National Toxicology Program
Toxicity Report Series
Number 56

NTP Technical Report on the Toxicity Studies of

Carisoprodol

(CAS No. 78-44-4)

Administered by Gavage to F344/N Rats and B6C3F$_1$ Mice

August 2000

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 and Prevention. 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 Toxicity Study 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.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

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August 2000

NIH Publication No. 00-4404

U.S. Department of Health and Human Services
Public Health Service
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PEER REVIEW

The draft report on the toxicity studies of carisoprodol was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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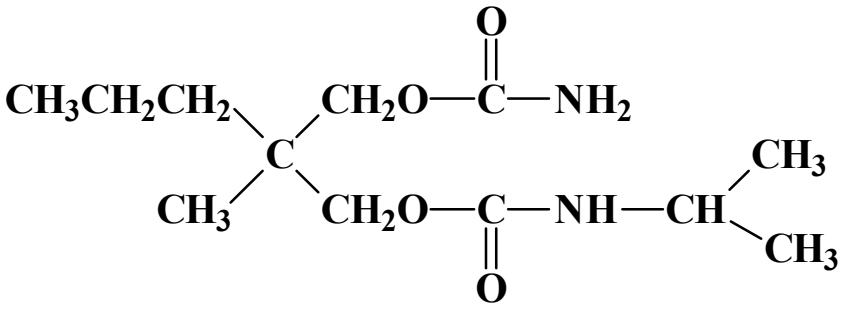
ABSTRACT



CARISOPRODOL

CAS No. 78-44-4

Chemical Formula: $C_{12}H_{24}N_2O_4$ Molecular Weight: 260.33

Synonyms: Carisoprodate; isobamate; isopropyl meprobamate; N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate; (1-methylethyl)carbamic acid 2-[[(aminocarbonyl)oxy]methyl]-2-methylpentyl ester
Trade names: Apesan; Arusal; Caprodat; Carisoma; Domarax; Flexal; Flexartal; Miolisodal; Mioril; Rela; Relasom; Sanoma; Soma; Somadril; Somalgit

Carisoprodol is a widely used skeletal muscle relaxant and analgesic and is available as a prescription drug. Comparative studies were conducted to determine the toxicity of carisoprodol administered in corn oil and in 0.5% methylcellulose by gavage. Carisoprodol plasma concentrations of rats and mice were measured at the end of the 13-week studies; single-dose plasma carisoprodol analyses were also performed. Genetic toxicity studies were conducted in *Salmonella typhimurium*, L5178Y mouse lymphoma cells, cultured Chinese hamster ovary cells, and peripheral blood erythrocytes of mice.

Groups of 10 male and 10 female F344/N rats received 0, 100, 200, 400, 800, or 1,600 mg carisoprodol per kilogram body weight in corn oil by gavage or 0, 100, 200, 400, or 800 mg/kg carisoprodol in 0.5% methylcellulose by gavage for 13 weeks. Groups of 10 male and 10 female B6C3F$_1$ mice received 0, 75, 150, 300, 600, or 1,200 mg/kg carisoprodol in corn oil by gavage or 0, 600, 1,200, or 1,600 mg/kg carisoprodol in 0.5% methylcellulose by gavage for 13 weeks.

Among rats that received carisoprodol in corn oil, survival was similar to that of the vehicle controls. Survival of rats administered carisoprodol in 0.5% methylcellulose was also similar to that of the vehicle controls after adjustment for deaths (two males and one female in the 800 mg/kg group and two females in the 400 mg/kg group). The final mean body weight gain of males administered 1,600 mg/kg carisoprodol in corn oil was significantly less than that of the vehicle controls; the final mean body weights and body weight gains of female rats in the 800 and 1,600 mg/kg groups were significantly greater. In the carisoprodol in 0.5% methylcellulose study, males in the 200 mg/kg group and females in the 100 and 800 mg/kg groups had significantly greater mean body weights and body weight gains than did the vehicle controls. Clinical findings in rats administered carisoprodol in corn oil or in 0.5% methylcellulose included lethargy, ataxia, diarrhea, and prostration; the incidences were dose-related, and females were more sensitive than males to the effects of carisoprodol.

In the carisoprodol in corn oil study, differences in hematology and clinical chemistry parameters occurred with no consistent patterns. The effects of carisoprodol in 0.5% methylcellulose on hematology and clinical chemistry parameters were not studied.

