2,500, 5,000, and 10,000 ppm females were less than those of the controls during block 2. There appeared to be no differences between exposure groups with regard to the inhibitory effect of a prepulse. The lack of any group differences may have been due to the greater prepulse inhibition occurring in control females at week 12 (−50%) than at week 2 (−28%). The 625, 1,250, and 10,000 ppm groups showed greater startle response than controls during block 3. Similar to their male counterparts, habituation was greater for control than exposed females at week 12.

2-Year Study

Grip strength. At the 6-month neurobehavioral evaluation in the 2-year study of $B6C3F_1$ mice, mean forelimb grip strength appeared to be reduced in exposed mice, but there was no statistically significant difference between exposure groups (Table G19). Males receiving 5,000 ppm showed increased hindlimb grip strength compared to control animals (Table G20). No significant changes in forelimb or hindlimb grip strength were observed in females.

At the 12-month evaluation, decreases in forelimb grip strength were evident for males in all exposure groups (Table G19). This decreased forelimb grip strength did not appear to be an indirect effect (i.e., due to body weight changes), based on further analysis of covariance results. However, it was uncertain if the decreased forelimb grip strength was a direct chemical effect because a dose-related trend was not apparent. Control data were similar to the 6-month values. Hindlimb grip strength in male mice was not affected by exposure. No significant changes in forelimb or hindlimb grip strength were observed in females at 12 months.

The 18-month evaluation revealed decreases in forelimb grip strength in both sexes in the 2,500 and 5,000 ppm groups (Table G19); these were not thought to be related to body weight changes. The 18-month data provided somewhat more certainty that the decreased forelimb grip strength was a chemical-related effect because a dose-related trend was more apparent than at 12 months. On the other hand, the abdominal swelling and neoplasia seen in many of the animals in these exposure groups may have contributed, in part, to the grip strength deficit. Oxazepam exposure did not affect hindlimb grip strength in either sex (Table G20).

Motor activity. At 6 and 12 months, motor activity in both sexes was increased compared to controls (Table G21). Motor activity of 5,000 ppm males and females was significantly reduced at 18 months, probably indicating the debilitating effect of the drug or of the drug-induced neoplasia at this point in time.

Thermal sensitivity and startle response. Paw lick latencies (Table G22) and startle response (data not shown) were relatively unchanged by oxazepam at 6 and 12 months. At the 18-month interval, paw lick latencies were decreased in the 125 ppm group while startle response remained relatively unchanged.

No neurobehavioral changes were noted at the 24-month testing interval. Due to high mortality in the 2,500 and 5,000 ppm groups, testing was limited to the controls and 125 ppm groups. Neurobehavioral study mice were observed daily for clinical signs of withdrawal during an eight-day observation period after completion of dosing. No symptoms of withdrawal were observed.

DISCUSSION

Neurobehavioral results suggest two pharmacologically distinct actions of oxazepam. A nonspecific muscle relaxant or depressant effect was particularly evident as reflected by deficits in grip strength. These were more prevalent in males than females of each strain, and only temporary as differences in grip strength

tended to diminish by week 12 of the 14-week studies. This is consistent with published reports that repeated dosing leads to an attenuation of the depressant effects of the benzodiazepines. A disinhibitory action of oxazepam was indicated by a facilitatory effect on motor activity. Increases in motor activity may be due to the anxiety-reducing effect of oxazepam. These effects were seen at weeks 2 and 12 of exposure with each sex and strain, although they were diminished somewhat at week 12 in exposed male Swiss-Webster mice. As reported in the literature, repeated dosing often leads to an enhancement of the stimulatory effects of low-dose benzodiazepine treatment. In all exposed female $B6C3F_1$ groups, especially, motor activity was increased to a greater extent at week 12 than at week 2.

The tactile startle response assesses somatosensory integrity, the ability of an animal to transform sensory input to motor output. In general, when increases in initial startle response were reported, they were evident at week 2 in low- to middle-dose groups. This could represent the presence of a hyperarousal state, or perhaps reflect a disinhibitory action as was proposed to account for the increase in motor activity. Continued exposure led to significant reductions in initial startle reactivity.

The inhibitory effect of an auditory prepulse on tactile startle responding was generally augmented in exposed $B6C3F_1$ mice, but not in the Swiss-Webster mice, compared to controls. Alone, an auditory startle response is usually 3 to 4 times weaker than a tactile one. Thus, greater prepulse inhibition seen in exposed mice may be due to increased sensitivity to acoustic stimuli. Alternatively, exposed mice may be better able to distinguish acoustic and tactile stimuli. For whatever reason, these findings suggest that effects of oxazepam on the sensory component of the startle reflex circuit may account for the differences observed. Habituation of the startle response appeared to be variably affected by oxazepam.

Effects of oxazepam on the sensory system, in general, and arousal mechanisms, specifically, may also be involved in the changes observed during the thermal sensitivity measurements. An increased sense of awareness by exposed mice may have contributed to decreased paw lick latencies seen in some exposure groups.

The purpose of long-term neurobehavioral studies was to determine if chronic exposure caused a change in the types or severity of neurobehavioral signs from those observed in the 14-week studies. The chronic studies were designed to include neurobehavioral evaluations at study initiation and at 6-month intervals, using the same animals at each time point. Because of high mortality in the 2,500 and 5,000 ppm groups in both strains, a complete assessment was not possible and replacement animals had to be used.

There was evidence of decreased forelimb grip strength in dosed Swiss-Webster mice at 6 months, and in $B6C3F_1$ mice at 12 and 18 months. As with other measures, it was difficult to determine if the changes seen in $B6C3F_1$ mice at 18 months were a direct effect of oxazepam exposure or an indirect effect due to the deteriorating condition of animals that weighed less and were developing liver neoplasia. Mice in the 125 ppm group that survived to 24 months did not develop deficits in forelimb or hindlimb grip strength.

Dosed Swiss-Webster mice showed decreased paw lick latencies at 6 months, as was noted in the 13-week studies, but consistent changes were not seen in the $B6C3F_1$ mice at any time.

In contrast, increased motor activity was noted in $B6C3F_1$ mice, but not in Swiss-Webster mice. Decreased motor activity in dosed mice of both strains late in the study was attributed to their poor condition. Thus, the direct effects of oxazepam exposure on motor activity appeared to be strain dependent, as Swiss-Webster mice showed an increase in activity only at week 2 in the 13-week study, while the effect in $B6C3F_1$ mice persisted for at least a year.

Although observation of noticeable behavioral changes in response to chronic oxazepam treatment was diminished by poor survival, no novel behavioral effects or significant enhancement of behavioral effects were apparent with 57-week and 2-year oxazepam exposure.

TABLE G1
Mean Forelimb Grip Strength of Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	Week 2 (g)	Week 12 (g)
Male			
0	103.16 ± 6.72	125.50 ± 4.86	147.72 ± 6.15
625	109.38 ± 5.67	118.84 ± 3.21	126.78 ± 3.69**
1,250	107.60 ± 4.24	117.22 ± 2.82	136.50 ± 3.22
2,500	111.36 ± 4.21	117.64 ± 5.50	140.46 ± 4.37
5,000	105.30 ± 3.68	107.10 ± 4.16*	126.92 ± 2.95**
10,000	105.90 ± 2.67	106.92 ± 4.75*	129.06 ± 3.10**
Female			
0	91.38 ± 3.37	109.10 ± 2.83	119.22 ± 5.08
625	85.54 ± 3.77	106.76 ± 3.07	124.64 ± 3.55
1,250	89.16 ± 5.17	108.70 ± 4.38	126.93 ± 8.91
2,500	88.84 ± 4.05	103.00 ± 4.30	110.50 ± 6.62
5,000	89.24 ± 4.23	93.76 ± 4.64*	113.62 ± 4.92
10,000	90.76 ± 5.17	95.62 ± 3.09*	109.04 ± 6.43

* Significantly different (P≤0.05) from the control group by Dunnett's test; data are given as grams of force needed to break grip
** P≤0.01
[a] Mean ± standard error; n=10 except for 625 ppm males and 1,250 ppm females in Week 12, where n=9

TABLE G2
Mean Hindlimb Grip Strength of Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	Week 2 (g)	Week 12 (g)
Male			
0	77.14 ± 4.59	88.96 ± 3.05	106.42 ± 3.31
625	78.52 ± 3.76	85.44 ± 1.39	102.13 ± 3.16
1,250	80.28 ± 3.50	84.36 ± 2.21	106.06 ± 2.37
2,500	82.08 ± 2.64	85.52 ± 3.09	105.96 ± 2.63
5,000	74.92 ± 3.72	79.10 ± 3.19	98.06 ± 1.79
10,000	74.82 ± 2.80	71.38 ± 4.39**	98.22 ± 1.96
Female			
0	67.46 ± 2.08	77.52 ± 3.17	90.32 ± 3.17
625	64.40 ± 3.00	78.14 ± 1.96	95.08 ± 2.29
1,250	66.50 ± 3.08	74.36 ± 1.94	94.40 ± 4.24
2,500	65.48 ± 3.41	69.70 ± 4.15	83.76 ± 4.65
5,000	66.64 ± 3.36	59.22 ± 4.24**	86.44 ± 3.43
10,000	67.44 ± 2.71	62.06 ± 3.06**	84.52 ± 4.75

** Significantly different (P≤0.01) from the control group by Dunnett's test; data are given as grams of force needed to break grip
[a] Mean ± standard error; n=10 except for 625 ppm males and 1,250 ppm females in Week 12, where n=9

TABLE G3
Mean Paw Lick Latency of Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (sec.)	Week 2 (sec.)	Week 12 (sec.)
Male			
0	14.62 ± 1.94	13.58 ± 1.49	17.29 ± 2.47
625	11.81 ± 0.72	9.98 ± 1.22	7.34 ± 0.55**
1,250	13.46 ± 1.28	12.78 ± 1.68	12.73 ± 1.76
2,500	15.10 ± 1.63	12.25 ± 1.92	11.72 ± 2.34
5,000	15.56 ± 1.55	16.30 ± 2.45	9.94 ± 0.73*
10,000	15.29 ± 1.71	17.81 ± 2.62	9.88 ± 0.67*
Female			
0	13.29 ± 1.06	11.55 ± 0.53	13.04 ± 1.83
625	13.36 ± 1.27	10.26 ± 0.80	8.35 ± 0.77*
1,250	13.01 ± 1.52	10.94 ± 0.96	8.43 ± 0.79*
2,500	12.18 ± 0.95	10.52 ± 0.98	8.57 ± 0.91*
5,000	12.34 ± 0.88	12.79 ± 1.45	8.47 ± 0.72*
10,000	13.41 ± 0.90	16.34 ± 2.69	11.33 ± 1.32

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard error; n=10 except for 625 ppm males and 1,250 ppm females in Week 12, where n=9

TABLE G4
Mean Total Motor Activity Count of Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy	Week 2	Change from Prestudy (%)	Week 12	Change from Prestudy (%)
Male					
0	380 ± 19	370 ± 30	–3	463 ± 32	+22
625	352 ± 14	555 ± 32**	+58	449 ± 12	+28
1,250	370 ± 23	545 ± 49**	+47	472 ± 43	+28
2,500	354 ± 19	508 ± 23*	+44	424 ± 38	+20
5,000	359 ± 21	545 ± 21**	+52	447 ± 19	+25
10,000	369 ± 15	551 ± 27**	+49	478 ± 26	+30
Female					
0	386 ± 17	392 ± 14	+2	428 ± 27	+11
625	377 ± 25	503 ± 35*	+33	513 ± 27	+36
1,250	364 ± 12	539 ± 29**	+48	564 ± 16*	+55
2,500	382 ± 11	538 ± 28**	+41	504 ± 35	+32
5,000	352 ± 18	504 ± 25*	+43	489 ± 38	+39
10,000	366 ± 17	558 ± 33**	+52	548 ± 30*	+50

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard error; n=10 except for 625 ppm males and 1,250 ppm females in Week 12, where n=9

TABLE G5
Startle Response Profiles Prior to Exposure of Swiss-Webster Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Block 1[b]	Block 2[c]	Prepulse Inhibition[d] (%)	Block 3[e]	Habituation[f] (%)
Male					
0	55 ± 3	28 ± 1	–49	56 ± 3	+2
625	67 ± 5	30 ± 2	–55	47 ± 3	–30
1,250	72 ± 5	35 ± 2*	–51	61 ± 4	–15
2,500	60 ± 6	26 ± 1	–57	46 ± 4	–23
5,000	54 ± 4	31 ± 2	–43	58 ± 4	+7
10,000	47 ± 4	28 ± 2	–40	37 ± 2**	–21
Females					
0	56 ± 3	34 ± 3	–39	46 ± 3	–18
625	44 ± 2*	28 ± 1	–36	35 ± 2*	–20
1,250	60 ± 3	26 ± 2*	–57	44 ± 3	–27
2,500	79 ± 4**	48 ± 2**	–39	58 ± 4*	–27
5,000	54 ± 2	31 ± 1	–43	43 ± 3	–20
10,000	63 ± 2	35 ± 2	–44	45 ± 2	–29

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Mean ± standard error of average responses (amplitudes) for each trial; n=10
[b] Block 1 = mean of first 10 trials (tactile stimulus without auditory prepulse)
[c] Block 2 = mean of trials 11-70 (tactile stimulus preceded by auditory prepulse); 80-90 dB
[d] Percent prepulse inhibition = $\frac{\text{block 1 - block 2}}{\text{block 1}} \times 100$
[e] Block 3 = mean of last 10 trials (tactile stimulus without auditory prepulse)
[f] Percent habituation = $\frac{\text{block 1 - block 3}}{\text{block 1}} \times 100$

TABLE G6
Startle Response Profiles of Swiss-Webster Mice at Week 2 in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Block 1[b]	Block 2[c]	Prepulse Inhibition[d] (%)	Block 3[e]	Habituation[f] (%)
Male					
0	65 ± 4	33 ± 1	–49	59 ± 5	–9
625	39 ± 3**	24 ± 1**	–38	37 ± 2**	–5
1,250	54 ± 6	26 ± 1**	–52	53 ± 6	–2
2,500	55 ± 5	22 ± 1**	–60	44 ± 3*	–20
5,000	47 ± 5*	19 ± 0**	–60	31 ± 2**	–34
10,000	29 ± 2**	19 ± 0**	–34	27 ± 2**	–7
Female					
0	36 ± 3	22 ± 1	–39	35 ± 2	–3
625	37 ± 4	22 ± 1	–41	39 ± 3	+5
1,250	37 ± 2	20 ± 0	–46	45 ± 2*	+22
2,500	38 ± 2	24 ± 1	–37	46 ± 3*	+21
5,000	36 ± 2	21 ± 1	–42	42 ± 3	+17
10,000	28 ± 2	16 ± 0**	–43	26 ± 1*	–7

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Mean ± standard error of average responses (amplitudes) for each trial; n=10
[b] Block 1 = mean of first 10 trials (tactile stimulus without auditory prepulse)
[c] Block 2 = mean of trials 11-70 (tactile stimulus preceded by auditory prepulse); 80-90 dB
[d] Percent prepulse inhibition = $\frac{\text{block 1 - block 2}}{\text{block 1}} \times 100$
[e] Block 3 = mean of last 10 trials (tactile stimulus without auditory prepulse)
[f] Percent habituation = $\frac{\text{block 1 - block 3}}{\text{block 1}} \times 100$

TABLE G7
Startle Response Profiles of Swiss-Webster Mice at Week 12 in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Block 1[b]	Block 2[c]	Prepulse Inhibition[d] (%)	Block 3[e]	Habituation[f] (%)
Male					
0	36 ± 3	15 ± 0	–58	30 ± 2	–17
625	31 ± 3	12 ± 0**	–61	27 ± 2	–13
1,250	28 ± 2*	12 ± 0**	–57	26 ± 2	–7
2,500	14 ± 1**	11 ± 0**	–21	16 ± 1**	+14
5,000	25 ± 2**	11 ± 0**	–56	22 ± 2**	–12
10,000	20 ± 1**	11 ± 0**	–45	17 ± 1**	–15
Female					
0	30 ± 2	14 ± 0	–53	21 ± 1	–30
625	25 ± 2	13 ± 0*	–48	23 ± 1	–8
1,250	22 ± 2*	11 ± 0**	–50	24 ± 2	+9
2,500	23 ± 2	13 ± 0*	–43	25 ± 2	+9
5,000	30 ± 3	13 ± 0*	–57	16 ± 1*	–47
10,000	23 ± 2	12 ± 0*	–48	15 ± 0*	–35

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Mean ± standard error of average responses (amplitudes) for each trial; n=10
[b] Block 1 = mean of first 10 trials (tactile stimulus without auditory prepulse)
[c] Block 2 = mean of trials 11-70 (tactile stimulus preceded by auditory prepulse); 80-90 dB
[d] Percent prepulse inhibition = $\frac{\text{block 1 - block 2}}{\text{block 1}} \times 100$
[e] Block 3 = mean of last 10 trials (tactile stimulus without auditory prepulse)
[f] Percent habituation = $\frac{\text{block 1 - block 3}}{\text{block 1}} \times 100$

TABLE G8
Mean Forelimb Grip Strength of Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	6 Months (g)	12 Months (g)
Male			
0	89 ± 3	122 ± 5[b]	103 ± 7[c]
2,500	86 ± 3	108 ± 4	84 ± 8[d]
5,000	88 ± 4	102 ± 5*	78 ± 10[d]
Female			
0	70 ± 6	106 ± 4	84 ± 6[b]
2,500	75 ± 4	93 ± 5[b]	78 ± 3[c]
5,000	69 ± 3[b]	93 ± 4[b]	74 ± 7[d]

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Mean ± standard error; n=10 except where indicated; data are given as grams of force needed to break grip strength
[b] n=9
[c] n=7
[d] n=4

TABLE G9
Mean Hindlimb Grip Strength of Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	6 Months (g)	12 Months (g)
Male			
0	108 ± 4	105 ± 4[b]	96 ± 4[c]
2,500	108 ± 4	115 ± 4	86 ± 8[d]
5,000	110 ± 5	115 ± 3	102 ± 2[d]
Female			
0	81 ± 4	100 ± 4	85 ± 3[b]
2,500	77 ± 3	95 ± 3[b]	78 ± 3[c]
5,000	77 ± 3[b]	100 ± 5[b]	77 ± 3[d]

[a] Mean ± standard error; n=10 except where indicated; data are given as grams of force needed to break grip strength; differences from the control group are not significant by Dunnett's test.
[b] n=9
[c] n=7
[d] n=4

TABLE G10
Mean Paw Lick Latency of Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (seconds)	6 Months (seconds)	12 Months (seconds)
Male			
0	15.0 ± 1.4	17.0 ± 2.8[b]	14.1 ± 2.9[c]
2,500	13.5 ± 2.1	12.1 ± 2.5	7.2 ± 1.7[d]
5,000	13.2 ± 1.2	8.9 ± 0.9*	17.8 ± 4.4[d]
Female			
0	13.2 ± 2.1	14.6 ± 2.5	14.1 ± 2.6[b]
2,500	12.8 ± 1.2	7.1 ± 0.6**[b]	10.9 ± 1.8[c]
5,000	11.2 ± 1.2[b]	6.8 ± 0.5**[b]	8.7 ± 1.6[d]

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard error; n=10 except where indicated
[b] n=9
[c] n=7
[d] n=4

TABLE G11
Mean Total Horizontal Activity Count of Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy	6 Months	12 Months
Male			
0	278 ± 11	279 ± 13[b]	266 ± 22[c]
2,500	233 ± 14	234 ± 15	166 ± 21[d]
5,000	261 ± 19	273 ± 27	279 ± 47[d]
Female			
0	265 ± 14	287 ± 18	266 ± 29[b]
2,500	264 ± 17	294 ± 27[b]	218 ± 31[c]
5,000	277 ± 17	314 ± 18[b]	125 ± 32*[d]

* Significantly different (P≤0.05) from the control group by Dunnett's test
[a] Mean ± standard error; n=10 except where indicated
[b] n=9
[c] n=7
[d] n=4

TABLE G12
Mean Forelimb Grip Strength of B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	Week 2 (g)	Week 12 (g)
Male			
0	114.28 ± 3.66	135.48 ± 3.62	164.60 ± 3.79
625	115.14 ± 3.55	120.30 ± 2.96*	160.58 ± 3.39
1,250	111.72 ± 3.46	122.00 ± 3.93*	157.04 ± 4.73
2,500	112.10 ± 2.65	117.24 ± 3.48**	156.48 ± 4.79
5,000	112.32 ± 3.25	116.28 ± 2.90**	152.48 ± 3.14
10,000	113.76 ± 3.31	112.38 ± 3.48**	156.90 ± 1.98
Female			
0	103.48 ± 2.59	108.54 ± 2.08	145.98 ± 2.14
625	99.10 ± 3.18	107.12 ± 1.91	143.90 ± 1.82
1,250	95.84 ± 2.06	103.80 ± 3.23	143.80 ± 3.38
2,500	102.54 ± 2.79	102.60 ± 3.44	145.76 ± 2.52
5,000	99.02 ± 2.66	104.14 ± 3.33	140.06 ± 1.58
10,000	100.10 ± 2.76	99.02 ± 2.67*	141.60 ± 2.34

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard error; n=10; data are given as grams of force needed to break grip strength

TABLE G13
Mean Hindlimb Grip Strength of B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	Week 2 (g)	Week 12 (g)
Male			
0	80.18 ± 3.43	91.78 ± 1.65	101.76 ± 2.01
625	79.28 ± 3.80	79.18 ± 2.49**	107.82 ± 1.44
1,250	82.86 ± 3.26	75.42 ± 2.42**	102.62 ± 1.36
2,500	81.88 ± 2.82	72.54 ± 2.89**	104.96 ± 2.36
5,000	81.16 ± 2.89	68.58 ± 2.08**	106.54 ± 2.20
10,000	80.90 ± 3.70	71.72 ± 2.38**	98.84 ± 1.82
Female			
0	70.10 ± 2.36	73.20 ± 2.08	96.28 ± 3.10
625	67.34 ± 3.33	67.14 ± 1.20	99.08 ± 1.85
1,250	70.20 ± 2.36	64.18 ± 1.56*	94.68 ± 2.11
2,500	72.26 ± 2.60	64.20 ± 1.81*	97.82 ± 1.75
5,000	67.42 ± 1.91	62.36 ± 3.26**	96.36 ± 1.31
10,000	68.44 ± 2.68	61.50 ± 2.42**	96.24 ± 2.04