In the corn oil study, the kidney and liver weights of male and female rats administered 200 mg/kg carisoprodol or greater were generally significantly greater than those of the vehicle controls. In the 0.5% methylcellulose study, liver weights were significantly greater in male rats administered 400 or 800 mg/kg and in female rats administered 800 mg/kg carisoprodol compared to the vehicle controls; however, a consistent effect on the kidney weights was not observed.

Nephropathy was observed in male rats administered 400 mg/kg carisoprodol or greater in corn oil; the livers of four males in the 1,600 mg/kg group had centrilobular hypertrophy of hepatocytes. No lesions were observed histopathologically in female rats administered carisoprodol in corn oil. In the carisoprodol in 0.5% methylcellulose study, the severity of nephropathy in males administered 200 mg/kg or greater was enhanced, and the incidence of nephropathy in female rats in the 800 mg/kg group was slightly greater than that in the vehicle controls.

Plasma carisoprodol concentrations at the end of 13 weeks generally increased with increasing dose in rats administered carisoprodol in corn oil or in 0.5% methylcellulose. The plasma carisoprodol concentrations in rats administered a single gavage dose of carisoprodol in corn oil also increased with increasing dose.

In the carisoprodol in corn oil mouse study, two females each in the vehicle control and 75 mg/kg groups and one female each in the 150 and 600 mg/kg groups were accidentally killed; all males survived to the end of the

study. One male and one female administered 1,600 mg/kg carisoprodol in 0.5% methylcellulose died; seven mice were accidentally killed. The mean body weights and body weight gains of mice administered carisoprodol in corn oil were generally similar to those of the vehicle controls. The final mean body weights and body weight gains of all groups of males and females administered carisoprodol in 0.5% methylcellulose were significantly less.

Clinical findings in the carisoprodol in corn oil study included lethargy, ataxia, tremors, and prostration in male and female mice. Ataxia, lethargy, convulsions, and prostration were observed in all dosed groups of males and females administered carisoprodol in 0.5% methylcellulose. In the carisoprodol in corn oil study, liver weights were significantly greater in males administered 300 mg/kg or greater and in females administered 150 mg/kg or greater than in the vehicle controls.

In the carisoprodol in corn oil study, no gross or microscopic lesions were considered related to carisoprodol administration. Minimal to mild centrilobular hypertrophy was observed in the liver of all dosed groups of males and in females in the 1,200 and 1,600 mg/kg groups in the carisoprodol in 0.5% methylcellulose study.

The testis weights of males administered 1,200 mg/kg carisoprodol in corn oil were significantly less than those of the vehicle controls; the sperm motility of males in this group was also significantly less than that of the vehicle controls. There were no significant differences in vaginal cytology parameters between dosed and vehicle control females.

At the end of the carisoprodol in corn oil study, the concentration of carisoprodol was above the limit of detection in the plasma of only one male mouse each in the 300 and 1,200 mg/kg groups and in four females in the 1,200 mg/kg group. In mice administered a single gavage dose of carisoprodol in corn oil, plasma concentrations increased with increasing dose; peak plasma concentrations occurred at 20 to 120 minutes in males and 60 to 120 minutes in females. In the carisoprodol in 0.5% methylcellulose study, plasma carisoprodol concentrations of female, but not male, mice increased with increasing dose; peak plasma carisoprodol concentrations occurred at 30 minutes postdosing in all groups of males and females.

Results of proportionality and bioavailability studies indicated that single gavage doses of 200 to 800 mg/kg carisoprodol in 0.5% methylcellulose in rats or 300 to 1,200 mg/kg in mice were dose proportional; absolute bioavailability values increased with increasing dose, ranging from 15% to 32% for rats and from 18% to 38% for mice. For rats, the bioavailability of carisoprodol in 0.5% methylcellulose was approximately fivefold that of carisoprodol in corn oil; the C_{max} values of the dose in 0.5% methylcellulose were approximately threefold

those of the dose in corn oil. For mice, no significant difference was observed in the bioavailability of carisoprodol between the vehicles; however, the C_{max} values of the dose in 0.5% methylcellulose were 1.5 to 1.75 times those of the dose in corn oil.

Carisoprodol was not mutagenic in any of four strains of *Salmonella typhimurium*, with or without S9 metabolic activation. It did induce mutations in L5178Y mouse lymphoma cells in the absence of S9; with S9, no mutagenic activity was noted in this assay. Results of the sister chromatid exchange test with carisoprodol in cultured Chinese hamster ovary cells were considered equivocal with and without S9. Chromosomal aberrations in cultured Chinese hamster ovary cells were clearly increased by carisoprodol treatment, particularly in the presence of S9. No significant increases in the frequency of micronucleated erythrocytes were observed in peripheral blood samples from male and female mice administered carisoprodol by gavage for 13 weeks.

In conclusion, carisoprodol induced ataxia and prostration in rats and mice, increases in liver weights in rats and mice, and nephropathy in male rats. The bioavailability of carisoprodol in 5% methylcellulose was greater than in corn oil. The no-observed-adverse-effect (NOAEL) level of carisoprodol administered in corn oil or in 0.5% methylcellulose was determined to be 100 mg/kg, compared to the clinical dose of 20 mg/kg per day for adults and 5 to 7.5 mg/kg per day for children.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

Carisoprodol, an odorless, white, crystalline powder with a slightly bitter taste, is an aliphatic dicarbamate and a chemical derivative of meprobamate. It is stable in dilute acids and alkalies. It is soluble in many organic solvents, sparingly soluble in water, and insoluble in vegetable oils. The melting point of carisoprodol is 92° to 93° C (*Merck Index*, 1996; AHFS, 1998). When heated, carisoprodol emits hazardous fumes (Lewis, 1993).

PRODUCTION, USE, AND HUMAN EXPOSURE

Carisoprodol is produced by reacting phosgene with 2-methyl-2-propyl-1,3-propanediol in the presence of dimethylaniline, followed by reaction with isopropylamine and then sodium cyanate in the presence of hydrogen chloride (HSDB, 1990). More than 1,000 pounds of carisoprodol were produced in the United States in 1979 (personal communication, IMS America, Ambler, PA); current production figures are not available. Carisoprodol is widely used as a skeletal muscle relaxant that acts by blocking interneuronal activity in the descending reticular formation and spinal cord and as an analgesic; it is available as a prescription drug in 250 mg capsules and 350 mg tablets, with or without additional analgesics such as aspirin (Franz, 1975). In 1984, approximately 70 million units of carisoprodol were prescribed; this did not include combinations of carisoprodol and other drugs (personal communication, IMS America, Ambler, PA).

PHARMACOKINETICS, ABSORPTION, DISTRIBUTION, AND METABOLISM

The American Hospital Formulary Service (1998) reported a 4.0-hour time to peak serum concentration in humans after a single oral dose of 350 mg carisoprodol, with the onset of action occurring at 0.5 hours and a duration of 4 to 6 hours. The peak serum concentration is 4 to 7 μg/mL; the half-life is 8.0 hours. Carisoprodol is rapidly absorbed from the gastrointestinal tract. A single gavage dose of carisoprodol produced a peak blood concentration in approximately 30 to 60 minutes in rats and approximately 15 minutes in mice (Bossoni *et al.*, 1979). Approximately 55% of carisoprodol (10 or 20 μg/mL human blood plasma) was bound to plasma proteins (Douglas *et al.*, 1964).

In mice administered intravenous injections of radiolabeled carisoprodol, the compound was taken up by the central nervous system within 40 seconds. The injected carisoprodol was distributed throughout the body within 10 minutes. The highest concentrations were found in the liver, myocardium, pituitary gland, and adrenal cortex, followed by the blood, lungs, and skeletal muscles. Radioactivity was uniformly distributed throughout the fetuses of pregnant mice 15 minutes after the dose was administered (van der Kleijn, 1969). Intravenously administered, ^{14}C-labeled carisoprodol was readily eliminated from the blood of dogs; the plasma half-life was approximately 15 minutes (Douglas *et al.*, 1962).