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard error; n=10; data are given as grams of force needed to break grip strength

TABLE G14
Mean Paw Lick Latency of B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	Week 2 (g)	Week 12 (g)
Male			
0	12.22 ± 0.80	14.91 ± 2.07	12.07 ± 1.17
625	10.24 ± 0.76	11.38 ± 1.20	10.58 ± 1.06
1,250	14.01 ± 1.53	15.87 ± 2.32	11.05 ± 1.22
2,500	14.40 ± 1.96	17.00 ± 1.91	13.47 ± 1.05
5,000	15.09 ± 2.09	15.27 ± 1.90	13.35 ± 1.37
10,000	13.47 ± 1.09	17.71 ± 2.15	15.64 ± 2.11
Female			
0	10.66 ± 0.95	13.46 ± 2.30	13.29 ± 1.22
625	10.60 ± 1.25	13.22 ± 1.47	8.92 ± 0.48*
1,250	12.12 ± 0.44	13.63 ± 1.72	9.71 ± 1.07*
2,500	11.89 ± 1.11	13.82 ± 1.73	11.23 ± 0.82
5,000	9.29 ± 0.59	17.94 ± 2.32	12.01 ± 1.12
10,000	12.24 ± 0.83	15.22 ± 2.21	9.61 ± 0.84*

* Significantly different (P≤0.05) from the control group by Dunnett's test
[a] Mean ± standard error; n=10; data are given as grams of force needed to break grip strength

TABLE G15
Mean Total Motor Activity Count of B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy	Week 2	Change from Prestudy[b] (%)	Week 12	Change from Prestudy[c] (%)
Male					
0	168 ± 22	316 ± 12	+88	378 ± 14	+125
625	207 ± 12	451 ± 22**	+118	459 ± 21*	+122
1,250	185 ± 11	467 ± 16**	+152	449 ± 19*	+143
2,500	205 ± 10	499 ± 22**	+143	473 ± 19**	+131
5,000	184 ± 11	507 ± 25**	+176	460 ± 9*	+150
10,000	192 ± 11	501 ± 20**	+161	477 ± 23**	+148
Females					
0	176 ± 6	325 ± 19	+85	386 ± 21	+119
625	195 ± 6	467 ± 22**	+139	521 ± 29**	+167
1,250	187 ± 15	434 ± 29*	+132	545 ± 20**	+191
2,500	189 ± 11	500 ± 28**	+165	512 ± 24**	+171
5,000	202 ± 12	479 ± 33**	+137	533 ± 41**	+164
10,000	180 ± 6	495 ± 22**	+175	571 ± 15**	+217

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Mean ± standard error; n=10
[b] Percent change from prestudy for Week 2 = $\frac{\text{Week 2 (activity count) - Prestudy (activity count)}}{\text{Prestudy (activity count)}} \times 100$
[c] Percent change from prestudy for Week 12 = $\frac{\text{Week 12 (activity count) - Prestudy (activity count)}}{\text{Prestudy (activity count)}} \times 100$

TABLE G16
Startle Response Profiles Prior to Exposure of B6C3F$_1$ Mice in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Block 1[b]	Block 2[c]	Prepulse Inhibition[d] (%)	Block 3[e]	Habituation[f] (%)
Male					
0	63 ± 4	57 ± 1	−10	54 ± 3	−14
625	71 ± 4	58 ± 2	−18	64 ± 4	−10
1,250	65 ± 4	47 ± 2**	−28	52 ± 3	−20
2,500	70 ± 4	50 ± 2*	−29	61 ± 4	−13
5,000	64 ± 3	44 ± 1**	−31	54 ± 3	−16
10,000	71 ± 4	56 ± 1	−21	63 ± 4	−11
Female					
0	55 ± 3	40 ± 1	−27	53 ± 3	−4
625	57 ± 3	41 ± 1	−28	60 ± 4	+5
1,250	62 ± 3	48 ± 1**	−23	64 ± 4	+3
2,500	68 ± 3*	48 ± 1**	−29	53 ± 2	−22
5,000	63 ± 4	49 ± 1**	−22	61 ± 3	−3
10,000	60 ± 3	44 ± 1*	−27	60 ± 4	0

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Mean ± standard error of average responses (amplitudes) for each trial; n=10
[b] Block 1 = mean of first 10 trials (tactile stimulus without auditory prepulse)
[c] Block 2 = mean of trials 11-70 (tactile stimulus preceded by auditory prepulse); 80-90 dB
[d] Percent prepulse inhibition = $\frac{\text{block 1 - block 2}}{\text{block 1}} \times 100$
[e] Block 3 = mean of last 10 trials (tactile stimulus without auditory prepulse)
[f] Percent habituation = $\frac{\text{block 1 - block 3}}{\text{block 1}} \times 100$

TABLE G17
Startle Response Profiles of B6C3F$_1$ Mice at Week 2 in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Block 1[b]	Block 2[c]	Prepulse Inhibition[d] (%)	Block 3[e]	Habituation[f] (%)
Male					
0	67 ± 4	51 ± 1	–24	70 ± 5	+4
625	82 ± 3*	45 ± 1**	–45	62 ± 3	–24
1,250	92 ± 4**	58 ± 1**	–37	85 ± 4*	–8
2,500	69 ± 3	41 ± 1**	–41	52 ± 3**	–25
5,000	71 ± 3	45 ± 1**	–37	63 ± 3	–11
10,000	66 ± 4	38 ± 1**	–42	53 ± 3**	–20
Female					
0	60 ± 4	43 ± 1	–28	52 ± 3	–13
625	62 ± 4	43 ± 1	–31	64 ± 4*	+3
1,250	70 ± 4	34 ± 1**	–51	49 ± 3	–30
2,500	82 ± 4**	50 ± 1**	–39	60 ± 4	–27
5,000	60 ± 3	39 ± 1**	–35	49 ± 3	–18
10,000	55 ± 4	30 ± 1**	–45	46 ± 2	–16

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Mean ± standard error of average responses (amplitudes) for each trial; n=10
[b] Block 1 = mean of first 10 trials (tactile stimulus without auditory prepulse)
[c] Block 2 = mean of trials 11-70 (tactile stimulus preceded by auditory prepulse); 80-90 dB
[d] Percent prepulse inhibition = $\frac{\text{block 1 - block 2}}{\text{block 1}} \times 100$
[e] Block 3 = mean of last 10 trials (tactile stimulus without auditory prepulse)
[f] Percent habituation = $\frac{\text{block 1 - block 3}}{\text{block 1}} \times 100$

TABLE G18
Startle Response Profiles of B6C3F$_1$ Mice at Week 12 in the 14-Week Feed Study of Oxazepam[a]

Dose (ppm)	Block 1[b]	Block 2[c]	Prepulse Inhibition[d] (%)	Block 3[e]	Habituation[f] (%)
Male					
0	38 ± 2	19 ± 1	–50	25 ± 2	–34
625	35 ± 2	14 ± 0**	–60	27 ± 2	–23
1,250	46 ± 2	18 ± 0	–61	43 ± 3**	–7
2,500	39 ± 3	15 ± 0**	–62	32 ± 2	–18
5,000	39 ± 3	18 ± 1	–54	34 ± 3*	–13
10,000	42 ± 3	15 ± 0**	–64	34 ± 3*	–19
Female					
0	40 ± 3	20 ± 0	–50	28 ± 2	–30
625	38 ± 2	18 ± 0	–53	39 ± 3*	+3
1,250	42 ± 3	18 ± 0	–57	40 ± 3**	–5
2,500	33 ± 2	17 ± 0*	–48	32 ± 2	–3
5,000	32 ± 2	15 ± 0**	–53	31 ± 2	–3
10,000	29 ± 2**	16 ± 0**	–45	38 ± 3*	+31

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard error of average responses (amplitudes) for each trial; n=10
[b] Block 1 = mean of first 10 trials (tactile stimulus without auditory prepulse)
[c] Block 2 = mean of trials 11-70 (tactile stimulus preceded by auditory prepulse); 80-90 dB
[d] Percent prepulse inhibition = $\frac{\text{block 1 - block 2}}{\text{block 1}} \times 100$
[e] Block 3 = mean of last 10 trials (tactile stimulus without auditory prepulse)
[f] Percent habituation = $\frac{\text{block 1 - block 3}}{\text{block 1}} \times 100$

TABLE G19
Mean Forelimb Grip Strength of B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	6 Months (g)	12 Months (g)	18 Months (g)	24 Months (g)
Male					
0	86 ± 5	128 ± 6	130 ± 3	104 ± 4	88 ± 3[b]
125	83 ± 4	115 ± 4	111 ± 3**	97 ± 2	86 ± 5[b]
2,500	87 ± 4	111 ± 5	108 ± 2**	77 ± 4**	–
5,000	85 ± 5	118 ± 4	114 ± 3**	65 ± 2**[c]	–
Female					
0	79 ± 4	116 ± 2	110 ± 3[b]	102 ± 4[b]	97 ± 3[d]
125	80 ± 3	109 ± 2	110 ± 3	98 ± 4	94 ± 5[b]
2,500	81 ± 3	105 ± 2	100 ± 2	86 ± 3*	–
5,000	80 ± 2	113 ± 4	102 ± 3	78 ± 6**[e]	–

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard error; n=10 except where indicated; data are given as grams of force needed to break grip strength
[b] n=9
[c] n=7
[d] n=8
[e] n=4

TABLE G20
Mean Hindlimb Grip Strength of B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (g)	6 Months (g)	12 Months (g)	18 Months (g)	24 Months (g)
Male					
0	59 ± 3	103 ± 5	83 ± 4	80 ± 4	65 ± 3[b]
125	59 ± 2	107 ± 3	92 ± 4	83 ± 5	71 ± 3[b]
2,500	61 ± 3	101 ± 4	89 ± 2	73 ± 2	–
5,000	63 ± 5	116 ± 3	96 ± 4	70 ± 5[c]	–
Female					
0	65 ± 4	98 ± 4	94 ± 4[b]	80 ± 3[b]	71 ± 4[d]
125	70 ± 4	104 ± 4	98 ± 4	85 ± 7	86 ± 6[b]
2,500	67 ± 3	98 ± 3	84 ± 4	75 ± 4	–
5,000	66 ± 3	101 ± 4	88 ± 4	68 ± 7[e]	–

[a] Mean ± standard error; n=10 except where indicated; data are given as grams of force needed to break grip strength; differences from the control group are not significant by Dunnett's test.
[b] n=9
[c] n=7
[d] n=8
[e] n=4

TABLE G21
Mean Total Motor Activity Count of B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy	6 Months	12 Months	18 Months	24 Months
Male					
0	183 ± 15	164 ± 12	154 ± 10	146 ± 13	153 ± 12[b]
125	180 ± 14	194 ± 12	162 ± 7	151 ± 9	151 ± 13[b]
2,500	190 ± 16	286 ± 11**	219 ± 13**	168 ± 10	–
5,000	176 ± 18	246 ± 13**	217 ± 13**	62 ± 14**[c]	–
Female					
0	189 ± 24	148 ± 14	155 ± 10[b]	158 ± 9[b]	159 ± 11[d]
125	189 ± 16	231 ± 10**	171 ± 9	173 ± 14	173 ± 6[b]
2,500	201 ± 19	270 ± 10**	215 ± 8**	171 ± 9	–
5,000	178 ± 19	291 ± 11**	223 ± 13**	86 ± 17**[d]	–

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard error; n=10 except where indicated
[b] n=9
[c] n=7
[d] n=8

TABLE G22
Mean Paw Lick Latency of B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam[a]

Dose (ppm)	Prestudy (seconds)	6 Months (seconds)	12 Months (seconds)	18 Months (seconds)	24 Months (seconds)
Male					
0	11.8 ± 0.7	13.7 ± 2.2	17.0 ± 3.0	19.2 ± 2.8	22.4 ± 2.4[b]
125	9.4 ± 0.5	10.2 ± 0.9	10.9 ± 1.0	10.6 ± 0.9*	17.3 ± 2.3[b]
2,500	12.3 ± 0.8	11.4 ± 0.9	12.5 ± 0.8	13.2 ± 1.6	–
5,000	11.5 ± 0.8	11.2 ± 0.7	14.0 ± 2.1	14.2 ± 1.7[c]	–
Female					
0	11.4 ± 0.9	13.4 ± 2.2	10.7 ± 1.4[b]	11.2 ± 2.4[b]	18.0 ± 2.9[d]
125	9.0 ± 0.7	7.9 ± 0.4	8.4 ± 0.6	12.8 ± 1.8	17.5 ± 3.1[b]
2,500	11.3 ± 1.1	11.0 ± 2.1	9.4 ± 0.7	15.4 ± 2.3	–
5,000	11.6 ± 1.1	9.8 ± 0.5	9.8 ± 0.9	14.4 ± 1.6[e]	–

* Significantly different (P≤0.05) from the control group by Dunnett's test
[a] Mean ± standard error; n=10 except where indicated
[b] n=9
[c] n=7
[d] n=8
[e] n=4

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF OXAZEPAM

Oxazepam was obtained from Rousel Corporation (Englewood Cliffs, NJ) in one lot (86017.01), which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). The reports on analyses performed in support of the oxazepam studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a white, powdered solid, was identified as oxazepam by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra (Florey, 1974) of oxazepam (Figures H1 and H2). The observed melting point of 204.5 °C was consistent with the literature reference (*Merck*, 1983).

The purity of oxazepam was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Functional group titration was performed by dissolving a sample in dimethylformamide and titrating with 0.1 N tetrabutylammonium hydroxide. TLC was performed on Silica Gel 60 F-254 plates with two solvent systems: A) chloroform:methanol (10:1) and B) ethyl acetate:methanol:glacial acetic acid (80:20:10). One μL of a 5 mg/mL solution of anthracene in methanol was used as an internal standard. Visualization was accomplished with ultraviolet light (254 and 366 nm) and a spray of 37% formaldehyde solution in concentrated sulfuric acid. HPLC was performed with a Hewlett-Packard RP-18 column (200 × 4.6 mm ID) using a solvent system consisting of 1) water containing 1% (v/v) glacial acetic acid and 2) methanol containing 1% (v/v) glacial acetic acid, with a solvent ratio of 50:50, at a flow rate of 1 mL/minute. Detection was with ultraviolet light at 254 nm.

Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were in agreement with the theoretical values for oxazepam. Karl Fischer analysis indicated less than 0.03% water. Functional group titration indicated a purity of 101.4% ± 0.5%. TLC analysis using system A indicated a major spot and one trace impurity; using system B, a major spot was observed. HPLC resolved a major peak with no impurity peaks with areas 0.1% or greater relative to the major peak. Major peak comparison between this lot and a United States Pharmacopeia XXI (USP) standard indicated a relative purity of 103% ± 1%. The overall purity was determined to be greater than 99%.

Stability studies were performed by the analytical chemistry laboratory. HPLC was performed using the system described above except with a solvent ratio of 35:65. These studies indicated that oxazepam was stable for 2 weeks when stored protected from light at temperatures up to 60° C. The stability of the bulk chemical was monitored periodically at the study laboratory using HPLC as described above. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing oxazepam and feed (Table H1). The mixture was stored in sealed, labeled, plastic buckets for up to 3 weeks at 5° C.

Homogeneity and dose formulation stability analyses of the 500 ppm concentration were performed by the analytical chemistry laboratory. Aliquots were mixed with 5 mL of internal standard solution (acetophenone, 0.03 mg/mL in methanol) and 15 mL of methanol, then diluted to 50 mL with deionized water. After mixing, HPLC analysis was performed using the following system: a Burdick and Jackson C_{18} column (250 × 4.6 mm ID) and a mobile phase solvent system consisting of water:methanol:glacial acetic

acid (43:57:1) (v/v/v), at a flow rate of 1 mL/minute. Detection was with a Waters 440 detector at 254 nm. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 3 weeks when stored protected from light at 5° C.

Periodic analyses of the dose formulations of oxazepam were conducted at the study laboratory using HPLC. Periodic analyses of the dose formulations of oxazepam were conducted at the analytical chemistry laboratory using HPLC. During the 14-week studies, all of the dose formulations for Swiss-Webster and B6C3F$_1$ mice were within 10% of the target concentrations (Table H2). During the chronic studies, dose formulations were analyzed approximately every 8 weeks; all analyzed dose formulations for Swiss-Webster and B6C3F$_1$ mice were within 10% of the target concentrations except for two 125 ppm formulations for B6C3F$_1$ mice. These dose formulations were remixed. Results of the dose formulation analyses for the chronic studies are presented in Table H3. Results of periodic referee analyses performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table H4).

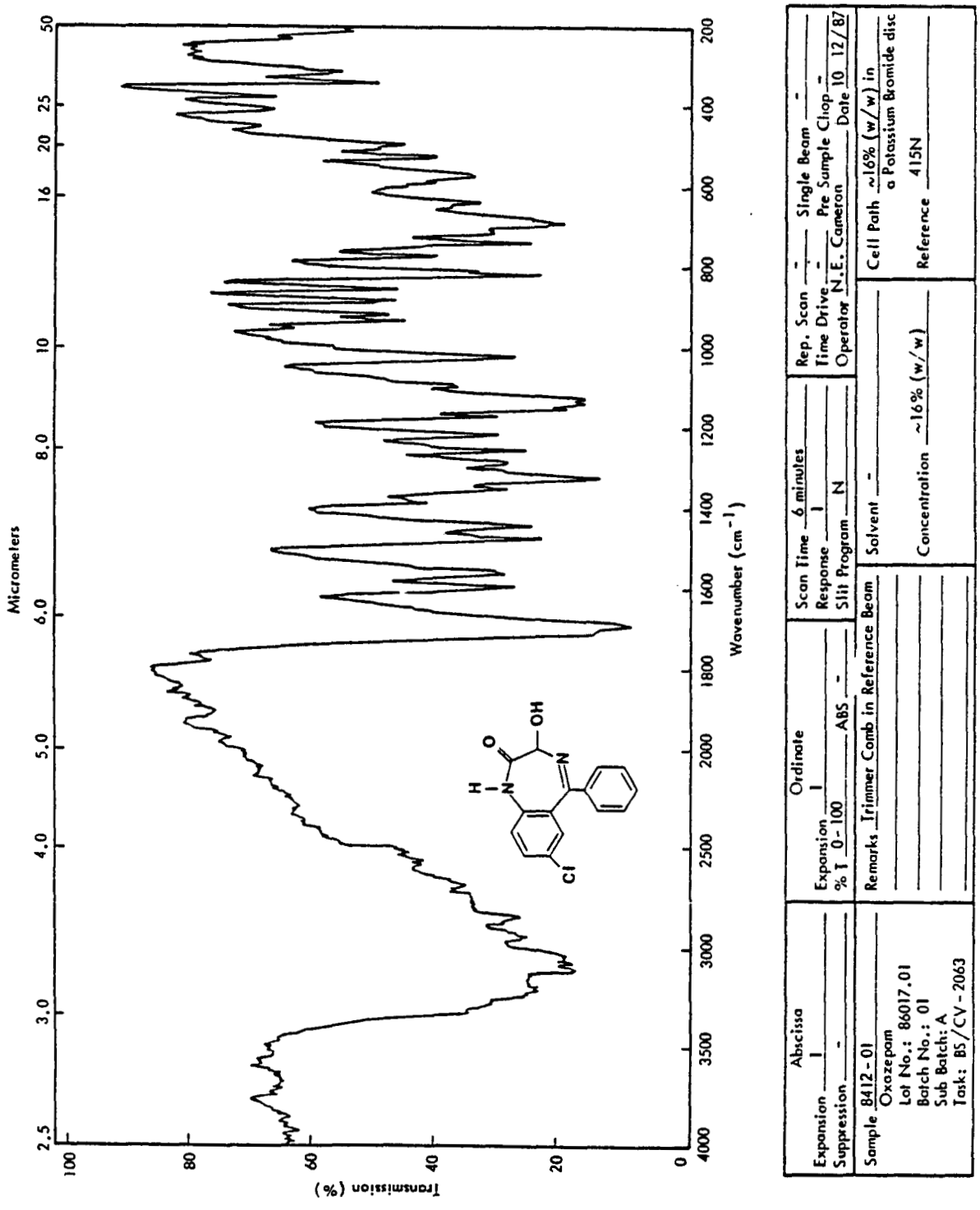

FIGURE H1
Infrared Absorption Spectrum of Oxazepam

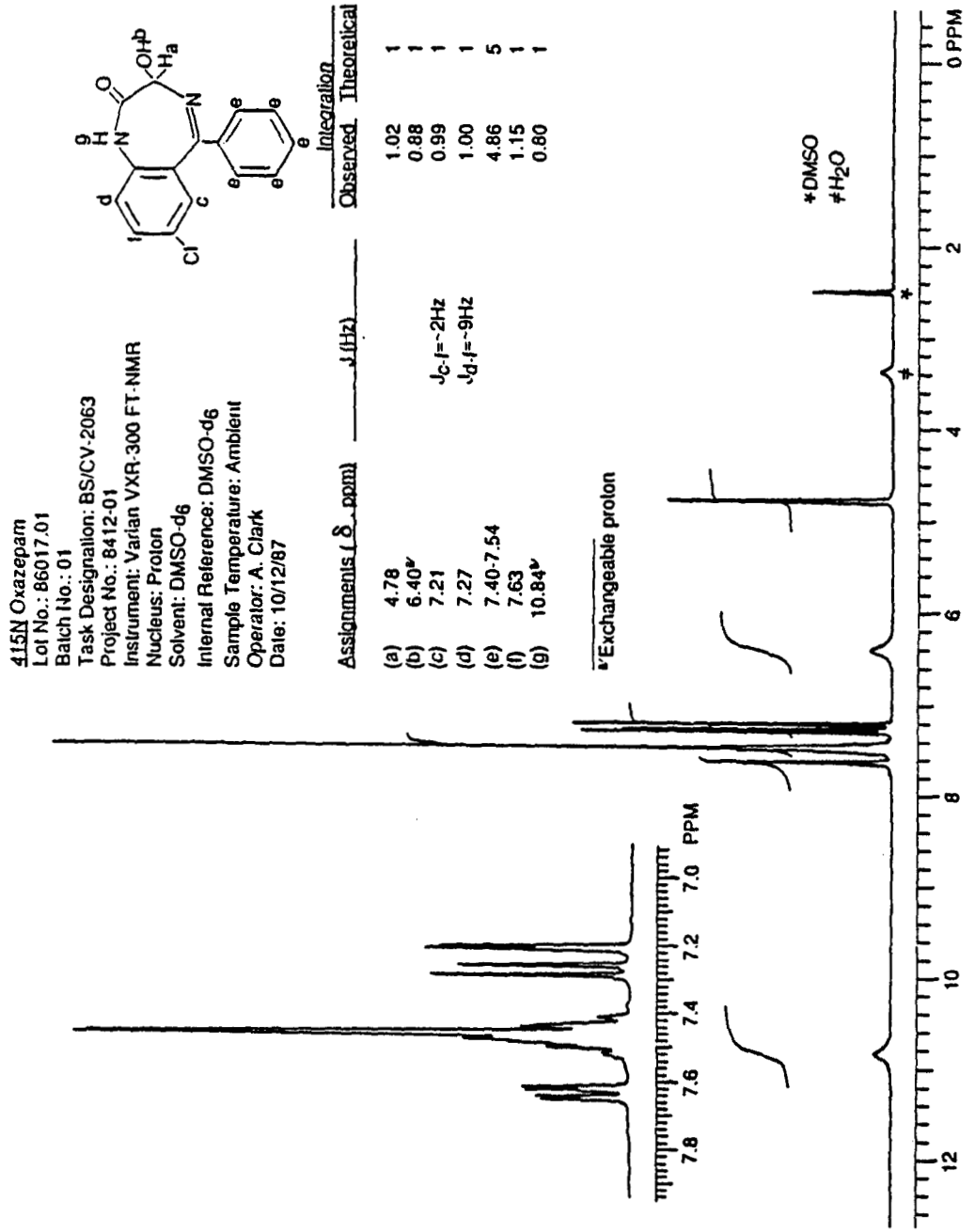

FIGURE H2
Nuclear Magnetic Resonance Spectrum of Oxazepam

TABLE H1
Preparation and Storage of Dose Formulations in the Feed Studies of Oxazepam