Carisoprodol is biotransformed by the cytochrome P450 drug metabolizing system in the liver (PDR, 1989). Doses of 200 mg/kg carisoprodol to male Wistar-derived Alderley Park rats (Topham *et al.*, 1972) or 180 mg/kg carisoprodol to Sprague-Dawley female rats (Kato, 1966) caused increases in the hepatic enzymes of the microsomal NADPH-electron transport chain; in the male rats, no increases in liver weights were observed (Topham *et al.*, 1972). Induction of the microsomal drug metabolizing enzymes increased carisoprodol metabolism and shortened the duration of paralysis induced by carisoprodol (Kato and Takanaka, 1968); this induction of metabolizing enzymes may be related to the development of tolerance to carisoprodol with chronic exposure. The rate of metabolism of carisoprodol by microsomal enzymes may be age related; old rats (600 days) metabolized carisoprodol more slowly than young rats (100 days). Consequently, the retention of carisoprodol in the brain and the duration of carisoprodol-induced paralysis were longer in old rats than in young rats (Kato and Takanaka, 1968).

Carisoprodol undergoes dealkylation and oxidation in the liver (Figure 1). Hydroxymeprobamate, meprobamate, and hydroxycarisoprodol were the major metabolites identified in the blood and urine; trace quantities of unchanged carisoprodol were also detected in the urine (AHFS, 1998). In mongrel dogs administered carisoprodol (Douglas *et al.*, 1962), the urinary metabolites were hydroxycarisoprodol (40%), meprobamate (15%), hydroxymeprobamate (40%), unchanged carisoprodol (<1%), and glucuronide condensate (1% to 2%). Meprobamate was the principal metabolite detected in the serum, urine, and gastric contents of a child who ingested approximately 3,500 mg carisoprodol (Adams *et al.*, 1975). Meprobamate was also the major metabolite identified in mice administered carisoprodol (van der Kleijn, 1969). Carisoprodol and its metabolites are excreted by the kidney.