14-Week Studies	Chronic Studies
Preparation Dose formulations were prepared monthly. Premix was prepared by mixing feed and oxazepam (w/w); premix and remaining feed were layered in a Patterson-Kelley twin-shell blender and mixed for 15 minutes with the intensifier bar in operation for the first 5 minutes.	Same as 14-week studies except that dose formulations were prepared every 2 weeks.
Chemical Lot Number 86017.01	Same as 14-week studies
Maximum Storage Time 3 weeks	Same as 14-week studies
Storage Conditions In sealed, labeled, plastic buckets, stored at 5° C	Same as 14-week studies
Study Laboratory Battelle Columbus Laboratories Columbus, OH	Same as 14-week studies
Referee Laboratory Midwest Research Institute, Kansas City, MO	Same as 14-week studies

TABLE H2
Results of Analysis of Dose Formulations Administered to Swiss-Webster and B6C3F$_1$ Mice in the 14-Week Feed Studies of Oxazepam

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Swiss-Webster Mice				
3 May 1988	5 May 1988	625	633[b]	+1
		625	656[c]	+5
		625	660[d]	+6
		10,000	9,784[b]	–2
		10,000	10,004[c]	0
		10,000	9,999[d]	0
1 June 1988	2 June 1988	625	617	–1
		1,250	1,214	–3
		2,500	2,435	–3
		5,000	4,968	–1
		10,000	9,975	0
6 July 1988	8 July 1988	625	655	+5
		1,250	1,242	–1
		2,500	2,484	–1
		5,000	4,941	–1
		10,000	10,196	+2
24 August 1988	25 August 1988	625	665	+6
		1,250	1,311	+5
		2,500	2,519	+1
		5,000	5,013	0
		10,000	10,022	0
B6C3F$_1$ Mice				
3 May 1988	5 May 1988	625	633[b]	+1
		625	656[c]	+5
		625	660[d]	+6
		10,000	9,784[b]	–2
		10,000	10,004[c]	0
		10,000	9,999[d]	0
10 May 1988	12 May 1988	625	584	–7
		1,250	1,230	–2
		2,500	2,537	+1
		5,000	5,063	+1
		10,000	10,405	+4
1 June 1988	2 June 1988	625	617	–1
		1,250	1,214	–3
		2,500	2,435	–3
		5,000	4,968	–1
		10,000	9,975	0

TABLE H2
Results of Analysis of Dose Formulations Administered to Swiss-Webster and B6C3F$_1$ Mice in the 14-Week Feed Studies of Oxazepam (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
B6C3F$_1$ Mice (continued)				
6 July 1988	8 July 1988	625	655	+5
		1,250	1,242	−1
		2,500	2,484	−1
		5,000	4,941	−1
		10,000	10,196	+2
1 August 1988	4 August 1988	626	628	0
		1,250	1,251	0
		2,500	2,504	0
		5,000	5,014	0
		10,000	9,983	0

[a] Results of duplicate analyses
[b] Sample selection from top right of twin-shell blender
[c] Sample selection from top left of twin-shell blender
[d] Sample selection from bottom of twin-shell blender

TABLE H3
Results of Analysis of Dose Formulations Administered to Swiss-Webster and B6C3F$_1$ Mice in the Chronic Feed Studies of Oxazepam

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Swiss-Webster Mice				
1 May 1989	4 May 1989	5,000	5,086[b]	+2
		5,000	4,916[c]	–2
		5,000	5,125[d]	+3
30 June 1989	6-7 July 1989	2,500	2,541	+2
		5,000	4,972	–1
11 August 1989	15-16 August 1989	2,500	2,482	–1
		5,000	4,928	–1
6 October 1989	11-12 October 1989	2,500	2,604	+4
		5,000	5,050	+1
1 December 1989	5 December 1989	2,500	2,493	0
		5,000	5,056	+1
26 January 1990	31 January 1990	2,500	2,432	–3
		5,000	4,984	0
6 April 1990	11 April 1990	2,500	2,530	+1
		5,000	5,080	+2
4 June 1990	5 June 1990	2,500	2,527	+1
		5,000	5,020	0
27 July 1990	1 August 1990	2,500	2,488	0
		5,000	4,978	0
B6C3F$_1$ Mice				
1 May 1989	3 May 1989	125	128[b]	+2
		125	130[c]	+4
		125	132[d]	+6
	4 May 1989	5,000	5,086[b]	+2
		5,000	4,916[c]	–2
		5,000	5,125[d]	+3
19 June 1989	20-21 June 1989	125	133	+6
		2,500	2,656	+6
		5,000	5,031	+1
30 June 1989	6-7 July 1989	125	137	+10
		2,500	2,541	+2
		5,000	4,972	–1
11 August 1989	15-16 August 1989	125	138	+10
		2,500	2,482	–1
		5,000	4,928	–1

TABLE H3
Results of Analysis of Dose Formulations Administered to Swiss-Webster and B6C3F$_1$ Mice in the Chronic Feed Studies of Oxazepam (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
B6C3F$_1$ Mice (continued)				
16 August 1989	16-17 August 1989[e]	125	132	+6
6 October 1989	11-12 October 1989	125	126	+1
		2,500	2,604	+4
		5,000	5,050	+1
1 December 1989	5 December 1989	125	140	+12
		2,500	2,493	0
		5,000	5,056	+1
6 December 1989	6 December 1989[e]	125	126	+1
26 January 1990	31 January 1990	125	127	+2
		2,500	2,432	−3
		5,000	4,984	0
6 April 1990	11 April 1990	125	128	+2
		2,500	2,530	+1
		5,000	5,080	+2
4 June 1990	5 June 1990	125	122	−2
		2,500	2,527	+1
		5,000	5,020	0
27 July 1990	1 August 1990	125	135	+8
		2,500	2,488	0
		5,000	4,978	0
28 September 1990	3 October 1990	125	125	0
		2,500	2,467	−1
		5,000	4,878	−2
16 November 1990	19 November 1990	125	124	−1
		2,500	2,487	−1
		5,000	5,034	+1
11 January 1991	15 January 1991	125	120	−4
		2,500	2,525	+1
		5,000	4,950	−1
8 March 1991	11 March 1991	125	124	−1
		2,500	2,495	0
6 May 1991	7 May 1991	125	126	+1
		2,500	2,386	−5

[a] Results of duplicate analyses
[b] Sample selection from bottom of twin-shell blender
[c] Sample selection from top right of twin-shell blender
[d] Sample selection from top left of twin-shell blender
[e] Analysis results of remix

TABLE H4
Results of Referee Analysis of Dose Formulations Administered in the 14-Week and Chronic Feed Studies of Oxazepam

Date Prepared	Target Concentration (ppm)	Determined Concentration (mg/mL)	
		Study Laboratory[a]	Referee Laboratory[b]
14-Week Studies			
Swiss-Webster Mice			
1 June 1988	10,000	9,999	10,100 ± 58
24 August 1988	625	665	628 ± 18
B6C3F$_1$ Mice			
10 May 1988	1,250	1,230	1,216 ± 6
6 July 1988	5,000	4,941	4,889 ± 104
Chronic Studies			
Swiss-Webster Mice			
30 June 1989	2,500	2,541	2,520 ± 60
26 January 1990	5,000	4,984	5,070 ± 10
B6C3F$_1$ Mice			
19 June 1989	125	133	132 ± 2
30 June 1989	2,500	2,541	2,520 ± 60
26 January 1990	5,000	4,984	5,070 ± 10

[a] Results of duplicate analysis
[b] Results of triplicate analysis; mean ± standard deviation

APPENDIX I
FEED AND COMPOUND CONSUMPTION IN THE CHRONIC FEED STUDIES

TABLE I1
Feed and Compound Consumption by Male Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

Week	0 ppm		2,500 ppm			5,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	4.6	27.9	4.2	28.6	364	4.0	28.7	690
6	5.1	31.9	4.5	32.4	351	4.7	33.4	700
10	4.6	34.3	4.3	34.5	310	4.5	35.2	642
13	4.7	36.0	4.1	36.0	286	4.4	36.2	605
17	4.7	38.4	3.9	37.5	262	4.0	37.4	532
21	4.1	40.3	3.8	38.7	246	4.0	38.2	522
25	4.4	41.6	3.8	39.8	242	3.9	39.2	502
29	4.4	42.2	3.9	40.8	241	4.0	39.4	506
33	4.5	42.8	4.1	40.7	251	4.2	39.2	530
41	4.1	42.2	3.8	40.3	238	4.2	39.2	532
45	4.1	41.7	3.7	39.4	236	4.1	37.8	542
49	4.4	41.5	3.9	38.6	253	4.1	37.4	549
53	4.4	41.7	3.7	37.9	247	3.8	36.4	521
Mean for weeks								
1-13	4.8	32.5	4.3	32.9	328	4.4	33.4	659
14-52	4.4	41.3	3.9	39.6	246	4.1	38.5	527
53	4.4	41.7	3.7	37.9	247	3.8	36.4	521

[a] Grams of feed consumed per animal per day; study terminated at week 57
[b] Milligrams of oxazepam consumed per day per kilogram body weight

TABLE I2
Feed and Compound Consumption by Female Swiss-Webster Mice in the 57-Week Feed Study of Oxazepam

Week	0 ppm		2,500 ppm			5,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	4.8	22.8	3.9	25.4	381	3.6	24.8	732
6	5.6	26.3	5.1	29.6	428	5.1	29.3	863
10	5.3	28.3	5.0	31.1	402	4.8	31.2	762
13	5.0	29.4	4.5	31.8	352	4.4	31.9	695
17	5.0	31.4	4.3	33.8	317	4.2	33.6	620
21	4.4	32.4	4.1	34.9	291	4.2	33.9	625
25	4.8	33.3	4.0	35.9	276	4.2	34.7	611
29	4.9	34.5	4.1	36.3	281	4.1	34.9	591
33	4.9	34.8	4.3	36.8	295	4.5	35.2	642
37	4.6	35.0	4.0	37.4	267	4.3	35.5	610
41	4.5	34.8	4.2	36.6	286	4.8	34.6	690
45	4.8	34.3	4.1	36.4	279	4.4	34.7	632
49	4.9	34.8	4.2	36.7	286	4.5	34.3	661
53	5.0	35.0	4.3	36.3	299	4.3	34.4	619
Mean for weeks								
1-13	5.1	26.7	4.6	29.5	391	4.5	29.3	763
14-52	4.7	33.9	4.1	36.1	287	4.4	34.6	631
53	5.0	35.0	4.3	36.3	299	4.3	34.4	619

[a] Grams of feed consumed per animal per day; study terminated at week 57
[b] Milligrams of oxazepam consumed per day per kilogram body weight

TABLE I3
Feed and Compound Consumption by Male B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

Week	0 ppm		125 ppm			2,500 ppm			5,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	3.3	22.8	3.4	23.7	18	3.0	23.8	313	2.8	23.4	589
6	4.3	27.5	4.3	29.3	18	4.5	29.2	382	4.8	28.7	836
10	4.4	32.0	4.2	32.9	16	4.5	31.7	352	4.9	31.5	780
13	4.2	34.6	4.2	35.4	15	4.4	33.5	332	4.7	33.3	702
17	4.1	38.5	4.0	38.2	13	4.2	35.9	290	4.3	34.9	616
21	4.0	41.2	4.1	39.9	13	4.0	36.7	276	4.4	35.2	624
25	4.0	43.5	3.9	41.8	12	4.0	38.1	266	4.4	36.0	605
29	3.9	44.3	3.7	43.0	11	3.6	38.8	234	4.0	36.7	547
33	4.1	46.3	4.1	44.4	12	4.4	39.4	277	4.6	37.1	625
37	4.0	46.6	3.9	45.1	11	4.2	40.1	259	4.4	37.6	589
41	4.0	47.1	3.9	46.1	10	4.1	40.8	250	4.6	38.0	611
45	4.3	47.4	4.3	46.3	12	4.3	40.8	266	4.8	37.8	641
49	4.3	47.5	4.1	46.9	11	4.3	41.0	264	4.8	37.7	639
53	4.5	47.3	4.2	47.5	11	4.5	41.1	274	5.2	37.1	705
57	4.5	48.0	4.4	47.9	11	4.8	41.4	289	5.7	36.4	788
61	4.4	48.5	4.4	48.3	11	4.5	41.3	273	5.2	35.8	726
65	4.6	48.7	4.4	48.3	12	4.7	40.5	288	5.7	34.7	816
69	4.5	48.3	4.4	48.2	11	4.6	39.7	291	5.4	33.9	792
73	4.6	48.8	4.5	48.4	12	4.7	38.4	303	5.4	33.6	803
77	4.4	48.8	4.1	48.9	11	5.0	37.1	338	4.8	33.4	724
81	4.3	48.6	4.2	48.6	11	4.6	36.1	319	4.3	33.0	648
85	4.3	49.4	4.3	49.6	11	3.9	35.4	275	4.3	32.8	659
89	4.6	49.4	4.2	48.9	11	5.4	34.5	388	4.7	32.6	724
93	4.7	49.1	4.4	48.7	11	5.1	33.1	386			
97	4.8	48.3	4.1	48.2	11	5.3	33.0	401			
101	4.4	47.7	4.2	47.8	11	4.4	33.2	332			
104	4.4	48.3	4.2	47.6	11	4.4	33.5	329			
Mean for weeks											
1-13	4.1	29.2	4.0	30.3	17	4.1	29.6	345	4.3	29.2	727
14-52	4.1	44.7	4.0	43.5	11	4.1	39.1	265	4.5	36.8	611
53-104	4.5	48.5	4.3	48.4	11	4.7	36.7	324	5.1	34.0	742

[a] Grams of feed consumed per animal per day
[b] Milligrams of oxazepam consumed per day per kilogram body weight

TABLE I4
Feed and Compound Consumption by Female B6C3F$_1$ Mice in the 2-Year Feed Study of Oxazepam

Week	0 ppm		125 ppm			2,500 ppm			5,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	3.6	19.1	3.0	20.7	18	2.3	21.0	271	1.8	20.5	444
6	5.9	23.3	5.8	25.1	29	5.1	25.1	512	5.4	24.7	1,085
10	5.8	27.3	5.7	29.2	24	5.4	28.2	482	5.1	27.2	939
13	6.1	30.3	5.6	31.8	22	5.2	30.3	429	5.2	28.5	907
17	5.6	35.5	5.1	35.9	18	4.7	33.4	348	4.6	31.5	732
21	5.6	38.7	5.1	38.4	17	4.6	34.6	334	4.6	32.5	709
25	5.2	41.7	4.8	40.3	15	4.6	36.6	313	4.6	33.6	689
29	5.1	43.9	4.6	42.0	14	4.3	37.7	285	4.6	34.4	674
33	5.3	45.9	5.0	43.2	14	4.9	38.4	317	5.1	35.2	728
37	4.9	47.8	4.5	44.2	13	4.4	39.4	280	4.6	36.0	640
41	4.9	48.7	4.5	45.3	12	4.5	40.3	282	4.9	36.8	662
45	5.1	49.9	5.0	45.3	14	4.7	39.9	297	5.2	36.2	723
49	5.0	50.8	4.8	46.2	13	4.8	40.3	298	5.2	36.9	706
53	5.2	51.0	4.7	46.3	13	4.8	40.6	295	5.4	36.4	743
57	5.2	51.9	5.1	46.5	14	5.4	40.6	331	6.1	35.8	849
61	5.1	53.2	5.0	47.0	13	4.9	40.9	301	6.0	35.1	848
65	5.2	53.4	5.1	46.6	14	5.2	41.0	315	6.3	34.2	926
69	5.1	53.6	4.8	46.8	13	5.2	40.7	318	6.3	33.9	934
73	5.0	54.4	4.9	47.5	13	5.6	39.3	356	6.2	33.5	920
77	5.0	54.4	4.8	47.3	13	5.7	38.5	373	5.8	33.3	870
81	5.0	55.1	4.7	47.3	12	5.5	37.8	366	4.9	33.1	745
85	4.7	56.0	4.8	47.2	13	5.4	36.8	366	4.2	33.4	624
89	5.0	54.6	4.8	47.1	13	5.9	36.1	411			
93	4.8	55.2	4.9	46.8	13	5.4	35.2	384			
97	4.8	54.1	4.8	46.5	13	5.6	35.6	390			
101	4.9	53.2	4.9	45.5	14	5.2	35.0	374			
Mean for weeks											
1-13	5.3	25.0	5.0	26.7	23	4.5	26.2	423	4.4	25.2	844
14-52	5.2	44.8	4.8	42.3	14	4.6	37.8	306	4.8	34.8	696
53-101	5.0	53.9	4.9	46.8	13	5.4	38.3	352	5.7	34.3	829

[a] Grams of feed consumed per animal per day
[b] Milligrams of oxazepam consumed per day per kilogram body weight

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE J1
Ingredients of NIH-07 Rat and Mouse Ration[a]

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

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

TABLE J2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

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

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

TABLE J3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	23.5 ± 0.88	21.30 – 25.20	18
Crude Fat (% by weight)	5.2 ± 0.29	4.80 – 5.80	18
Crude Fiber (% by weight)	3.6 ± 0.56	2.60 – 4.80	18
Ash (% by weight)	6.5 ± 0.20	6.20 – 6.97	18
Amino Acids (% of total diet)			
Arginine	1.308 ± 0.060	1.210 – 1.390	8
Cystine	0.306 ± 0.084	0.181 – 0.400	8
Glycine	1.150 ± 0.047	1.060 – 1.210	8
Histidine	0.576 ± 0.024	0.531 – 0.607	8
Isoleucine	0.917 ± 0.029	0.881 – 0.944	8
Leucine	1.946 ± 0.055	1.850 – 2.040	8
Lysine	1.270 ± 0.058	1.200 – 1.370	8
Methionine	0.448 ± 0.128	0.306 – 0.699	8
Phenylalanine	0.987 ± 0.140	0.665 – 1.110	8
Threonine	0.877 ± 0.042	0.824 – 0.940	8
Tryptophan	0.236 ± 0.176	0.107 – 0.671	8
Tyrosine	0.676 ± 0.105	0.564 – 0.794	8
Valine	1.103 ± 0.040	1.050 – 1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 ± 0.258	1.830 – 2.570	7
Linolenic	0.280 ± 0.040	0.210 – 0.320	7
Vitamins			
Vitamin A (IU/kg)	7,425 ± 1,737	5,060 – 12,540	18
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 – 6,300	4
α-Tocopherol (ppm)	37.95 ± 9.406	22.5 – 48.90	8
Thiamine (ppm)	18.72 ± 1.72	15.0 – 22.0	18
Riboflavin (ppm)	7.92 ± 0.87	6.10 – 9.00	8
Niacin (ppm)	103.38 ± 26.59	65.0 – 150.0	8
Pantothenic acid (ppm)	29.54 ± 3.60	23.0 – 34.0	8
Pyridoxine (ppm)	9.55 ± 3.48	5.60 – 14.0	8
Folic acid (ppm)	2.25 ± 0.73	1.80 – 3.70	8
Biotin (ppm)	0.25 ± 0.04	0.19 – 0.32	8
Vitamin B_{12} (ppb)	38.45 ± 22.01	10.6 – 65.0	8
Choline (ppm)	3,089 ± 328	2,400 – 3,430	8
Minerals			
Calcium (%)	1.21 ± 0.08	1.08 – 1.37	18
Phosphorus (%)	0.95 ± 0.04	0.88 – 1.03	18
Potassium (%)	0.883 ± 0.078	0.772 – 0.971	6
Chloride (%)	0.526 ± 0.092	0.380 – 0.635	8
Sodium (%)	0.313 ± 0.390	0.258 – 0.371	8
Magnesium (%)	0.168 ± 0.010	0.151 – 0.181	8
Sulfur (%)	0.280 ± 0.064	0.208 – 0.420	8
Iron (ppm)	360.54 ± 100	255.0 – 523.0	8
Manganese (ppm)	91.97 ± 6.01	81.70 – 99.40	8
Zinc (ppm)	54.72 ± 5.67	46.10 – 64.50	8
Copper (ppm)	11.06 ± 2.50	8.09 – 15.39	8
Iodine (ppm)	3.37 ± 0.92	1.52 – 4.13	6
Chromium (ppm)	1.79 ± 0.36	1.04 – 2.09	8
Cobalt (ppm)	0.681 ± 0.14	0.490 – 0.780	4

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.36 ± 0.18	0.06 – 0.60	18
Cadmium (ppm)	<0.10		18
Lead (ppm)	0.20 ± 0.07	0.10 – 0.30	18
Mercury (ppm)	<0.05		18
Selenium (ppm)	0.33 ± 0.11	0.10 – 0.52	18
Aflatoxins (ppb)	<5.00		18
Nitrate nitrogen (ppm)[b]	14.48 ± 4.36	5.0 – 21.0	18
Nitrite nitrogen (ppm)[b]	0.20 ± 0.21	<0.10 – 1.00	18
BHA (ppm)[c]	1.33 ± 0.77	<1.00 – 4.00	18
BHT (ppm)[c]	1.55 ± 1.42	<1.10 – 7.00	18
Aerobic plate count (CFU/g)[d]	121,222 ± 91,920	25,000 – 380,000	18
Coliform (MPN/g)[e]	20.91 ± 20.22	<3.00 – 75.00	18
E. coli (MPN/g)	3.38 ± 1.61	<3.00 – 9.00	18
Total nitrosoamines (ppb)[f]	7.02 ± 2.87	2.00 – 13.70	18
N-Nitrosodimethylamine (ppb)[f]	5.30 ± 2.30	1.00 – 11.00	18
N-Nitrosopyrrolidine (ppb)[f]	1.72 ± 1.14	1.00 – 4.30	18
Pesticides			
α-BHC[g]	<0.01		18
β-BHC	<0.02		18
γ-BHC	<0.01		18
δ-BHC	<0.01		18
Heptachlor	<0.01		18
Aldrin	<0.01		18
Heptachlor epoxide	<0.01		18
DDE	<0.01		18
DDD	<0.01		18
DDT	<0.01		18
HCB	<0.01		18
Mirex	<0.01		18
Methoxychlor	<0.05		18
Dieldrin	<0.01		18
Endrin	<0.01		18
Telodrin	<0.01		18
Chlordane	<0.05		18
Toxaphene	<0.1		18
Estimated PCBs	<0.2		18
Ronnel	<0.01		18
Ethion	<0.02		18
Trithion	<0.05		18
Diazinon	<0.1		18
Methyl parathion	<0.02		18
Ethyl parathion	<0.02		18
Malathion	0.20 ± 0.15	0.05 – 0.48	18
Endosulfan 1	<0.01		18
Endosulfan 2	<0.01		18
Endosulfan sulfate	<0.03		18

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Sources of contamination: alfalfa, grains, and fish meal
[c] Sources of contamination: soy oil and fish meal
[d] CFU = colony forming unit
[e] MPN = most probable number
[f] All values were correct for % recovery.
[g] BHC = hexachlorocyclohexane or benzene hexachloride

APPENDIX K
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 all 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.

Swiss-Webster Mice

For the 14-week study, samples were obtained from five male and five female control mice at terminal sacrifice. These samples were processed appropriately and were submitted to Microbiological Associates (Bethesda, MD) for viral titer screening. The following tests were performed:

Method of Analysis	Time of Analysis
ELISA	
Ectromelia virus	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
Mouse adenoma virus	Study termination
MHV (mouse hepatitis virus)	Study termination
MVM (minute virus of mice)	Study termination
PVM (pneumonia virus of mice)	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
K (papovavirus)	Study termination
Polyoma virus	Study termination
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Reovirus 3	Study termination

For the 57-week study, serum samples for viral screening were collected from up to five male and five female sentinel mice at 6, 12, and 13 months into the study. Additional samples were collected at 10, 11, and 12 months from mice exhibiting neurological signs; test results were negative. Blood from each collection was processed appropriately, shipped to Microbiological Associates, and screened for the following:

Method of Analysis	Time of Analysis
ELISA	
Ectromelia virus	6, 12, and 13 months
GDVII	6, 12, and 13 months
EDIM	12 and 13 months
LCM	6, 12, and 13 months
Mouse adenoma virus	6, 12, and 13 months
MHV	6, 12, and 13 months
PVM	6, 12, and 13 months
Reovirus 3	6, 12, and 13 months
Sendai	6, 12, and 13 months
Hemagglutination Inhibition	
K	6, 12, and 13 months
Polyoma virus	6, 12, and 13 months
Immunofluorescence Assay	
EDIM	6 months
MVM	6, 12, and 13 months

B6C3F$_1$ Mice

For the 14-week study, samples were obtained from five male and five female control mice at terminal sacrifice. These samples were processed appropriately and were submitted to Microbiological Associates for viral titer screening. The following tests were performed:

Method of Analysis	Time of Analysis
ELISA	
Ectromelia virus	Study termination
GDVII	Study termination
Mouse adenoma virus	Study termination
MHV	Study termination
MVM	Study termination
PVM	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
K	Study termination
Polyoma virus	Study termination
Immunofluorescence Assay	
EDIM	Study termination
LCM	Study termination
Reovirus 3	Study termination

For the 2-year study, serum samples for viral screening were collected from up to five male and five female sentinel mice at 6, 12, and 18 months into the study. Serum for the 24-month screening was obtained from four male and two female mice in the 2,500 ppm groups and one male and three female mice in the 125 ppm groups. Blood from each collection was processed appropriately, shipped to Microbiological Associates, and screened for the following:

Method of Analysis	Time of Analysis
ELISA	
Ectromelia virus	6, 12, 18, and 24 months
EDIM	12 and 18 months
GDVII	6, 12, 18, and 24 months
LCM	6, 12, and 18 months
Mouse adenoma virus	6, 12, 18, and 24 months
MHV	6, 12, 18, and 24 months
Mycoplasma arthritidis	24 months
Mycoplasma pulmonis	24 months
PVM	6, 12, 18, and 24 months
Reovirus 3	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months
Hemagglutination Inhibition	
K	6, 12, 18, and 24 months
MVM	18 and 24 months
Polyoma virus	6, 12, 18, and 24 months
Immunofluorescence Assay	
EDIM	6 and 24 months
LCM	24 months
Mouse adenoma virus	24 months
MHV	24 months
MVM	6 and 12 months
Reovirus 3	18 months

All test results were negative.

APPENDIX L
RAS PROTO-ONCOGENE ACTIVATION OF LIVER NEOPLASMS FROM B6C3F$_1$ MICE ADMINISTERED OXAZEPAM IN FEED FOR 2 YEARS

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RAS PROTO-ONCOGENE ACTIVATION OF LIVER NEOPLASMS FROM B6C3F$_1$ MICE ADMINISTERED OXAZEPAM IN FEED FOR 2 YEARS

INTRODUCTION

Liver neoplasms are commonly seen in B6C3F$_1$ mice in 2-year studies, occurring with a typical incidence of 30% to 40% in control males and 10% to 20% in control females. Chemical-induced liver neoplasms in mice have a high frequency of proto-oncogene activation, particularly by point mutations of H-, K-, or N-*ras* genes (Barbacid, 1987). The frequency of *ras* activation in these neoplasms is often greater than that detected in neoplasms occurring in control animals (Reynolds *et al.*, 1987), and there is evidence for chemical specificity in the pattern of oncogene activation (Wiseman *et al.*, 1986). The specific types of oncogene-activating mutations induced by a chemical carcinogen often agree with what is expected based on the DNA adducts formed by that agent (Wiseman *et al.*, 1986; You *et al.*, 1989). Even for "nongenotoxic" carcinogens, the patterns of *ras* gene mutations in neoplasms can give clues about the mechanism of tumorigenesis (Fox *et al.*, 1990; Devereux *et al.*, 1993).

The purpose of this study was to examine liver neoplasms from male and female B6C3F$_1$ mice administered oxazepam for the presence of activated *ras* genes. Oncogene activation in chemical-induced and "spontaneous" neoplasms was compared to identify mechanisms that may be involved in the induction of hepatic neoplasia in these animals.

MATERIALS AND METHODS

Neoplasm isolation. The liver neoplasms analyzed in this study were collected from mice in the 2-year Battelle Columbus (Columbus, OH) study at moribund or terminal sacrifice. Upon necropsy, sections of each neoplasm were fixed and processed for histological examination, and the remainder of each neoplasm was frozen in liquid nitrogen and stored at -70° C. Tissues were transferred to NIEHS where the oncogene study was performed.

DNA isolation. DNA was isolated from small (<0.1 g) pieces of 20 or more liver neoplasms from each group receiving oxazepam. The DNA isolation procedure (Marmur, 1961) was scaled down and performed in 1.5 mL Eppendorf tubes.

DNA amplification. DNA was amplified by the polymerase chain reaction (PCR) (Saiki *et al.*, 1988), and details on the use of nested primers have been described previously (Smit *et al.*, 1988; Devereux *et al.*, 1991). Reactions were carried out in volumes of 20 to 50 μL and consisted of 0.2 to 0.5 μg genomic DNA, two amplification primers (1μM), 200μM dNTPs (dATP, dCTP, dGTP, dTTP), reaction buffer [50mM KCl, 10mM Tris (pH 8.4 at room temperature), 1mM $MgCl_2$], and 0.5 units of Taq polymerase (Promega, Madison, WI). The primers used for amplification of the different exons of the K-*ras* and H-*ras* genes are listed in Devereux *et al.* (1991) and Devereux *et al.* (1993). Incubations containing DNA from normal tissue and no DNA controls were run with all sets of reactions. For those samples to be sequenced, asymmetric PCR (one primer at normal concentration and one primer diluted 1:20) was performed with the outer PCR reaction as the source of DNA template for this amplification reaction. Amplified DNA was desalted and unused primers and dNTPs were removed by spin dialysis in a Centricon 30 tube (Amicon, Danvers, MA). The amount of DNA was estimated by measuring optical density at a wavelength of 260 nm, and then the samples were evaporated and stored at -20° C until further use.

Slot-blot oligonucleotide hybridization. Amplified DNA samples (10 to 50 ng) were denatured in 0.4M NaOH/3M NaCl and applied to Nytran nylon filters (0.45 μm mesh) using a slot-blot apparatus (Schleicher and Schuell, Keene, NH). The filters were hybridized to 5′-^{32}P end labeled 19 base

oligonucleotides centered on the second base of codon 61 (or other codons of interest) of the H-*ras* or K-*ras* genes. The 19-oligomer probes contained either the wild type sequence (CAA for codon 61) or mutant sequence (AAA, CGA, or CTA for codon 61). Following hybridization, the blots were washed according to the method of Saiki *et al.* (1986) and exposed to X-ray film for 2 to 24 hours.

Direct sequencing. Direct sequencing of the amplified exon 2 of the H-*ras* gene was performed as described by Tindall and Stankowski (1989). The sequences of the primers used for the different regions of the *ras* genes are also given by these authors, and the primers were end labeled with γ-^{33}P labeled ATP. Following the sequencing reaction, samples were electrophoresed on 8% acrylamide gels containing urea. Gels were dried and exposed to X-ray film overnight.

Single stranded conformation polymorphism. Single stranded conformation polymorphism (SSCP) of amplified exons 1, 2, or 3 of the H-*ras* gene and exon 1 of the K-*ras* gene in some liver neoplasm samples was performed to screen neoplasms for mutations according to the method of Orita *et al.* (1989). Briefly, DNA was amplified by PCR in 20 μL volumes (20 cycles - 94° C, 1 min.; 50° C, 30 sec.; 72° C, 30 sec.) with 200μM dNTPs using outer amplification primers (4 pmol each); a second amplification was performed with inner amplification primers (20 pmol each) (30 cycles - 94° C, 1 min; 52° C, 1 min; 72° C, 30 sec). One μL from the first reaction was added to 19 μL to start the second set of cycles. At this point, a mini-agarose gel was run with 5 μL of the PCR reaction to check for successful amplification. A third PCR reaction with α-^{33}P labeled dATP (0.2 μCi), 2μM dNTPs, and 20 pmol each inner primer was incubated with 1 μL from the second reaction as the template DNA (15 cycles - 94° C, 1 min; 52° C, 1 min; 72° C, 30 sec). The crude product (2 μL) was mixed with 50 μL of a 0.1% sodium dodecyl sulfate-10mM EDTA mixture, followed by 1:1 dilution with a 95% formamide-20mM EDTA-0.05% bromophenol blue-0.05% xylene cyanol loading solution. Diluted samples were heat denatured by boiling for 2 to 5 minutes and then placed on ice and loaded on a 12% non-denaturing acrylamide gel containing 5% glycerol. Electrophoresis was carried out at 4° C with constant power at 20 watts for 16 hours on a Model S2 sequencing gel apparatus (BRL, Gaithersburg, MD). The gels were dried and exposed to film for 4 to 24 hours at room temperature.

RESULTS

In order to determine if the oxazepam-induced neoplasms contained an H-*ras* mutation profile similar to that observed with "spontaneous" neoplasms, similar sample groups of 20 or more adenomas and carcinomas from each exposure group were screened by PCR amplification of H-*ras* exon 2 followed by selective oligonucleotide hybridization with slot blots of the amplified DNA for the three common codon 61 mutations (Table L1). SSCP was used as an alternative screening method for detection of mutations in DNA from some of the neoplasm samples. Results from the screening were confirmed by direct sequencing. In neoplasms from animals in the 125 ppm exposure group, 13/37 (35%) exhibited H-*ras* mutations in codon 61. Nine of these showed C to A transversions in base 1, and four had A to G transitions in base 2. Mutations occurred in 58% of the neoplasms taken from control mice and examined in the present study and in 65% of historical control B6C3F$_1$ mouse liver neoplasms. While the frequency of codon 61 mutations in the 125 ppm group was only about half that observed with control liver neoplasms, the mutation spectrum of the H-*ras* genes detected was similar to that in the "spontaneous" neoplasms. In the liver neoplasms from the 2,500 ppm exposure group, only one H-*ras* codon 61 mutation was identified, and no mutations were detected in codon 61 in neoplasms from the 5,000 ppm exposure group.

In subsequent analyses, neoplasm DNA from the 125 and the 2,500 or 5,000 ppm groups was analyzed for mutations in codons 12, 13, or 117 of the H-*ras* gene (Table L1) and codons 12 or 13 of the K-*ras* gene (not shown), the other known hotspots for *ras* activation in mouse liver neoplasms. However, no mutations in these regions of the genes were detected in any of the neoplasms.

DISCUSSION

The formation of both "spontaneous" and chemical-induced liver neoplasms in B6C3F$_1$ mice has often been associated with activation of the H-*ras* gene (Wiseman *et al.*, 1986; Reynolds *et al.*, 1987; Fox *et al.*, 1990; Devereux *et al.*, 1993). In this mouse strain, activated H-*ras* genes have been detected in "spontaneous" liver neoplasms at a high frequency, which suggests that this gene is important in liver neoplasm formation. Despite the prevalence of H-*ras* mutations in liver neoplasms in this mouse strain, distinct specific mutation spectra have been identified in neoplasms induced by certain genotoxic agents. For example, specific mutation patterns in codon 61 of the H-*ras* gene, which are different from the profile in control liver neoplasms, have been detected in neoplasms induced by treatment with N-hydroxy-1-acetylaminofluorene, vinyl carbamate, and 1-hydroxy-2,3-dehydroestragole (Wiseman *et al.*, 1986). For many genotoxic carcinogens, the specific *ras* mutation pattern identified in murine neoplasms is associated with DNA adducts derived from the chemicals (Wiseman *et al.*, 1986; Belinsky *et al.*, 1989; You *et al.*, 1989). These studies indicated that *ras* activation is an early event in the development of these neoplasms and that *ras* gene mutation patterns in neoplasm sets may be important in understanding the mechanisms by which certain chemicals cause cancer.

In addition to studies with genotoxic carcinogens, recent studies have analyzed B6C3F$_1$ mouse liver neoplasms induced by "nongenotoxic" chemicals for *ras* activation. Recently it was reported that the frequency and the pattern of H-*ras* gene mutations identified in methylene chloride-induced liver neoplasms did not differ significantly from those detected in control neoplasms (Devereux *et al.*, 1993). Results from that study suggest that the activation of the H-*ras* gene in the chemical-induced neoplasms is not directly related to chemical exposure. In another study (Fox *et al.*, 1990), detection of H-*ras* mutations in phenobarbital-, chloroform-, or ciprofibrate-induced liver neoplasms was significantly lower than that detected in neoplasms in control animals. Results from that study suggested that pathways other than H-*ras* activation are involved in the development of liver neoplasms induced by those compounds. Furthermore, those "nongenotoxic" compounds appear to give a selective growth advantage to cells that lack mutations in the H-*ras* gene.

In the present study, codon 61 mutations in the H-*ras* gene were detected in 13/37 (35%) of the hepatocellular neoplasms from the 125 ppm group as compared to 58% of the neoplasms in control mice, only one neoplasm (2.5%) in the 2,500 ppm group, and none in the 5,000 ppm group of mice. Of those 13 neoplasms from the 125 ppm group of mice with an activated H-*ras* gene, the mutation spectrum identified was similar to that detected in liver neoplasms that had an activated H-*ras* gene in control mice from both this study and others. These data indicate that oxazepam treatment fosters the formation of neoplasms that do not contain H-*ras* mutations, and thus, it is likely that the neoplasms in the 125 ppm group include some related to chemical exposure. These findings are significant because the neoplasm incidence data alone are insufficient to determine if the neoplasms that formed in the 125 ppm group were influenced in any way by oxazepam administration. These findings are also relevant when considering that the 125 ppm dietary level of oxazepam resulted in serum oxazepam concentrations very close to the targeted range for human therapeutic use.

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TABLE L1
H-*ras* Mutations in Oxazepam-Induced Liver Neoplasms from B6C3F$_1$ Mice

A. Data Summary

Treatment	Neoplasms with Mutations	Codon 61[a]			Codons	Codon
		AAA	CGA	CTA	12, 13	117
Control-historical[b]	102/156 (65%)	58	30	13	0	1
Control	11/19 (58%)	6	4	1	ND	ND
Oxazepam-125 ppm	13/37 (35%)	9	4	0	0	0
Oxazepam-2,500 ppm	1/20 (5%)	0	0	1	0	ND
Oxazepam-5,000 ppm	0/20 (0%)	0	0	0	ND	0

B. Mutations by Sex and Neoplasm Types for Oxazepam (125 ppm) Liver Neoplasms

Neoplasm Type	Sex of Mice	Neoplasms with Mutations	Codon 61	
			AAA	CGA
Adenomas	Female	6/20 (30%)	4	2
	Male	2/9 (22%)	1	1
Carcinomas	Female	3/4 (75%)	2	1
	Male	2/4 (50%)	2	0

[a] AAA, CGA, CTA=mutant sequences for codon 61; CAA=wild type sequence.
[b] Fox *et al.* (1990); Reynolds *et al.* (1987); and Devereux (unpublished). H-*ras* mutation profile not significantly different between liver neoplasms from male and female B6C3F$_1$ mice. ND=not done; 0=not detected.

APPENDIX M
MEASURES OF REPLICATIVE DNA SYNTHESIS IN LIVER IN A 90-DAY FEED STUDY OF OXAZEPAM IN MALE B6C3F$_1$ MICE

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MEASURES OF REPLICATIVE DNA SYNTHESIS IN LIVER IN A 90-DAY FEED STUDY OF OXAZEPAM IN MALE B6C3F$_1$ MICE

INTRODUCTION

The 14-week toxicity studies of oxazepam conducted by Battelle Columbus (Columbus, OH) in Swiss-Webster and B6C3F$_1$ mice gave evidence of marked compound-related increases in liver weight and hepatocyte hypertrophy without significant evidence of cytotoxicity. Because studies have shown a "promoter" type activity of oxazepam for hepatocellular carcinogenesis (Diwan *et al.*, 1986), and effects of oxazepam on the liver of rodents similar to those of chemicals such as phenobarbital and mirex (Cattley and Popp, 1989; Schulte-Hermann *et al.*, 1983; Ward *et al.*, 1988; Yarbrough *et al.*, 1991), the potential of oxazepam to stimulate cell replication in the liver was studied.

Male B6C3F$_1$ mice received diets containing oxazepam at the concentrations used in the 2-year Battelle study (125, 2,500, and 5,000 ppm) as well as a lower concentration (25 ppm) that was expected to produce blood levels within or below the therapeutic dose range for humans. Mice were evaluated for liver cell replication at 15, 30, 45, and 90 days by measuring scheduled DNA synthesis using a bromodeoxyuridine (BrdU) labeling method. Livers were also evaluated histopathologically for evidence of hepatocellular hypertrophy and cytotoxicity. Blood was taken for analysis of oxazepam concentrations, and serum was evaluated for clinical evidence of cytotoxicity.

MATERIALS AND METHODS

Oxazepam was acquired from Roussel Corporation (Englewood Cliffs, NJ), and was the same material used in the chronic Battelle Columbus (Columbus, OH) studies. Dosed feed was prepared biweekly, by mixing ground feed and oxazepam in a Patterson-Kelley blender for 15 minutes with the intensifier bar in operation for the first 5 minutes. Aliquots were collected and analyzed by high performance liquid chromatography (HPLC) using acetophenone as internal standard. Measured concentrations of oxazepam in feed were within 10% of target concentrations. Powdered, dosed feed was delivered to mice in DataMAX 50 mouse feeders (Lambert Associates, Flourtown, PA) to allow accurate estimation of feed consumption with limited spillage.

Male B6C3F$_1$ mice (Charles River Breeding Laboratories, Raleigh, NC), weighing approximately 24 g, received water and standard NIH-07 feed *ad libitum* and were maintained under alternating 12-hour periods of light and dark at 21° to 23° C and 40% to 60% relative humidity. The animals were acclimated to this environment for 2 weeks prior to beginning the experiment. Mice were randomly assigned to treatment groups of 10 animals each per time point. All animals were identified by tail tattoos with indelible ink.

Mice were observed twice daily, and feed consumption was measured three times per week. Mice were sacrificed by carbon dioxide asphyxiation and exsanguination. Blood samples were collected from the retroorbital sinus and allowed to clot for approximately 30 minutes at room temperature, after which they were centrifuged at 13,600 × *g* for 5 minutes. Serum was collected, an aliquot frozen for oxazepam analysis, and the remainder immediately analyzed for several clinical pathology parameters (Appendix N).