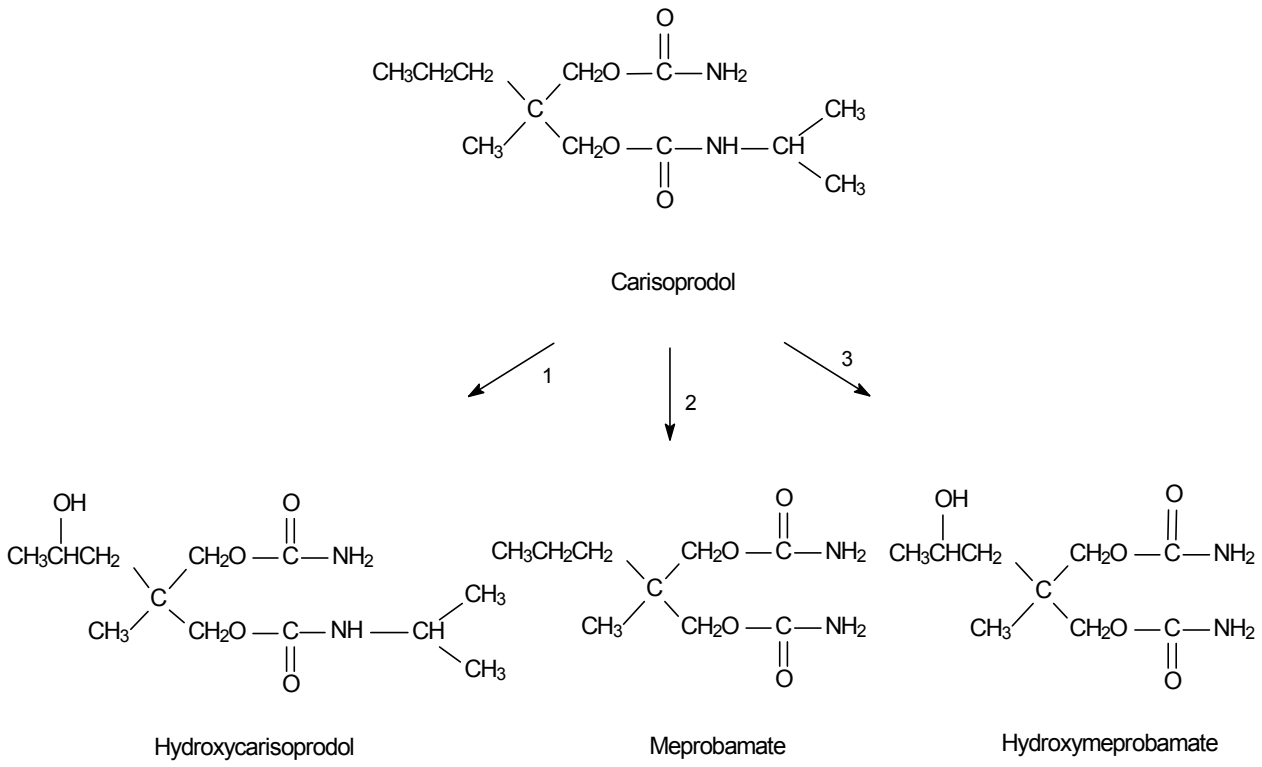


FIGURE 1
Metabolism of Carisoprodol in the Liver (1=oxidation hydroxylation; 2=oxidative dealkylation; 3=oxidation hydroxylation + oxidative dealkylation)

TOXICITY

Experimental Animals

The LD_{50} values of carisoprodol for rats are 450 mg/kg for intravenous and intraperitoneal injection and 1,320 mg/kg for gavage dosing. For mice, the LD_{50} values are 165 mg/kg for intravenous injection, 980 mg/kg for intraperitoneal injection, and 2,340 mg/kg for gavage dosing. The LD_{50} value for rabbits administered carisoprodol by intravenous injection is 124 mg/kg (Berger *et al.*, 1959; Sax, 1984).

In mice (strain not specified), intraperitoneal injections of a 5% aqueous gum acacia solution of 180 mg/kg carisoprodol or greater per kilogram body weight produced a reversible flaccid paralysis of voluntary muscles; animals were excited before the onset of paralysis (Berger *et al.*, 1959). No tremors or twitching were noted during the paralysis, and respiration and heart rate were not impaired. Mice responded to painful stimuli, which suggests that the peripheral nerve, interneuronal junction, and muscle were not significantly affected.

The corneal reflex was blocked and the righting reflex abolished; higher doses of carisoprodol suppressed the pinna reflex. Very large doses (>1 g/kg) of carisoprodol caused death from respiratory paralysis. Similar effects were observed in other animal species (Ludwig and Potterfield, 1971). No body weight, feed consumption, or hematologic effects were noted in Sprague-Dawley rats fed diets containing 0.5%, 1%, or 2% carisoprodol for 1 year (Berger *et al.*, 1959). Rats in the 2% group had slightly enlarged kidneys and livers.

Humans

Carisoprodol has been shown to block interneuronal activity in the reticular formation and spinal cord (PDR, 1996). Its efficacy, however, may be due in part to central nervous system depression; a blood concentration of 3.3 mg/dL carisoprodol produces central nervous system depression in humans. Carisoprodol is more effective than mephenesin, a muscle relaxant that depresses transmission through a number of spinal and supraspinal polysynaptic reflexes, but is a less effective antagonist of strychnine (Esplin, 1970). Carisoprodol is antipyretic and has weak anticholinergic activity. The most frequent side effects of carisoprodol are drowsiness and dizziness; higher concentrations may cause lethargy, double vision, nystagmus, and tachycardia (AHFS, 1998). Idiosyncratic reactions may occur after the first dose; side effects, which may last for a few hours, include weakness, lassitude or sedation, transient quadriplegia, ataxia, vertigo, temporary loss of vision, mydriasis, dysarthria, confusion, disorientation, agitation, and euphoria (*Remington's Pharmaceutical Sciences*, 1990; PDR, 1996). Skin reactions, including rashes and hives, have been reported (Jones, 1960). Respiratory depression, coma, hypothermia, and hypotension have occurred in severe cases of poisoning. Clear evidence for addictive properties of carisoprodol is lacking, although withdrawal symptoms have been observed after therapy ceased (*Remington's Pharmaceutical Sciences*, 1990).

The drowsiness and ataxia observed in patients treated with carisoprodol may be caused by the metabolite meprobamate, a centrally active agent that has no effects on the autonomic nervous system. Meprobamate also causes depression of the polysynaptic reflexes in the spinal cord without affecting monosynaptic reflexes; the compound is mildly tranquilizing and has some anticonvulsant and muscle relaxant properties. Hooper *et al.* (1961) reported that meprobamate caused increased serum prolactin concentrations and galactorrhea. Studies have also reported eczematous eruptions (Savin, 1970; Pasricha, 1979). The *Physicians' Desk Reference* (1989) reported sedation and gastrointestinal upset in the nursing infants of women treated with carisoprodol.