Serum oxazepam analysis. Frozen serum was allowed to thaw and 0.1 mL aliquots were made basic with 10 µL of 0.1 M NaOH. Oxazepam was extracted from the aqueous layer with 1 mL ethyl acetate. The organic layer containing the oxazepam was removed and evaporated to dryness under nitrogen. Samples were reconstituted in 100 µL of 60% methanol/40% phosphate buffer (0.05 M; pH 2.8). Analysis of oxazepam was conducted using an HPLC system (Waters Associates, Milford, MA) consisting of two

model 510 HPLC pumps, a 712 WISP multiple sample injector, a C_{18} column, and a 490E multiwavelength detector at 230 nm. Samples were run isocratically at 0.8 mL/minute at 40% phosphate buffer/60% methanol. Serum oxazepam was quantitated against an oxazepam standard curve created with spiked serum standards.

DNA synthesis measurements. Seven days prior to sacrifice, osmotic minipumps (Alza Corporation, Palo Alto, CA, model 2002) were implanted subcutaneously on the backs of the mice. These minipumps delivered bromodeoxyuridine (BrdU) (Sigma Chemical Co., St. Louis, MO) at 15 μg/hr. Seven days later, the animals were killed by CO_2 inhalation and blood was collected for clinical chemistry measurements and for analysis of oxazepam levels as described above. Livers were blotted and weighed. A mid-lobe radial section of the right anterior lobe was fixed in neutral buffered formalin for 24 hours. A cross section of small intestine was also fixed as a positive control for the proper operation of the minipump and the staining technique because intestinal crypt cells are constantly in S phase. Tissues were embedded in paraffin and serial sections mounted onto poly-*l*-lysine coated slides. Following deparaffination and rehydration, one set of slides was stained with hematoxylin and eosin for histopathological analysis and another set stained for BrdU incorporation by a variation on the method of Sugihara *et al.* (1986), as described previously (Cunningham and Matthews, 1991; Cunningham *et al.*, 1991, 1992). Slides were treated with 2 N HCl for 30 minutes at 37° C to allow the DNA to become single stranded. The acid treatment was quenched with boric acid buffer (pH 7.6) for 1 minute at room temperature, followed by digestion in 0.01% trypsin (Sigma, St. Louis, MO) and rinsed in PBT [phosphate buffer, pH 7.2, containing 1% bovine serum albumin (Sigma, St. Louis, MO), 0.05% Tween 20 (Bio-Rad, Richmond, CA) and 7.2% NaCl]. Nonspecific antibody binding was eliminated by blocking (20 minutes) with normal horse serum (1:20)(Vector Laboratories, Inc., Burlington, CA). The slides were then incubated with a 1:50 dilution of rat anti-BrdU monoclonal antibody (Accurate Corp., Westbury, NY) for 20 minutes at room temperature. Following two PBT washes, the slides were incubated with a 1:100 dilution of a biotinylated rabbit anti-rat antibody (Vector, Burlington, CA) for 20 minutes at room temperature and visualized with the avidin biotin peroxidase complex (ABC) method using a Vectastain (peroxidase standard) kit (Vector, PK4004, Burlington, CA). Nuclear binding of the ABC reagent (labeled nuclei) was visualized by staining for 6 minutes with 3,3′-diaminobenzidine (Sigma, St. Louis, MO) to give a dark brown color, and nonlabeled nuclei were stained with hematoxylin to yield a blue color. Random areas of the slides were chosen for counting stained and unstained hepatocyte nuclei (>1,000 hepatocytes/animal). Statistics were performed using a Student's *t*-test. For the purposes of this study, measures of replicative DNA synthesis are assumed to represent cell replication events. This may be an overestimation because changes in cell ploidy that commonly occur in rodent liver have not been distinguished and considered.

RESULTS

While there were no deaths considered to be compound related during the study (Table M1), animals in the 2,500 and 5,000 ppm groups appeared sedated and lethargic, and during the first 2 weeks some exposed mice became trapped in the feeders and died. The mice appeared to adapt to the marked pharmacologic effect of oxazepam after this period, and no further deaths occurred. At 15, 30, and 45 days, feed consumption by 2,500 and 5,000 ppm mice was generally significantly less than that by controls (Table M1). Mice in the 2,500 and 5,000 ppm groups gained less weight than those in the control, 25, or 125 ppm groups at 15, 30, and 45 days. At 90 days, feed consumption by 2,500 ppm mice was similar to that by controls as was the mean body weight of this group (Table M1). Feed consumption by the 25 ppm group at 90 days was slightly greater than controls and was reflected by greater body weight gain (Table M1).

Serum oxazepam concentrations increased with dose in the 25, 125, and 2,500 ppm groups, but concentrations observed in animals receiving 5,000 ppm were similar to those in mice receiving 2,500 ppm at each time point. The highest serum oxazepam concentrations in each exposure group occurred at the 15-day sacrifice. Serum oxazepam concentrations were also increased in all exposure groups at the 45-day

evaluation. Serum oxazepam concentrations at 30 and 90 days were somewhat lower than those at 15 and 45 days (Table M2).

Histopathologic evaluation of hematoxylin- and eosin-stained slides revealed little evidence of cytotoxicity or hepatocyte degeneration and no generalized or periportal inflammation. This was in basic agreement with the results of the clinical pathology studies which showed mild cholestasis without hepatocellular necrosis (Appendix N). The major histopathological feature in mice exposed to oxazepam was hepatocellular hypertrophy characterized by enlarged hepatocytes with pale pink, nonvacuolated cytoplasm consistent with proliferation of smooth endoplasmic reticulum. In the 25 and 125 ppm groups, the hypertrophy was minimal, was in the centrilobular region, and encompassed hepatocytes 2 to 6 cells deep emanating from the central vein. At 2,500 and 5,000 ppm, the hypertrophy became more extensive and the area of enlarged hepatocytes included the periportal region. Although the largest hepatocytes were usually found around the central vein, all hepatocytes in mice exposed to 2,500 and 5,000 ppm were markedly enlarged and pale, and there was generalized occlusion of hepatic sinusoids. There was no apparent change in hepatocyte ploidy.

The extent of hepatocellular replication was examined in relation to the amount of oxazepam consumed and the relative increase in liver growth over the course of this study. Male B6C3F$_1$ mice exposed to 125, 2,500, or 5,000 ppm oxazepam exhibited a significant increase in the rate of hepatocyte replication at 15 days (Table M3). By this point, (representing cumulative replicative synthesis during days 8 to 15), there were fourfold to fivefold increases in BrdU labeling indices at each exposure level compared to those of the controls. The mean labeling index at the 25 ppm level was almost twice the control value, but it was not statistically different from the control ($P<0.05$). Relative liver weights of 2,500 and 5,000 ppm mice were significantly greater than those of the controls at the 15-day time point and similar elevated ratios were seen in these groups throughout the 90-day study. Relative liver weights of 25 and 125 ppm mice were only slightly elevated and were significantly different only when control values appeared low. With the exception of a variable, but significantly elevated, cell replication rate at 45 days in the 2,500 ppm group, there were no other significant differences in labeling index between exposed and control animals.

DISCUSSION

A significant increase in the rate of liver cell replication was noted after 15 days of exposure to 125, 2,500, and 5,000 ppm oxazepam. This increase was dose related, although the rate in the 25 ppm group was similar to that of the control. The rate of cell replication returned to control levels after 30 days of exposure, although the control rate at 30 days was twice that seen at 15 days. Control values for hepatocellular labeling indices using BrdU administered to B6C3F$_1$ mice via osmotic minipumps for 7 days have been reported to vary from approximately 4% to 7% (Eacho *et al.*, 1992), which was the range observed for control mice in the present study. It is unlikely that this decline in DNA synthesis in oxazepam-treated mice could be due to the lower serum levels of oxazepam noted at 30 days because the levels in animals in the 2,500 and 5,000 groups still exceeded that sufficient to stimulate DNA replication in the 125 ppm group at 15 days.

The labeling indices of mice treated with higher levels of oxazepam for 15 days closely approximate the levels observed in mice treated with 500 mg phenobarbital/L drinking water for 14 days. Klaunig *et al.* (1991) observed a labeling index of approximately 30% in male B6C3F$_1$ mice as determined by ^{3}H-thymidine delivered by osmotic minipump for 7 days prior to sacrifice. Male CD-1 mice exposed to 0.1% phenobarbital in the diet produced a labeling index of approximately 30% after 1 week of exposure compared to about 2% in untreated controls as measured by 7-day BrdU labeling with osmotic minipumps. However, by 5 weeks of exposure to phenobarbital, the labeling indices were similar to those in controls (Smith *et al.*, 1991). In the current study with oxazepam, by 30 days of exposure, the labeling indices in the livers of all exposed groups of mice were similar to that of the control. A decrease in cell replication

has also been observed following chronic administration of phenobarbital to Wistar rats (Schulte-Hermann *et al.*, 1983). This effect is similar to that with trophic hormones and suggests that a feedback mechanism may exist to prevent excessive cell proliferation in the continued presence of a mitogenic stimulus. Jirtle and Meyer (1991) have shown that chronic administration of phenobarbital to rats results in a transient mitogenic effect on the liver, followed by a reduction in the ability of hepatocytes to respond to other mitogenic stimuli, such as epidermal growth factor. In addition, the periportal hepatocyte concentration of transforming growth factor β, a mitoinhibitory peptide, is increased with chronic phenobarbital administration. These mitoinhibitory effects apparently do not occur in preneoplastic foci, possibly accounting for their preferential growth during chronic phenobarbital treatment.

In *in vitro* studies using lymphoma cells, it was demonstrated that the mitogenic activity of prolactin is potentiated by the stimulation of peripheral benzodiazepine receptors at low concentrations (10^{-9} M) of agonist (Laird *et al.*, 1989). Activation of peripheral benzodiazepine receptors have also been reported to induce differentiation in a variety of cell types (Clarke and Ryan, 1980; Matthew *et al.*, 1981; Wang *et al.*, 1984a), and benzodiazepines have been shown to inhibit proliferation of mouse thymoma cells in culture, with a potency that correlates with their affinity to bind to the peripheral benzodiazepine receptor (Wang *et al.*, 1984b). Thus, there are several possible mechanisms by which oxazepam may influence hepatocellular replication. The GABAergic neurons in the central nervous system have binding sites for both the benzodiazepines and phenobarbital (Olsen *et al.*, 1986), however, there are no reports that the peripheral benzodiazepine receptor may also bind barbiturates.

In summary, oxazepam appears to stimulate replicative DNA synthesis in the mouse liver, producing an approximately 5-fold increase in cell replication within 15 days without associated cytotoxicity. This effect is dose dependent and is not sustained with chronic exposure.

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TABLE M1
Survival, Mean Body Weights, and Feed Consumption of Male B6C3F$_1$ Mice Administered Oxazepam in Feed for 90 Days

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption[c]
15 Days						
0	10/10	23.7 ± 1.5	28.5 ± 1.9	4.8 ± 0.8		3.07 ± 0.55
25	10/10	23.5 ± 0.8	29.3 ± 1.5	5.8 ± 1.3	102.8	2.77 ± 0.77
125	9/10	24.1 ± 1.6	29.8 ± 2.6	5.7 ± 1.1	104.6	2.92 ± 0.77
2,500	10/10	23.5 ± 1.8	25.5 ± 1.8**	2.0 ± 1.3**	89.5	1.77 ± 1.09**
5,000	10/10	22.9 ± 1.4	23.9 ± 1.8**	1.0 ± 1.5**	83.9	1.55 ± 0.91**
30 Days						
0	10/10	22.9 ± 0.8	29.4 ± 2.0	6.5 ± 2.3		3.27 ± 0.68
25	10/10	25.5 ± 1.9**	32.2 ± 2.2*	6.7 ± 2.2	109.5	3.45 ± 0.68
125	9/10	24.7 ± 1.6	30.0 ± 1.8	5.5 ± 1.2	102.0	2.98 ± 0.77
2,500	8/10	24.4 ± 1.4	27.9 ± 3.0	3.6 ± 2.4*	94.9	2.23 ± 1.23*
5,000	6/10	23.8 ± 2.3	27.6 ± 3.4	3.3 ± 1.3*	93.9	2.30 ± 1.16
45 Days						
0	10/10	23.6 ± 1.2	31.6 ± 2.1	8.0 ± 1.6		3.36 ± 0.72
25	8/10	24.7 ± 1.7	32.9 ± 2.0	8.0 ± 1.2	104.1	3.48 ± 0.70
125	9/10	24.4 ± 1.1	31.9 ± 1.6	7.5 ± 0.5	100.9	3.22 ± 1.14
2,500	9/10	24.2 ± 1.2	30.0 ± 1.3	5.8 ± 0.8**	94.9	2.29 ± 1.00*
5,000	5/10	24.3 ± 1.2	27.8 ± 2.4**	3.3 ± 1.7**	88.0	2.22 ± 1.03*
90 Days						
0	10/10	23.6 ± 1.3	33.4 ± 2.4	9.8 ± 1.4		3.03 ± 0.99
25	6/10	23.6 ± 0.7	35.1 ± 1.3	11.7 ± 0.8*	105.1	3.39 ± 1.08
125	10/10	24.1 ± 1.0	34.3 ± 1.6	10.2 ± 1.0	102.7	3.06 ± 0.96
2,500	9/10	25.1 ± 1.2	34.2 ± 1.5	9.1 ± 1.4	102.4	2.82 ± 0.95
5,000	8/10	24.1 ± 1.2	32.5 ± 1.4	8.2 ± 1.3*	97.3	2.69 ± 0.97

* Significantly different (P≤0.05) from the control group by 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 deviation
[c] Feed consumption is given as grams consumed per animal per day

TABLE M2
Serum Oxazepam Concentrations in Male B6C3F$_1$ Mice Administered Oxazepam in Feed for 90 Days[a]

Dose (ppm)	15 Days	30 Days	45 Days	90 Days
25	0.6 ± 0.06	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
125	5.9 ± 0.6	3.9 ± 0.4	4.9 ± 0.7	2.1 ± 0.3
2,500	27.8 ± 5.1	13.0 ± 7.7	23.9 ± 8.8	12.4 ± 3.3
5,000	27.9 ± 5.7	7.7 ± 4.5	21.0 ± 6.8	15.4 ± 3.9

[a] Values are expressed in μg/mL serum ± standard deviation; 6 to 10 animals per group were evaluated

TABLE M3
BrdU Labeling Indices and Liver Weight/Body Weight Ratios of Male B6C3F$_1$ Mice Administered Oxazepam in Feed for 90 Days[a]

Concentration (ppm)	Number of Mice per Group	Labeling Index[b]	Fold Increase Over Control	Liver Weight/ Body Weight (%)
15 Days				
0	10	3.63 ± 3.57		5.6 ± 0.7
25	10	6.65 ± 6.19	1.83	5.6 ± 0.3
125	9	12.97 ± 5.32**	3.57	5.6 ± 0.4
2,500	10	16.31 ± 5.18**	4.49	7.9 ± 0.5**
5,000	10	17.50 ± 4.13**	4.82	9.1 ± 0.6**
30 Days				
0	10	7.92 ± 4.15		4.4 ± 0.4
25	10	10.10 ± 3.48	1.28	5.1 ± 0.7**
125	9	6.37 ± 3.30	0.80	5.3 ± 0.2**
2,500	8	13.16 ± 8.65	1.66	8.0 ± 0.3**
5,000	6	6.17 ± 1.95	0.78	9.5 ± 0.5**
45 Days				
0	10	8.68 ± 2.64		5.1 ± 0.3
25	8	9.23 ± 3.07	1.06	5.1 ± 0.6
125	9	8.37 ± 7.50	0.96	6.0 ± 0.2**
2,500	9	21.46 ± 15.72*	2.47	8.4 ± 0.6**
5,000	5	6.28 ± 2.16	0.72	9.8 ± 0.5**
90 Days				
0	10	3.41 ± 2.57		4.1 ± 0.4
25	6	3.13 ± 1.84	0.92	5.3 ± 0.5**
125	10	3.73 ± 1.87	1.09	5.1 ± 0.5**
2,500	9	2.57 ± 2.06	0.75	9.0 ± 0.2**
5,000	8	3.56 ± 3.13	1.04	10.8 ± 0.7**

* Significantly different from the control (P≤0.05) by Dunnett's test
** P≤0.01
[a] Data are presented as means ± standard deviation
[b] Number of hepatocytes with labeled nuclei/1,000 hepatocytes scored

APPENDIX N
CLINICAL PATHOLOGY ANALYSES IN MALE B6C3F$_1$ MICE IN A 90-DAY FEED STUDY OF OXAZEPAM

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CLINICAL PATHOLOGY ANALYSES IN MALE B6C3F$_1$ MICE IN A 90-DAY FEED STUDY OF OXAZEPAM

INTRODUCTION

The 14-week toxicity studies of oxazepam conducted by Battelle Columbus (Columbus, OH) in Swiss-Webster and B6C3F$_1$ mice gave evidence of marked compound-related increases in liver weight and hepatocyte hypertrophy without significant necrosis. To further evaluate the extent of necrotic damage, a clinical pathology study was performed on the same animals used in the study of liver cell replication (Appendix M). Male B6C3F$_1$ mice received oxazepam in the diet at concentrations of 25, 125, 2,500, or 5,000 ppm for up to 90 days. Clinical pathology analyses were made on serum taken from animals killed after 15, 30, 45, and 90 days of treatment.

MATERIALS AND METHODS

Oxazepam was acquired from Roussel Corporation (Englewood Cliffs, NJ), and was the same material used in the 2-year Battelle studies. Dosed feed was prepared biweekly by adding ground feed and oxazepam and mixing in a Patterson-Kelley blender for 15 minutes with the intensifier bar in operation for the first 5 minutes. Aliquots were collected for analysis by high performance liquid chromatography (HPLC) using acetophenone as internal standard. Measured concentrations of oxazepam in feed were within 10% of target concentrations. Powdered, dosed feed was delivered to mice in DataMAX 50 mouse feeders (Lambert Associates, Flourtown, PA) to allow accurate estimation of feed consumption with limited spillage.

Male B6C3F$_1$ mice (Charles River Breeding Laboratories, Raleigh, NC) weighing approximately 24 g, received water and standard NIH-07 feed *ad libitum* and were maintained under alternating 12-hour periods of light and dark at 21° to 23° C, and 40% to 60% relative humidity. The animals were acclimated to this environment for 2 weeks prior to beginning the experiment. Mice were randomly assigned to treatment groups of 10 animals each per time point. All animals were identified by tail tattoos with indelible ink.

Mice were observed twice daily, and feed consumption was measured three times per week. At 15, 30, 45, and 90 days, mice were anesthetized with CO_2 and bled from the retroorbital sinus using heparinized microcapillary tubes. Blood was collected in plastic tubes containing a serum separator gel (Microtainer, Becton Dickinson, Rutherford, NJ). Samples were allowed to clot at room temperature and were centrifuged at 13,600 × *g* for 5 minutes. Biochemical analyses were performed using an automated analyzer (Monarch 2000, Instrumentation Laboratory Inc., Lexington, MA). Activities or concentrations of the following were determined: alkaline phosphatase (AP), alanine aminotransferase (ALT), creatine kinase (CK), sorbitol dehydrogenase (SDH), 5′nucleotidase (5′N), bile acids, albumin, total protein, creatinine, and cholesterol. Reagents and applications were from Instrumentation Laboratory except those for bile acids, SDH, and 5′N. For these assays, reagent kits were obtained from Sigma Chemical Company (St. Louis, MO) and adapted for the analyzer.

RESULTS

At each evaluation there were moderate increases in serum activities of AP and 5′N in the 2,500 and 5,000 ppm groups (Table N1). The increases were statistically significant with the exception of the AP activities measured at 15 days and 5′N at 90 days in the 2,500 ppm group. Mild, significant increases in activities of SDH occurred in 2,500 ppm mice at 15 and 90 days and in 5,000 ppm mice at 30, 45, and

90 days. Other significant changes included increases in cholesterol concentration in 2,500 and 5,000 ppm mice at 15 and 45 days, increases in bile acids in 2,500 and 5,000 ppm mice at 15 and 45 days and in the 5,000 ppm group at 90 days, and increases in total protein in 5,000 ppm mice at 30, 45, and 90 days. Changes in bile acids were inconsistent, and the magnitude of the changes overall was considered minimal.

DISCUSSION

The increased activities of AP and 5'N and concentrations of cholesterol and bile acids are compatible with mild cholestasis. Whether this represents a cellular or a physical mechanism of cholestasis cannot be determined based on these biochemical data. An example of a direct cellular effect that results in cholestasis is a decrease in hepatocellular membrane fluidity produced by treatment with some steroids (e.g., ethynyl estradiol) (Zimmerman and Lewis, 1987). A physical mechanism that can result in cholestasis is mild compression of canaliculi and bile ducts produced by increased numbers of parenchymal cells. The physical mechanism would be consistent with the histologic appearance of the livers, and the effects on liver weight (Appendix M). The minimal to mild increases in activities of SDH (without changes in ALT) are suggestive of leakage of the enzyme from intact rather than necrotic cells. As with indicators of cholestasis, this may have resulted from compression of cells related to increased rates of replication. The increases in total protein appear to be produced by globulins. A distinction was not made between immunoglobulins and globulins of hepatic origin. Overall, changes in clinical pathology parameters indicated mild cholestasis consistent with compression of bile canaliculi by the hypertrophied hepatocytes resulting in obstructed bile flow. There was little evidence of impaired liver function or significant hepatocyte injury.

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TABLE N1
Clinical Chemistry Data for Male B6C3F$_1$ Mice Administered Oxazepam in Feed for 90 Days[a]

Concentration (ppm)		AP IU/L	ALT IU/L	CK IU/L	SDH IU/L	5'N IU/L	Bile Acids μmol/L	Albumin g/dL	Protein g/dL	Creatinine mg/dL	Cholesterol mg/dL
15 Days											
0	(n=10)	60 ± 15	29 ± 7	307 ± 155	28 ± 4	17.7 ± 4.5	14 ± 3	3.3 ± 0.3	5.9 ± 0.3	0.63 ± 0.1	136 ± 6
25	(n=10)	51 ± 10	40 ± 17	212 ± 111	40 ± 10*	19.2 ± 3.8	11 ± 5	3.2 ± 0.4	5.8 ± 0.3	0.69 ± 0.1	129 ± 17
125	(n=9)	47 ± 10	36 ± 12	361 ± 231	31 ± 2	19.4 ± 2.7	11 ± 3	2.9 ± 0.3*	5.9 ± 0.3	0.64 ± 0.1	122 ± 14
2,500	(n=10)	81 ± 19	42 ± 17	207 ± 101	54 ± 15**	68 ± 13**	22 ± 4**	3.6 ± 0.2	6.2 ± 0.4	0.75 ± 0.1**	157 ± 15**
5,000	(n=10)	98 ± 29**	29 ± 7	255 ± 118	38 ± 6	97 ± 32**	19 ± 3**	3.5 ± 0.3	6.1 ± 0.4	0.64 ± 0.1	153 ± 14*
30 Days											
0	(n=10)	57 ± 9	34 ± 17	239 ± 158	31 ± 9	20 ± 6	16.1 ± 4.5	3.1 ± 0.2	5.9 ± 0.3	0.53 ± 0.1	135 ± 6
25	(n=10)	32 ± 5	33 ± 12	253 ± 153	32 ± 8	16 ± 6	14.0 ± 5.8	2.6 ± 0.2**	5.9 ± 0.2	0.54 ± 0.1	112 ± 15
125	(n=9)	37 ± 10	28 ± 8	300 ± 167	32 ± 3	23 ± 4	12.9 ± 1.3	2.8 ± 0.2	6.0 ± 0.2	0.53 ± 0.1	120 ± 12
2,500	(n=8)	81 ± 23*	32 ± 8	270 ± 115	33 ± 6	69 ± 28**	16.9 ± 4.2	3.0 ± 0.4	5.9 ± 0.3	0.54 ± 0.1	149 ± 41
5,000	(n=6)	133 ± 39**	30 ± 7	207 ± 80	44 ± 10**	126 ± 34**	16.6 ± 3.6	3.2 ± 0.5	6.3 ± 0.5*	0.52 ± 0.1	145 ± 15
45 Days											
0	(n=10)	46 ± 6	30 ± 8	514 ± 272	27.4 ± 4.2	17.2 ± 1.6	20.0 ± 2.4	3.1 ± 0.2	5.7 ± 0.3	0.62 ± 0.1	114 ± 11
25	(n=8)	37 ± 9	32 ± 9	325 ± 225	30.0 ± 4.0	16.5 ± 1.6	17.6 ± 2.2	2.7 ± 0.2**	5.6 ± 0.2	0.65 ± 0.1	113 ± 8
125	(n=9)	32 ± 5	28 ± 3	377 ± 273	27.4 ± 2.7	20.0 ± 4.7	17.9 ± 1.8	2.7 ± 0.2**	5.6 ± 0.4	0.60 ± 0.1	108 ± 13
2,500	(n=9)	71 ± 25**	25 ± 5	247 ± 278	32.0 ± 2.8	41.1 ± 9.4**	16.8 ± 1.0**	3.0 ± 0.3	5.6 ± 0.3	0.58 ± 0.1	133 ± 15**
5,000	(n=5)	138 ± 10**	28 ± 4	282 ± 122	42.0 ± 7.1**	110 ± 34**	16.7 ± 2.3*	3.4 ± 0.3	6.2 ± 0.2**	0.60 ± 0.1	149 ± 5**
90 Days											
0	(n=10)	42 ± 5	29 ± 4	330 ± 241	28.8 ± 4.7	19.4 ± 2.3	14.7 ± 1.3	3.0 ± 0.1	5.5 ± 0.2	0.68 ± 0.1	120 ± 5
25	(n=6)	41 ± 9	26 ± 8	148 ± 105	29.7 ± 3.8	17.0 ± 2.4	14.6 ± 3.1	2.8 ± 0.3	5.8 ± 0.2	0.60 ± 0.1	109 ± 4
125	(n=10)	37 ± 11	32 ± 9	312 ± 256	30.7 ± 5.2	19.1 ± 2.1	15.7 ± 3.1	2.8 ± 0.3	5.6 ± 0.3	0.60 ± 0.0	121 ± 9
2,500	(n=9)	93 ± 12**	47 ± 33	247 ± 85	45.7 ± 25*	39.8 ± 5.9**	17.5 ± 6.0	2.9 ± 0.2	5.8 ± 0.4	0.60 ± 0.1	129 ± 18
5,000	(n=8)	137 ± 19**	37 ± 9	287 ± 106	57.1 ± 10**	97.3 ± 39**	19.2 ± 2.2**	2.8 ± 0.1	6.2 ± 0.3**	0.66 ± 0.1	126 ± 10

* Significantly different from the control ($P \leq 0.05$) by Dunnett's test
** $P \leq 0.01$
[a] Data are presented as means ± standard deviations; AP=alkaline phosphatase; ALT=alanine aminotransferase; CK=creatine kinase; SDH=sorbitol dehydrogenase; 5'N=5'nucleotidase

APPENDIX O
TOXICOKINETICS OF OXAZEPAM IN B6C3F$_1$ AND SWISS-WEBSTER MICE

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TOXICOKINETICS OF OXAZEPAM IN B6C3F$_1$ AND SWISS-WEBSTER MICE

INTRODUCTION

In the 14-week Battelle Columbus (Columbus, OH) feed studies, B6C3F$_1$ and Swiss-Webster mice received diets containing oxazepam at concentrations of 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm, and blood samples were collected at weeks 2 and 12. Serum oxazepam concentrations ranged from 6 to 37 μg/mL at week 2 and 6 to 22 μg/mL at week 12. In the 2-year Battelle studies, mice received oxazepam in feed at 0, 125 (B6C3F$_1$ only), 2,500, or 5,000 ppm. Blood samples were collected at 66 weeks from B6C3F$_1$ mice and at 57 weeks from Swiss-Webster mice. The serum oxazepam concentrations ranged from about 1 μg/mL in 125 ppm mice to 6 to 10 μg/mL in 2,500 and 5,000 ppm mice, and were significantly lower than those observed in the 14-week studies. The lower concentrations were likely due to typically lower feed consumption by older animals, and to an increased capability of the mice to metabolize oxazepam.

Toxicokinetic studies of oxazepam were conducted in B6C3F$_1$ and Swiss-Webster mice to aid interpretation of the results of the mouse carcinogenicity studies and to improve the use of the studies for risk assessment. The studies were designed to define: 1) the elimination profiles of oxazepam after intravenous (IV) administration, 2) the equivalent single gavage doses that would produce peak plasma concentrations similar to those observed in the dosed feed studies, 3) the linear absorption and elimination range of oxazepam after oral gavage doses, and 4) the bioavailability of oxazepam from dosed feed. Because the ultimate goal is to describe the kinetics of serum oxazepam in the dosed feed studies, the pharmacokinetics were simulated using a previously developed model (Yuan, 1993), and the simulation results were verified by measurements of actual serum oxazepam concentrations during a short dosed feed study.

MATERIALS AND METHODS

Oxazepam was procured from Roussel Corporation (Englewood Cliffs, NJ). Identity and purity were confirmed by independent analyses. The oxazepam IV formulations were prepared by dissolving oxazepam in 80% aqueous dimethyl acetamide at 10 mg/mL. In order to reduce the potential hemolytic side effects of the IV vehicle, a small injection volume (2 mL/kg) was used. The gavage formulations (5, 20, and 40 mg/mL) were prepared by suspending oxazepam in 0.5% aqueous methylcellulose. The dosing volume was 10 mL/kg. The homogeneity and stability of the gavage formulations were confirmed prior to use. Oxazepam dosed feed formulations (125 and 2,500 ppm) were prepared by directly mixing oxazepam with powdered rodent feed (NIH-07). The concentrations of all dose formulations were independently confirmed using high performance liquid chromatography (HPLC).

For the IV studies, male and female B6C3F$_1$ and Swiss-Webster mice (11 weeks old) were obtained from Charles River Breeding Laboratories (Raleigh, NC) and were quarantined and acclimated to laboratory conditions for one week prior to study start. For each strain, 30 male and 30 female mice were administered 20 mg/kg oxazepam intravenously via the tail vein. Blood samples were collected from anesthetized (70% CO_2, 30% O_2) mice at 2, 15, and 30 minutes, and 1, 2, 3, 4, 6, 8, and 10 hours. Each mouse was sampled only once.

For the gavage studies, groups of 24 mice were administered a single oxazepam dose of 50, 200 or 400 mg/kg. Blood samples were collected from the orbital sinus of three animals per time point at the same time intervals as in the IV study. Plasma was separated from blood and stored at -20° C until analysis.

For dosed feed studies, male B6C3F$_1$ mice (6 weeks old) were obtained from Charles River Breeding Laboratories (Raleigh, NC) and were quarantined and acclimated to laboratory conditions for 2 weeks prior to study start. After randomization, mice were housed five per cage on hardwood chip bedding in polystyrene cages covered with polyester filter sheets. The animal room was maintained at 20° to 24° C, and 40% to 60% relative humidity, with a 12-hour light/dark cycle. Mice received oxazepam dosed feed formulations (125 and 2,500 ppm) and water *ad libitum*. Feed consumption data were collected during the study. Blood samples were collected at various time points from one mouse per time point. Each mouse was sampled only once. Plasma was separated for oxazepam analysis.

Oxazepam analysis. Plasma oxazepam concentrations were determined by an HPLC method. Briefly, an aliquot of 500 μL of acetonitrile containing an internal standard (acetophenone, ~ 23 μg/mL) was added to 200 μL of plasma for extraction. The mixture was then vortexed for 20 seconds. The content of each vial was decanted into separate 1 mL disposable syringes equipped with 13 mm filters (0.2 μm). The solutions were filtered directly into 500 μL polypropylene HPLC autosampler vials. The 100 μL acetonitrile extract was injected directly into a Waters 510 HPLC which was equipped with an autosampler and a Waters 486 UV detector operated at 238 nm. A Phenomenex Ultracarb 7 ODS 30 (250 × 4.6 mm ID) with a Whatman Pellicular ODS guard column (20 × 2 mm ID) was used. The mobile phase was 45% acetonitrile:55% water containing 0.5% (v/v) glacial acetic acid at flow rate of 1 mL/min. The method was validated to be linear over a range of 0.2 to 50 μg/mL. The recovery of oxazepam from plasma was complete. The limit of detection for the method was 0.04 μg/mL, which was three times the standard deviation obtained from triplicate determinations of the lowest concentration sample. The limit of quantitation, defined as the concentration at which the standard deviation and relative error are less than 10%, was 0.24 μg/mL. Stability studies of spiked plasma indicated that oxazepam was stable in plasma for at least 4 weeks.

Data analysis. Oxazepam plasma concentration-versus-time data sets obtained after IV administration were evaluated for estimation of toxicokinetic parameters using the program NONLIN® (Metzler *et al.*, 1974). The data were fitted to a two-compartment model:

$$C(t)=A\ \exp(-\lambda_1 t)+\ B\ \exp(-\lambda_2 t)$$

Where: C(t) stands for the plasma concentration at time t, λ_1 and λ_2 are the rate constants for the first and second phases of the decline, respectively, and A and B are the corresponding zero-time intercepts. These four parameters were estimated by nonlinear regression using a least-squares method and a weighting factor equal to the square of the reciprocal of the concentration calculated by the model. Clearance (Cl) was computed by dividing dose by area under the curve (AUC). Apparent volume of the central compartment (V_1) and volumes of distribution at steady state (V_{ss}) were calculated by Dose/(A+B) and $(A/\lambda_1^2 + B/\lambda_2^2)$/AUC, respectively. Half-life values for $t_{1/2,\lambda_1}$ and $t_{1/2,\lambda_2}$ were calculated as $(\ln 2)/\lambda_1$ and $(\ln 2)/\lambda_2$, respectively.

The initial values to be used in the NONLIN® program were estimated by a manual curve stripping method. Standard errors of the toxicokinetic parameters were obtained from the NONLIN® program output. Non-compartmental analysis methods were used to evaluate the gavage data. AUC values and their standard deviations after gavage dosing were estimated using the trapezoidal rule. Student's *t*-test was used whenever appropriate.

RESULTS

Intravenous studies. After IV injection of 20 mg/kg, the estimated half-life of distribution of oxazepam in B6C3F$_1$ and Swiss-Webster mice ranged from 12 to 19 minutes (Table O1). The terminal elimination half-life of oxazepam in Swiss-Webster mice was about 6 hours for males and 7 hours for females, while in B6C3F$_1$ mice, the estimated terminal elimination half-life was 7 hours for males and 5 hours for females.

A two-compartment model was found to be adequate to fit the IV data for both B6C3F$_1$ and Swiss-Webster mice (Figure O1). The estimated apparent volume of distribution at steady state (V_{ss}) in mice was larger than the vascular or the body water space, which indicates tissue distribution of oxazepam. At most time points, and in both strains, female mice had a higher plasma oxazepam concentration than males. This difference was also reflected by the smaller V_{ss} value for the females.

Gavage studies. Plasma concentrations of oxazepam after gavage administration were fit to a one-compartment model using a least-squares fit procedure. After gavage administration of 50, 200, and 400 mg/kg, peak plasma oxazepam concentrations (C_{max}) increased nonlinearly with dose and were less than or equal to 31 μg/mL (Table O2). Hypothermia and sedation were observed in mice after dosing with oxazepam at 200 and 400 mg/kg. Elimination profiles of oxazepam at all gavage doses were determined (Figures O2 and O3). The peak serum concentration achieved after a single gavage dose of 400 mg/kg was close to the maximum serum oxazepam concentration observed in the 14-week Battelle studies. The time to reach the maximum plasma oxazepam concentrations (T_{max}) in most mice was 2 to 3.5 hours. Swiss-Webster mice tended to have a later peak time, which may be due to a delay in stomach emptying. The calculated bioavailabilities of oxazepam in mice at most doses were less than 50% (Table O2). Again, female mice had higher plasma oxazepam concentrations than males.

Dosed feed study. The results of analyses of oxezapam concentration in the plasma of male B6C3F$_1$ mice exposed to 125 or 2,500 ppm are shown in Figure O4. Possibly because of the bitter taste and sedative effect of oxazepam, feed consumption by exposed groups was initially less than controls, but gradually increased (Table O3). As expected, plasma oxazepam concentrations in exposed mice showed individual variations and changed with the feeding circadian rhythm. A quasi-steady-state was achieved after 4 days *ad libitum* access to the dosed feed.

Based on mouse feeding habits (Duffy *et al.*, 1991), daily feed consumption (Table O3), and kinetic parameters generated with the gavage study at 50 mg/kg, the plasma oxazepam concentrations during the dosed feed study were predicted using a previously developed simulation model (Yuan, 1993). The predicted results are shown in Figure O4.

Simulation with the computer model further suggested that absorption of oxazepam from dosed feed between 125 and 2,500 ppm was proportionate and bioavailability (or that fraction of the total dose that appeared in the systemic circulation as parent drug) was about 43% for both levels. The simulated plasma concentrations for the second and third weeks of the study at 2,500 ppm were higher than the actual values (Figure O4). This may be due to an overestimate of the daily feed consumption (Table O3), and/or to increased metabolism or elimination of oxazepam.

DISCUSSION

The bioavailability of oxazepam in humans following a small oral dose (approximately 0.2 mg/kg) was reported to be about 93% and the peak serum concentration was reached in 1.7 to 2.8 hours (Sonne *et al.*, 1988). Metabolism studies conducted in mice indicated that 30% of a 22 mg/kg gavage dose of ^{14}C-oxazepam was recovered in urine during a 5-day collection period. The majority of the radioactivity (57.8% of dose) was recovered in the feces, but was not identified (Sisenwine *et al.*, 1987). If most of the radioactivity represented unabsorbed oxazepam, this would agree with current findings that the bioavailability of oxazepam in rodents after a gavage dose of 50 mg/kg is between 43% and 56%, due primarily to incomplete absorption. At higher doses, bioavailability tends to be even lower, which is also consistent with incomplete absorption. However, findings from the comparative metabolism studies (Appendix P) and those reported by Sisenwine and Tio (1986) for rats suggest that enterohepatic circulation of oxazepam, involving the cleavage of glucuronides in the gut, may be significant. This would mean that the amount of unchanged drug found in feces would be an overestimate of the unabsorbed

fraction of a given dose. Thus, until the extent of enterohepatic cycling is determined for these strains of mice, the determined numbers for bioavailability should be considered as apparent bioavailability.

Because of the smaller apparent volume of distribution of oxazepam in female mice, plasma oxazepam concentrations in females at most time points (including C_{max}) were significantly higher than in males. Therefore, females might be more vulnerable to adverse pharmacologic and possibly carcinogenic or toxic effects of the parent compound than males.

The T_{max} in Swiss-Webster mice occurred later than in $B6C3F_1$ mice, suggesting slower absorption of oxazepam. This difference in the rate of absorption may be caused by a strain difference in the pharmacological response to oxazepam. It is possible that the Swiss-Webster mouse may be more sensitive to the sedative effects of oxazepam, which would delay stomach emptying.

In conclusion, the toxicokinetics of oxazepam in $B6C3F_1$ and Swiss-Webster mice were similar. Differences in the elimination profiles of males and females after IV administration were observed in both strains of mice, with females having higher plasma concentrations. The terminal elimination half-life ranged from 5 to 7 hours in each strain and sex. Plasma oxazepam concentrations during prolonged administration via dosed feed could be reasonably simulated using pharmacokinetic parameters derived from single dose gavage and IV studies. The bioavailability of oxazepam from dosed feed in male $B6C3F_1$ mice was about 43% and the absorption of oxazepam was first order. Deviations from the predicted plasma concentrations at higher doses with long-term administration may be due to enhanced elimination.

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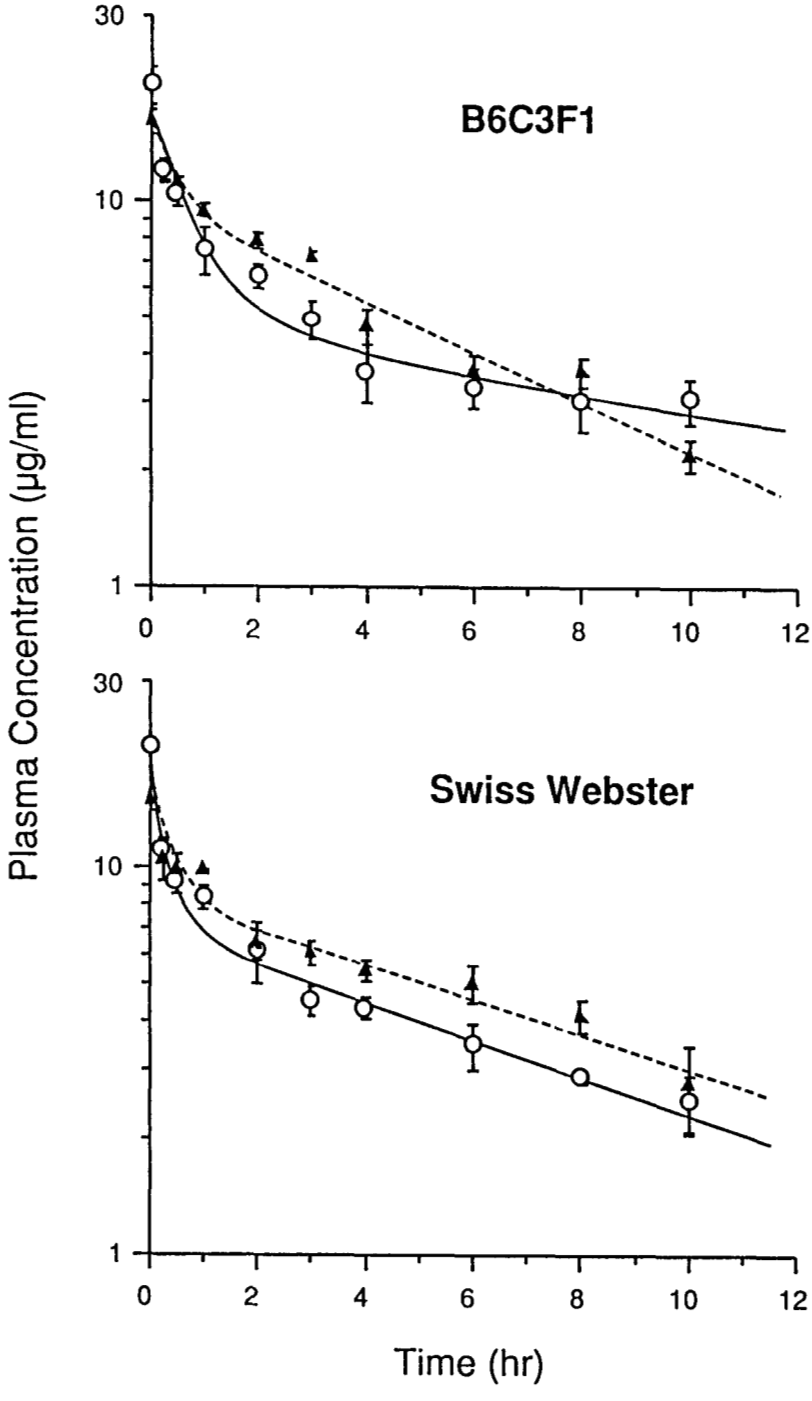

FIGURE O1
Plasma Concentrations of Oxazepam after IV Administration of 20 mg/kg to B6C3F$_1$ and Swiss-Webster Mice. Each data point [(○) male, (▲) female] represents the mean ± standard error for three mice. Solid (male) and dashed (female) lines are the two-compartment model results.

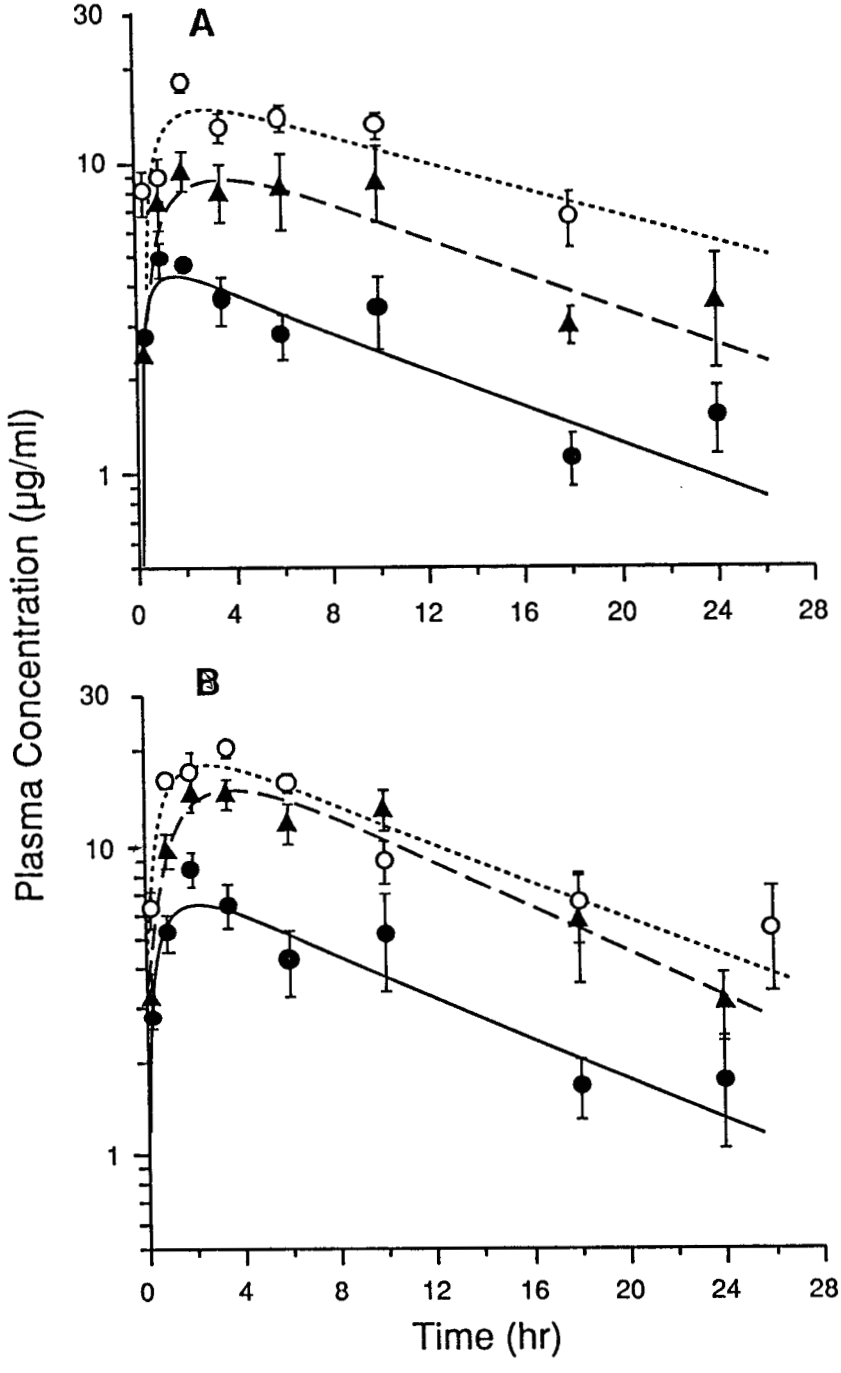

FIGURE O2
Plasma Concentrations of Oxazepam after Gavage Administration to B6C3F$_1$ Mice. Each data point [(●) 50 mg/kg, (▲) 200 mg/kg, (○) 400 mg/kg] represents the mean ± standard error for three mice. Solid (50 mg/kg), dashed (200 mg/kg), and dotted (400 mg/kg) lines are the one-compartment model results. Figure (A), males; Figure (B), females.

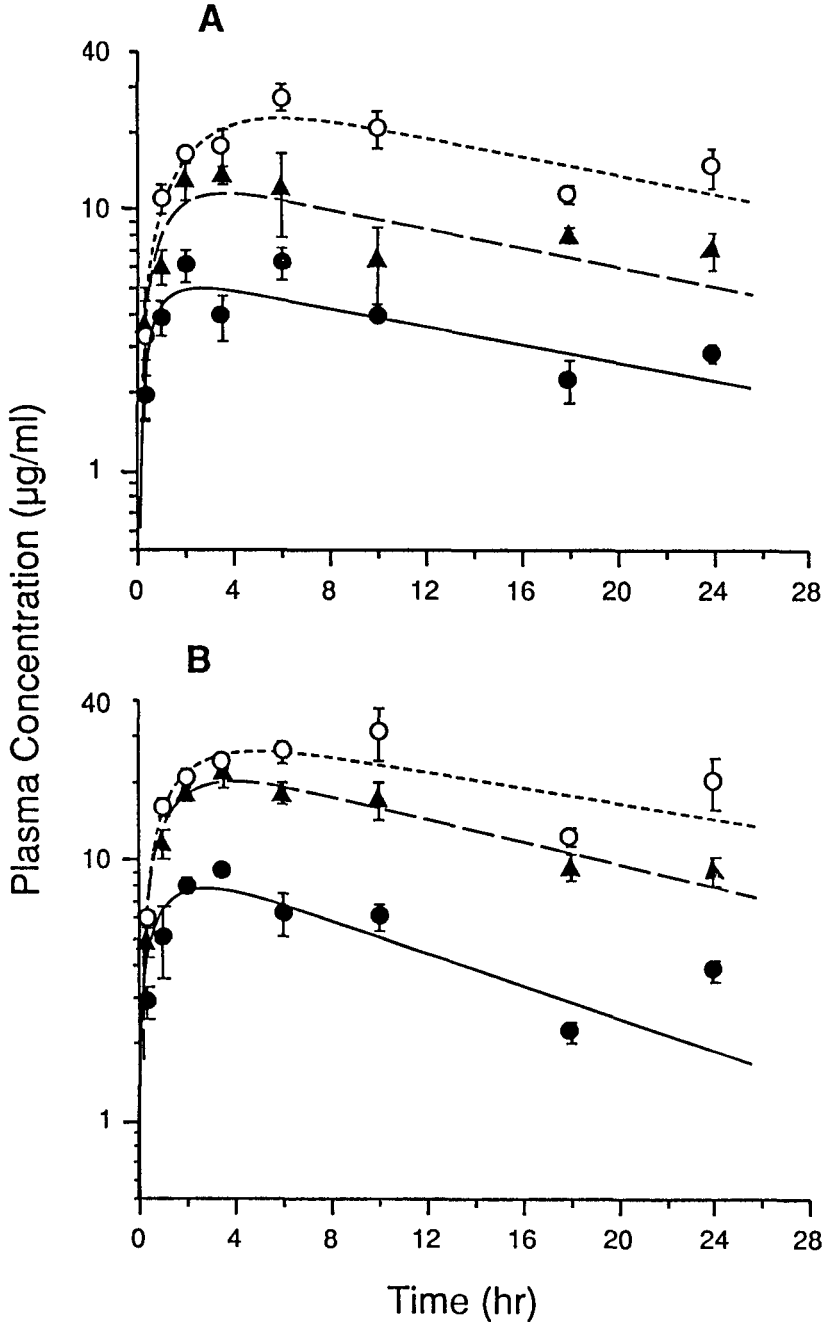

FIGURE O3
Plasma Concentrations of Oxazepam after Gavage Administration to Swiss-Webster Mice. Each data point [(●) 50 mg/kg, (▲) 200 mg/kg, (○) 400 mg/kg] represents the mean ± standard error for three mice. Solid (50 mg/kg), dashed (200 mg/kg), and dotted (400 mg/kg) lines are the one-compartment model results. Figure (A), males; Figure (B), females.

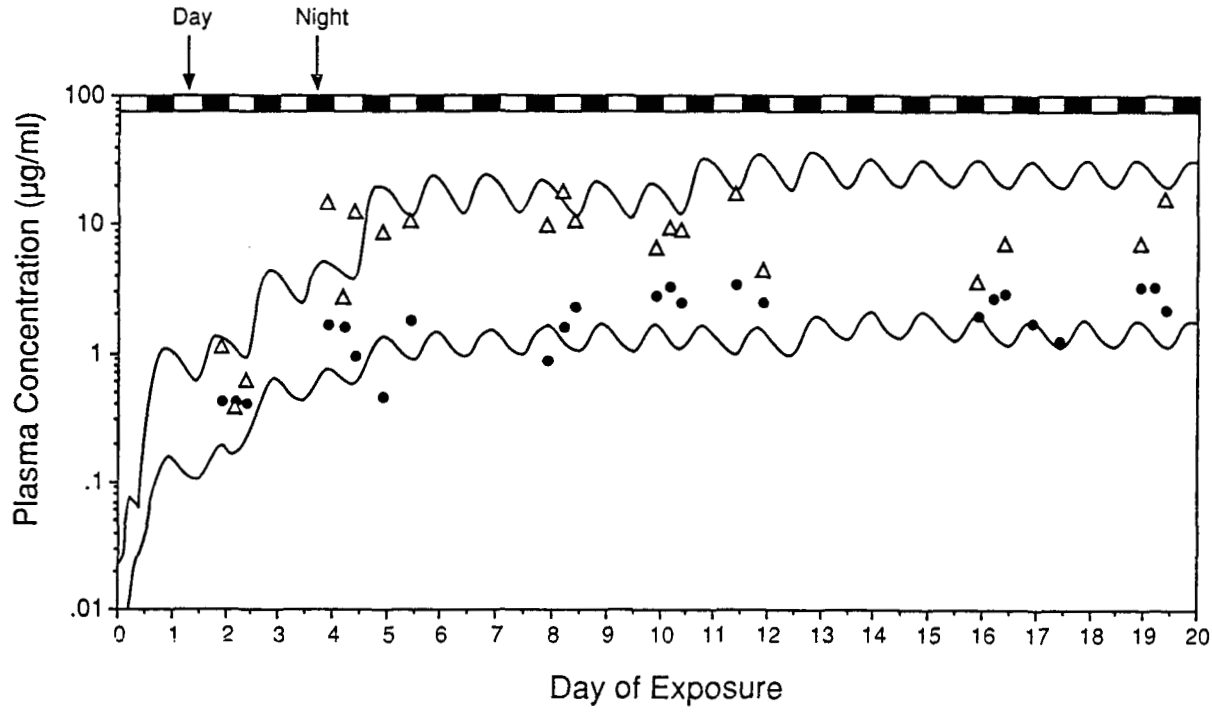

FIGURE O4
Plasma Concentrations of Oxazepam in Male B6C3F$_1$ Mice Administered Oxazepam in Feed for 3 Weeks. Each data point [(●) 125 ppm, (▲) 2,500 ppm] represents an individual mouse. Lines (bottom, 125 ppm; top, 2,500 ppm) are the prediction based on the developed dosed feed computer model.

TABLE O1
Toxicokinetic Parameters Obtained from B6C3F$_1$ and Swiss-Webster Mice after Intravenous Administration of Oxazepam

Strain	Sex	Dose (mg/kg)	Cl (L/hour/kg)	V_1[a] (L/kg)	V_{ss}[b] (L/kg)	$t_{1/2,\lambda_1}$[c] (hour)	$t_{1/2,\lambda_2}$[d] (hour)	AUC (μg×hour/mL)
B6C3F$_1$								
	M	20	0.28 ± 0.04	1.1 ± 0.17	2.61	0.30 ± 0.12	6.9 ± 1.9	71 ± 10
	F	20	0.29 ± 0.02	1.24 ± 0.17	1.86	0.26 ± 0.19	4.6 ± 0.6	69 ± 4
Swiss-Webster								
	M	20	0.30 ± 0.02	0.97 ± 0.13	2.50	0.19 ± 0.06	6.1 ± 0.9	67 ± 5
	F	20	0.24 ± 0.02	1.4 ± 0.17	2.23	0.32 ± 0.20	6.8 ± 1.1	85 ± 8

[a] V_1=apparent volume of the central compartment
[b] V_{ss}=volume of distrubution at steady-state
[c] $t_{1/2,\lambda_1}$=half-life at rate of decline during first phase
[d] $t_{1/2,\lambda_2}$=half-life at rate of decline during second phase

TABLE O2
Toxicokinetic Parameters Obtained from B6C3F$_1$ and Swiss-Webster Mice after Oral Gavage Administration of Oxazepam[a]

Sex	Dose (mg/kg)	C_{max}[b] (μg/mL)	T_{max}[c] (hour)	AUC[d] (μg×hour/mL)	Bioavailability (%)
B6C3F$_1$					
Male	50	4.9 ± 0.64	1.0	76 ± 4	43 ± 6
	200	9.4 ± 1.4	2.0	181 ± 14	25 ± 4
	400	18 ± 6	2.0	304 ± 25	21 ± 3
Female	50	8.4 ± 1.1	2.0	101 ± 11	51 ± 7
	200	14.8 ± 2	2.0	249 ± 22	36 ± 4
	400	20 ± 1.2	3.5	287 ± 26	21 ± 2
Swiss-Webster					
Male	50	6.1 ± 0.8	2	113 ± 5	51 ± 15
	200	13.6 ± 1.0	3.5	268 ± 24	40 ± 5
	400	17.4 ± 3	3.5	535 ± 40	40 ± 4
Female	50	9.2 ± 0.4	3.5	118 ± 6	56 ± 6
	200	22 ± 3	3.5	335 ± 21	40 ± 4
	400	31 ± 7	6	505 ± 44	30 ± 4

[a] All parameters listed were obtained using noncompartmental analysis method. Trapezoidal rule and endpoint correction were used to estimate AUC value.
[b] C_{max}=peak serum oxazepam concentration
[c] T_{max}=time at which peak plasma oxazepam concentration was reached
[d] AUC=area under the curve

TABLE O3
Estimated Average Daily Feed Consumption for Male B6C3F$_1$ Mice Administered Oxazepam in Feed for 3 Weeks[a]

Days	125 ppm	2,500 ppm
0 to 2	0.5	0.2
2 to 4	2.3	0.7
4 to 7	4.5	3.0
7 to 9	4.2	2.6
9 to 11	3.9	4.4
11 to 14	4.9	4.6
14 to 16	4.2	4.0
16 to 18	5.0	3.2

[a] Feed consumption is expressed as grams per animal per day.

APPENDIX P
COMPARATIVE METABOLISM STUDIES IN B6C3F$_1$ AND SWISS-WEBSTER MICE ADMINISTERED OXAZEPAM BY ORAL GAVAGE

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COMPARATIVE METABOLISM STUDIES IN B6C3F$_1$ AND SWISS-WEBSTER MICE ADMINISTERED OXAZEPAM BY ORAL GAVAGE

INTRODUCTION

The manner in which oxazepam is metabolized by humans has been determined by metabolite identification in urine (Sisenwine *et al.*, 1972). The metabolic fate of oxazepam in the male Charles River COBS CD-1 mouse has also been evaluated, and a brief comparison of the differences in the metabolism of oxazepam in several species has been reported (Sisenwine *et al.*, 1987). No comparisons of oxazepam metabolism in different strains of mice have appeared in the literature, and no studies using the Swiss-Webster mouse strain were located. Because of the observation of liver neoplasms in B6C3F$_1$ and Swiss-Webster mice in 2-year studies, and their very early occurrence in the latter strain, studies were carried out to identify the major pathways for metabolism of oxazepam in these two strains. In addition, elimination of the various metabolites by the two strains was compared, and the possibility that reactive intermediates might be formed as a consequence of oxidative metabolism in the mouse was explored.

MATERIALS AND METHODS

Oxazepam labeled with ^{14}C in the 3 position of the diazepine ring, was obtained from Amersham (UK). Standards used to establish the identities of metabolites were provided by Wyeth-Ayerst (Philadelphia, PA). These included 3′-hydroxyoxazepam, 4′-hydroxy-3′methoxyoxazepam, 6-chloro-4-phenyl2(1H)-quinazoline (CPQ), and R- and S- oxazepam glucuronides. In addition, 4′-hydroxyoxazepam was synthesized according to the procedure described by Sisenwine and Cesario (1986) for the synthesis of 3′-hydroxyoxazepam, by substituting *p*-methoxybenzycyanide for *m*-methoxybenzylcyanide in the first step. 6-Chloro-4-phenyl-2(1H)-quinazolinecarboxylic acid (CPQ-acid) was prepared according to Sternbach *et al.* (1964).

Male and female B6C3F$_1$ and Swiss-Webster mice (18 to 22 g) were obtained from Frederick Cancer Research Facility (Frederick, MD) and Charles River Breeding Laboratories (Portage, MI), respectively, and quarantined for 6 days. Animals were dosed orally (5 mL/kg) with ^{14}C-labeled oxazepam (5 microcuries) in carrier drug in a vehicle of water/emulphor/ethanol (8:1:1) at 25, 250, and 500 mg/kg.

Some animals received 2,500 ppm oxazepam in dosed feed for 2 weeks prior to dosing with labeled material. Dosed feed was removed 16 hours prior to study to allow clearance of the major portion of the drug. Immediately after oral gavage dosing, animals were placed in glass metabolism cages for 72 hours for collection of urine, feces, and expired carbon dioxide and then killed. Carbon dioxide was trapped in ethanolamine/ethylene glycol monomethyl ether (3:7).

Collected urine was centrifuged at 1,000 × *g* to sediment particulates, and directly injected into a Waters Associates (Milford, MA) HPLC. Separation was achieved on a C_{18}, 5 micron, Rainin Microsorb column (4.6 × 250 mm). The mobile phase was a linear gradient of 85% dibutylamine phosphate (pH 6.5, 10mM)/15% methanol, to 100% methanol, programmed over 23 minutes, with a flow rate of 1.2 mL/min. Detection was by UV absorption (Beckman model 163 Variable Wavelength Detector, Beckman Inst., Palo Alto, CA) and radiochemistry (Radiomatic Flo-One, Tampa, FL).

Feces were homogenized in sodium acetate buffer (pH 6.8, 100mM), precipitated in acetonitrile (1:3), vortexed, allowed to extract at 4° C overnight, and then centrifuged. The supernatant was analyzed by the high performance liquid chromatography (HPLC) method outlined above. Feces were dried and combusted for determination of total radioactivity. The extraction process generally removed more than

85% of the total radioactivity. Activity remaining in the carcass was determined following hydrolysis in 3 mL of tetraethylammonium hydroxide per gram.

Some urinary and fecal metabolites were identified by co-chromatography with known standards. Conjugates were identified after treatment with Type IX ß-glucuronidase or Type VII sulfatase and saccharolactone (all from Sigma Chemical Company, St. Louis, MO). Final identification of isolated peaks was confirmed by 1H-nuclear magnetic resonance (NMR) spectroscopy (General Electric GN-500 NMR Spectrometer, Fremont, CA), or by mass spectrometry (ZAB-4F, VG Analytical, Manchester, UK) with glycerol or dithiothreitol as a matrix.

Residual radioactivity assumed to represent covalently bound oxazepam metabolites was assayed in the livers of the animals killed 72 hours after receiving the radiolabel. Livers were removed and homogenized (100 mM sodium acetate, pH 6.8, 4 mL/g tissue) and aliquots were exhaustively extracted three times with 80% methanol, followed by trichloroacetic acid (0.4M), and a 3:1 mixture of ether and ethanol until no further radioactivity could be removed. The protein pellets were solubilized in 0.1 N NaOH, and radioactivity/mg protein was determined.

RESULTS

The recovery of administered radiolabel in the feces, urine, expired air, and the amount remaining in the carcass 72 hours after the oral dose is shown in Table P1. The values given are percent of administered radioactivity ± the standard error from four animals per dose group. Information is given for animals receiving a single oral gavage dose of 25, 250 or 500 mg/kg, and for animals pretreated for 14 days with feed containing 2,500 ppm oxazepam and then given 500 mg/kg by oral gavage. There did not appear to be major dose-, sex-, or strain-dependent differences in the proportion of materials excreted by the various routes, although females tended to excrete somewhat more label in expired breath than did males. Pretreatment with oxazepam did result in a consistent increase in the amount of label recovered in the urine and decreases in the amount in the feces and the amount remaining in the carcass.

Shown in Figure P1 are the separations of oxazepam metabolites in the urine and feces from male Swiss-Webster mice given a dose of 500 mg/kg. The major peaks in both the urine and feces are oxazepam glucuronide; CPQ-acid, a nonenzymatic degradation product of oxazepam; and unchanged oxazepam. The bulk of the material excreted in the feces is unchanged drug, possibly representing unabsorbed oxazepam. Additional peaks found in urine appear to comprise metabolites that have undergone oxidative metabolism, and include 4′-hydroxyoxazepam and its glucuronide, a single peak containing 3′-hydroxyoxazepam and 4′-hydroxy-3′-methoxyoxazepam, and peaks that are as yet unidentified. 4′-Hydroxyoxazepam was also identified in the feces, and a unique fecal metabolite remains unidentified.

The five main urinary metabolites, expressed as percent of total urinary radioactivity, are shown in Table P2. A number of strain-dependent differences were noted. B6C3F$_1$ mice excreted more oxazepam as the glucuronide and less as hydroxylated and other metabolites or unchanged drug than did Swiss-Webster mice. Females eliminated less unchanged oxazepam, and more CPQ-acid, 4′-hydroxyoxazepam and an unknown metabolite than did males. Pretreatment appeared to cause increased excretion of glucuronidated metabolites, more unknown metabolite, and less unchanged drug and CPQ-acid.

These five major metabolites were also determined in feces and the total urinary and fecal excretion patterns are given in Table P3. Overall, the manner in which oxazepam is metabolized appears similar in the two strains of mice. Pretreatment appeared to result in an increase in total glucuronidated metabolites in urine and a reduction in elimination of unchanged drug in the feces.

An evaluation of residual radioactivity in the livers of mice showed a dose-dependent accumulation that was quite similar for the two strains of mice (Table P4). Pretreatment for 14 days resulted in lower levels of residual radioactivity.

DISCUSSION

The data from numerous studies of oxazepam metabolism in humans indicate that a larger portion of an administered dose is excreted via the urine than was found in the current mouse studies (Knowles and Ruelius, 1972; Alvan *et al.*, 1977; Greenblatt, 1981; Sonne *et al.*, 1988). This could be due in part to the proportionately higher doses used in mice than are typically given to humans, and it could reflect a larger unabsorbed fraction. An alternative explanation for the large amount of unchanged drug in the feces of mice is that some of it may represent oxazepam glucuronides that entered via the bile and were cleaved by bacterial action. This is supported by the observation that about 50% of the radioactivity extracted from the liver of animals treated similarly to those described earlier was present as oxazepam glucuronide, a much higher fraction than appears in the urine.

Humans appear to excrete from 95% to 99% of urinary metabolites as the glucuronide (Figure P2), along with small amounts of the other indicated metabolites. However, three of these, CPQ, 4′-hydroxyoxazepam glucuronide, and 4′-hydroxy-3′-methoxyoxazepam glucuronide are common to those identified in the current mouse studies (Figure P3). The identification of the 3′- and 4′-hydroxylated forms indicates the involvement of oxidative metabolism in both humans and mice, and suggests the potential for formation of a reactive epoxide.

Residual radioactivity in the liver presumably represents protein bound metabolites, likely the result of oxidative metabolism. Binding increased nearly linearly with oral dose over the range 25 to 500 mg/kg, but was in the pmol/mg protein range. For comparison, following a 750 mg/kg dose of acetaminophen, binding to liver protein was reported to be approximately 20 nmol/mg (Jollow *et al.*, 1973). Thus, oxazepam should not be considered a particularly potent protein binding agent in mouse liver.

Prior and presumed chronic administration of oxazepam to mice would likely increase the relative fraction of the drug metabolized to the glucuronide and the total amount of metabolites excreted in the urine, thus increasing the resemblance of the metabolic pattern to that of humans. The origin of the diazepine ring condensation products CPQ and CPQ-acid is not clear, although along with the open ring forms found in the human studies, they are possible nonenzymatic reaction products that may be present in the tissue or that may form during the extraction and analysis procedures.

Overall, there was no marked difference in the metabolic profile or in residual hepatic protein binding of oxazepam between the B6C3F$_1$ and Swiss-Webster mice that would suggest a possible cause for the very early onset of hepatic neoplasia in the Swiss-Webster strain.

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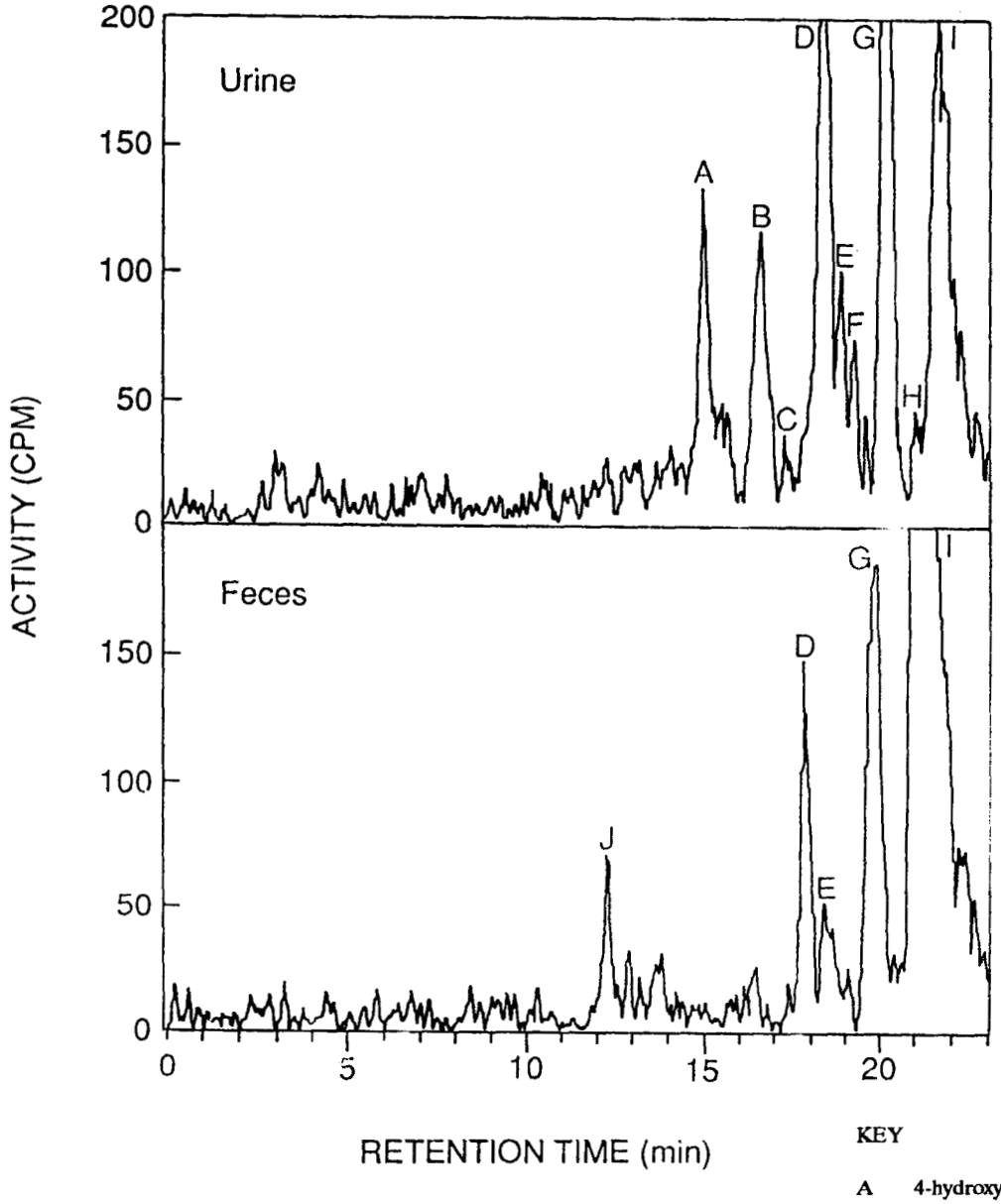

KEY

A	4-hydroxy-oxazepam-glucuronide
B	unknown RT-16.2
C	unknown RT-17.2
D	oxazepam-glucuronide
E	4-hydroxy-oxazepam
F	peak 19.2
G	CPQ-acid
H	unknown RT-20.8
I	oxazepam
J	unknown RT-12.6

FIGURE P1
Metabolites in Urine and Feces from Male Swiss-Webster Mice Administered 500 mg/kg Oxazepam

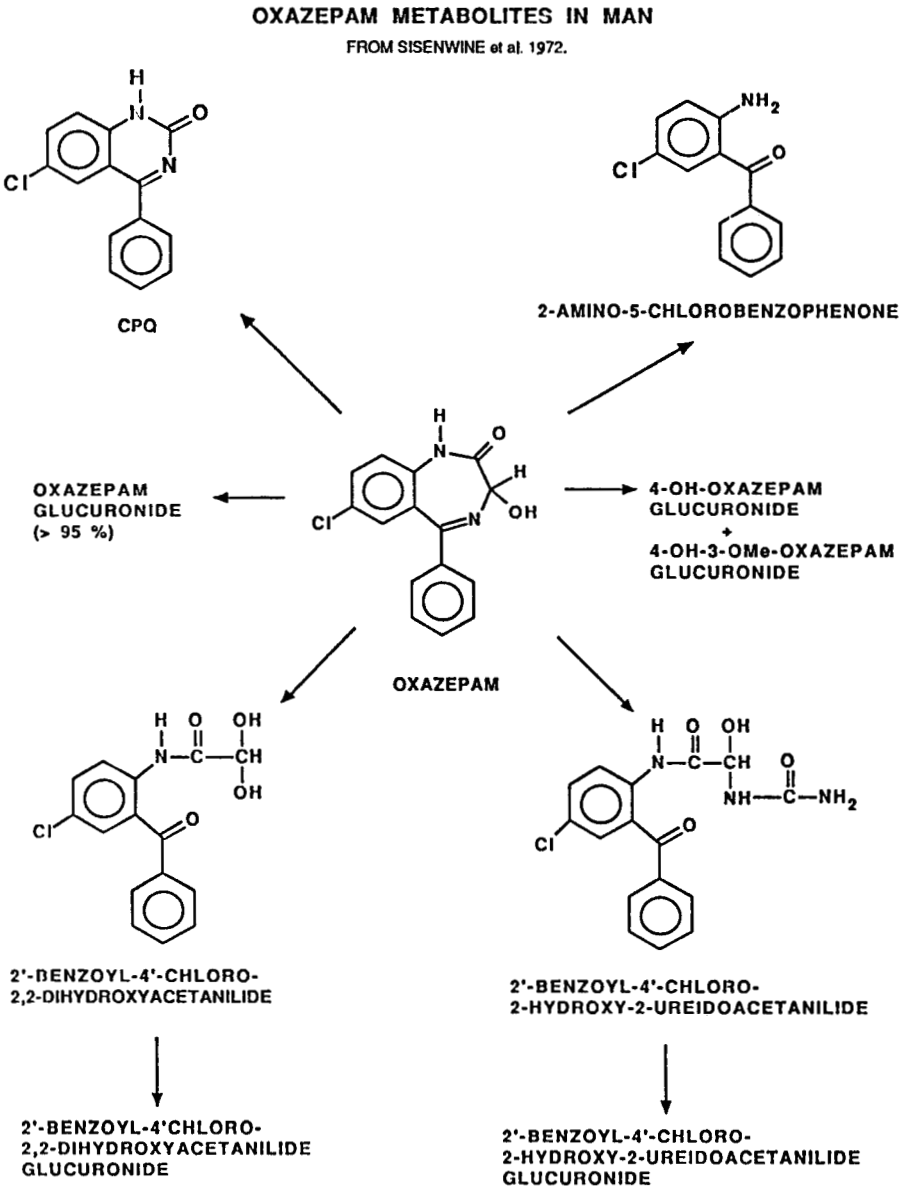

FIGURE P2
Oxazepam Metabolites in Man

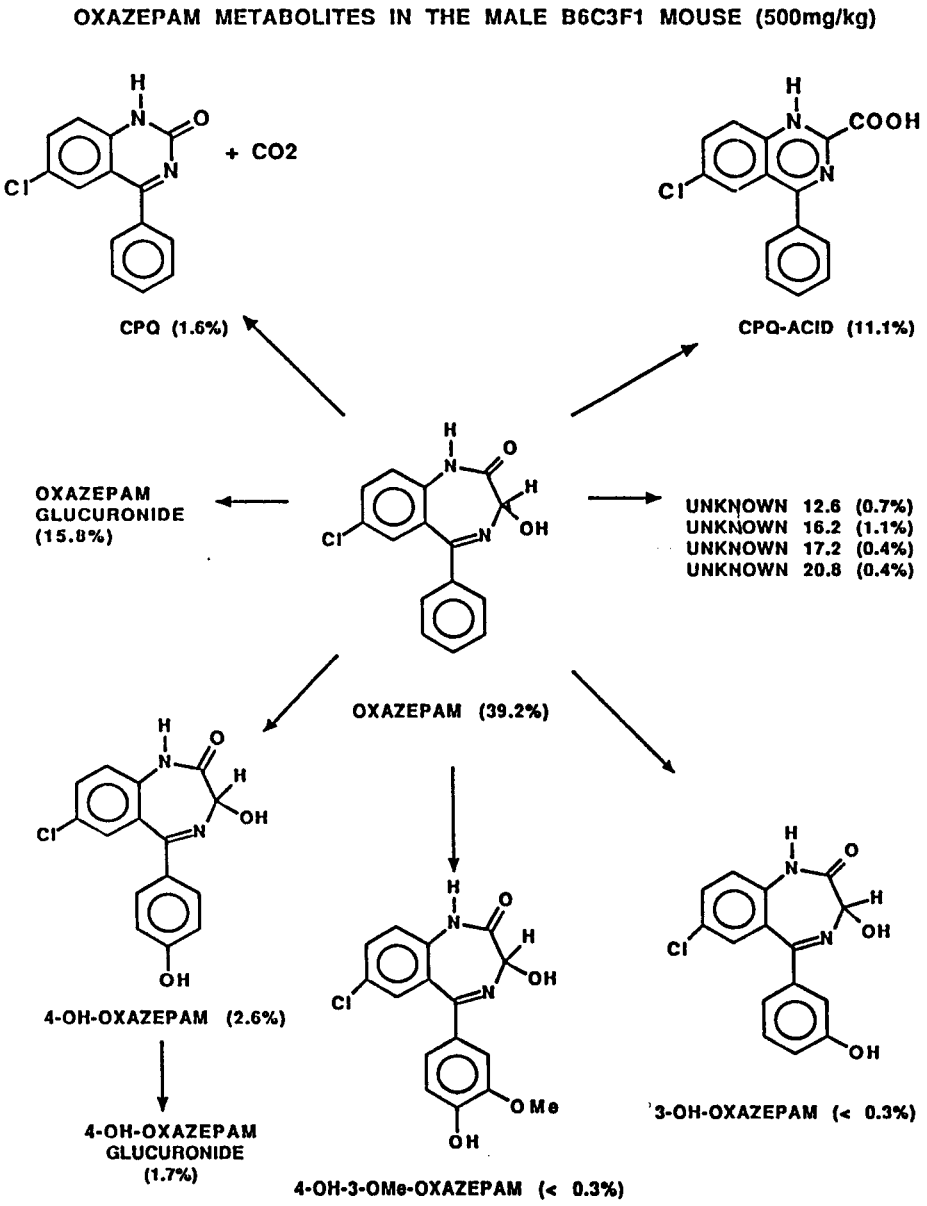

FIGURE P3
Oxazepam Metabolites in Male B6C3F$_1$ Mice (500 mg/kg)

TABLE P1
Recovery of Radiolabel from Mice Administered Oxazepam by Oral Gavage[a]

Strain	Gavage Dose (mg/kg)	Days Pretreated	Sex	Feces	Urine	Expiration	Carcass	Total
B6C3F$_1$								
	25	0	M	62.1 ± 7.0	23.6 ± 3.3	1.8 ± 0.1	2.3 ± 0.2	90.4 ± 4.6
	250	0	M	45.7 ± 0.6	37.2 ± 4.5	1.5 ± 0.1	1.5 ± 0.4	87.1 ± 4.6
	500	0	M	55.2 ± 4.2	22.8 ± 2.3	1.4 ± 0.3	2.0 ± 0.2	81.5 ± 2.7
	500	14	M	34.9 ± 3.8	29.1 ± 5.3	1.9 ± 0.1	1.1 ± 0.1	67.5 ± 2.4
	500	0	F	40.4 ± 4.5	29.5 ± 1.9	2.3 ± 0.1	1.6 ± 0.1	75.7 ± 6.0
	500	14	F	36.6 ± 1.9	41.8 ± 3.8	2.5 ± 0.1	0.9 ± 0.1	82.5 ± 2.7
Swiss-Webster								
	25	0	M	63.7 ± 1.9	25.2 ± 3.7	2.3 ± 0.2	3.2 ± 0.8	95.6 ± 1.2
	250	0	M	47.8 ± 0.9	27.1 ± 2.4	1.9 ± 0.1	1.7 ± 0.3	79.3 ± 2.2
	500	0	M	66.1 ± 9.4	24.1 ± 6.8	1.6 ± 0.4	3.3 ± 1.2	91.4 ± 4.2
	500	14	M	25.0 ± 1.1	40.4 ± 2.9	1.9 ± 0.1	1.2 ± 0.3	68.9 ± 1.8
	500	0	F	52.6 ± 6.1	18.5 ± 3.8	3.2 ± 0.7	3.4 ± 1.7	79.4 ± 4.4
	500	14	F	43.1 ± 2.9	42.6 ± 4.9	2.9 ± 0.1	1.2 ± 0.1	90.3 ± 2.2

[a] Data are presented as percent of administered radiolabel ± standard error; n=4

TABLE P2
Major Urinary Metabolites in Mice Administered Oxazepam by Oral Gavage[a]

Gavage Dose (mg/kg)	Days Pretreated	Sex	Metabolite					
			Oxazepam	CPQ-Acid	4OH-OX	OXAZ-GLUC	UNK-16.2	4OH-OX-GLUC
B6C3F$_1$								
25	0	M	15.3 ± 2.5	18.8 ± 0.3	3.4 ± 0.3	47.7 ± 1.0	3.2 ± 0.5	6.7 ± 0.5
250	0	M	10.1 ± 2.0	11.2 ± 1.1	1.6 ± 0.5	67.7 ± 2.9	2.3[b]	3.7 ± 0.4
500	0	M	19.7 ± 2.7	12.9 ± 1.0	3.3 ± 1.1	55.0 ± 4.8	1.7[b]	4.0 ± 0.5
500	14	M	1.3 ± 0.3	10.5 ± 0.7	2.9 ± 0.2	69.7 ± 1.3	2.3 ± 0.5	7.4 ± 0.3
500	0	F	4.2 ± 1.0	17.7 ± 0.7	1.2 ± 0.2	62.4 ± 1.9	3.7 ± 0.3	5.6 ± 0.5
500	14	F	1.0 ± 0.2	10.5 ± 0.5	2.0 ± 0.4	71.6 ± 0.6	3.0 ± 0.2	7.4 ± 0.3
Swiss-Webster								
25	0	M	36.1 ± 4.8	23.1 ± 1.8	5.8 ± 0.8	16.9 ± 1.3	5.0 ± 1.0	8.1 ± 1.1
250	0	M	22.2 ± 5.5	20.3 ± 1.5	2.5 ± 1.1	37.5 ± 6.4	6.1 ± 1.3	8.0 ± 0.7
500	0	M	23.5 ± 4.6	20.7 ± 5.3	4.3 ± 1.1	37.4 ± 12.2	4.5 ± 1.7	7.5 ± 1.7
500	14	M	4.5 ± 1.2	13.5 ± 0.8	ND[c]	72.1 ± 1.5	2.6 ± 0.4	7.3 ± 0.5
500	0	F	14.9 ± 3.2	30.6 ± 6.3	2.2 ± 0.8	32.1 ± 12.0	8.2 ± 2.5	6.7 ± 1.4
500	14	F	2.2 ± 0.4	22.3 ± 4.8	2.2 ± 0.6	60.0 ± 5.9	3.5 ± 0.4	8.9 ± 1.1

[a] Data are presented as percent of total urinary radioactivity ± standard error; n=4
[b] n=3; no standard error available
[c] ND=not detected

TABLE P3
Total Major Urinary and Fecal Metabolites in Mice Administered Oxazepam by Oral Gavage[a]

Gavage Dose (mg/kg)	Days Pretreated	Sex	Metabolite					
			Oxazepam	CPQ-Acid	4OH-OX	OXAZ-GLUC	UNK-16.2	4OH-OX-GLUC
B6C3F$_1$								
25	0	M	46.8 ± 5.2	11.1 ± 1.3	2.0 ± 0.1	13.0 ± 1.5	1.1 ± 0.6	1.6 ± 0.3
250	0	M	31.2 ± 3.3	8.7 ± 1.2	1.2 ± 0.1	29.7 ± 4.2	1.0[b]	1.4 ± 0.2
500	0	M	45.0 ± 3.4	5.8 ± 0.9	1.6 ± 0.4	14.7 ± 1.7	0.4[b]	0.9 ± 0.0
500	14	M	19.9 ± 1.6	7.3 ± 0.2	2.6 ± 0.3	23.7 ± 3.0	0.6 ± 0.1	2.1 ± 0.4
500	0	F	19.2 ± 1.4	13.4 ± 1.4	1.1 ± 0.1	23.0 ± 2.5	1.1 ± 0.1	1.6 ± 0.2
500	14	F	19.2 ± 1.5	8.7 ± 0.6	2.1 ± 0.1	35.8 ± 3.4	1.4 ± 0.2	3.1 ± 0.3
Swiss-Webster								
25	0	M	51.2 ± 1.5	13.6 ± 1.9	3.2 ± 0.6	4.5 ± 0.2	1.3 ± 0.4	2.1 ± 0.5
250	0	M	34.7 ± 3.4	11.6 ± 0.5	2.2 ± 0.8	11.9 ± 2.4	1.6 ± 0.2	2.3 ± 0.2
500	0	M	39.2 ± 7.2	11.1 ± 0.5	2.6 ± 0.4	15.8 ± 5.6	1.1 ± 0.3	1.7 ± 0.3
500	14	M	15.4 ± 1.4	8.5 ± 0.7	1.1 ± 0.3	31.0 ± 2.5	1.0 ± 0.1	2.9 ± 0.3
500	0	F	29.6 ± 3.8	13.1 ± 2.8	2.2 ± 0.7	12.8 ± 2.8	1.8 ± 0.5	1.2 ± 0.3
500	14	F	21.9 ± 2.8	12.9 ± 2.6	2.5 ± 0.6	32.2 ± 4.5	1.6 ± 0.1	3.6 ± 0.2

[a] Data are presented as percent of administered radiolabel ± standard error; n=4
[b] n=3; no standard error available

TABLE P4
Covalent Binding to Hepatic Protein Following Oxazepam Treatment in Mice[a]

Gavage Dose (mg/kg)	B6C3F$_1$ Males	B6C3F$_1$ Females	Swiss-Webster Males	Swiss-Webster Females
25	7.7 ± 1.2	ND[b]	8.8 ± 1.2	ND
250	63.9 ± 7.8	ND	68.4 ± 13.2	ND
500	129.4 ± 13.4	133.2 ± 19.6	124.4 ± 29.7	322.9 ± 105.8
500[c]	75.8 ± 4.6	93.7 ± 8.0	63.4 ± 5.1	107.7 ± 7.8

[a] Values are the mean of four samples ± the standard error of the mean; units are presented in (pmol bound/mg of protein)
[b] ND=not determined
[c] These groups were pretreated with oxazepam-dosed feed (2,500 ppm) for 2 weeks prior to receiving 500 mg/kg by oral gavage.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
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TR No.	CHEMICAL
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

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PRINTED AS OF AUGUST 1993 (CONT.)

TR No.	CHEMICAL
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
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

TR No.	CHEMICAL
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
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
394	Acetaminophen
395	Probenecid
396	Monochloroacetic Acid
397	C.I. Direct Blue 15
399	Titanocene Dichloride
401	2,4-Diaminophenol Dihydrochloride
402	Furan
403	Resorcinol
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
408	Mercuric Chloride
409	Quercetin
410	Naphthalene
411	C.I. Pigment Red 23
412	4,4-Diamino-2,2-Stilbenedisulfonic Acid
413	Ethylene Glycol
414	Pentachloroanisole
415	Polysorbate 80
416	*o*-Nitroanisole
417	*p*-Nitrophenol
418	*p*-Nitroaniline
419	HC Hellow 4
434	1,3-Butadiene

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