Overdoses of carisoprodol may cause stupor, coma, shock, respiratory depression, and, very rarely, death (AHFS, 1998). In one man who ingested 8.4 g carisoprodol and another who ingested 9.45 g, the maximum plasma concentrations of carisoprodol were 37 and 38 μg/mL, respectively. These men exhibited drowsiness, dizziness, headache, diplopia, and nystagmus on lateral gaze. The metabolite meprobamate has been associated

with hematologic effects in humans, including leukopenia, thrombocytopenia, acute nonthrombocytopenic purpura, eosinophilia, aplastic anemia, and bone marrow hyperplasia (Schiller *et al.*, 1969; Davies, 1981).

REPRODUCTIVE EFFECTS

Experimental Animals

Continuous breeding studies on reproduction and fertility were conducted with carisoprodol (0, 300, 750, or 1,200 mg/kg) administered by gavage to Swiss (CD-1®) mice. No toxic effects were observed in first- or second-generation mice except slightly decreased body weights; third-generation mice in the 1,200 mg/kg group had 22% fewer live pups per litter and a mean body weight that was 8% less than that of the controls (Grizzle *et al.*, 1995).

Humans

No information on the reproductive or developmental toxicity of carisoprodol was found in the literature. However, carisoprodol is excreted in breast milk at concentrations two to four times that in maternal plasma (PDR, 1989). Additionally, the carisoprodol metabolite meprobamate is associated with an increased risk of congenital malformations during the first trimester of pregnancy (PDR, 1996).

CARCINOGENICITY

Experimental Animals

No 2-year carcinogenesis studies were reported in the literature. No gross or microscopic lesions were observed in Sprague-Dawley rats fed diets containing 0.5%, 1%, or 2% carisoprodol for 1 year or in male mongrel dogs that were fed capsules containing 50 mg carisoprodol per kilogram body weight five times per week for 6 months (Berger *et al.*, 1959).

Humans

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

GENETIC TOXICITY

Carisoprodol at concentrations up to 10,000 μg/plate was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without S9 metabolic activation enzymes (Zeiger *et al.*, 1987). No additional mutagenicity test data for carisoprodol have been published.

The carisoprodol metabolite meprobamate has been tested for mutagenicity in a limited number of *in vitro* and *in vivo* assays; all results were negative. No induction of gene mutations was noted in *S. typhimurium* strain TA98 or TA100, with or without S9 (Takeda and Kanaya, 1981). Results of feeding and injection assays of meprobamate in *Drosophila* were negative for induction of sex-linked recessive lethal mutations (Lüers *et al.*, 1974; Filippova *et al.*, 1975) and chromosomal aberrations (Lüers *et al.*, 1974). In mammalian cell assays, no increases in the frequency of chromosomal aberrations were observed in human lymphocytes treated with meprobamate *in vitro* (Kamada *et al.*, 1971; Lüers *et al.*, 1974), and no dominant lethal mutations were induced in Swiss mice treated with up to 400 mg/kg meprobamate by intraperitoneal injection (Epstein *et al.*, 1972).

STUDY RATIONALE AND DESIGN

Carisoprodol was nominated by the National Cancer Institute for toxicity testing because it is widely used as a skeletal muscle relaxant and analgesic, because toxicity and carcinogenicity data for carisoprodol are not available, and because the carbamate moiety in its structure is susceptible to nitrosation to form an analogue of N-methyl-N-nitrosourethane, a rat liver carcinogen. Plasma carisoprodol concentration analysis was included in the study design to further characterize the toxic effects of carisoprodol. Gavage was chosen as the route of administration because the drug is taken orally by humans. Two studies were conducted: one used 0.5% methylcellulose and the other corn oil as the gavage vehicle.

Endpoints evaluated during these 13-week studies included histopathology (rats and mice) and clinical pathology (rats). The effects of carisoprodol on reproduction were assessed by the evaluation of testicular and spermatozoal parameters and by determination of the length of the estrous cycle. In addition, the genetic toxicity of carisoprodol was assessed in studies in *S. typhimurium*, L5178Y mouse lymphoma cells, and cultured Chinese hamster ovary cells and by determination of the frequency of micronucleated erythrocytes in peripheral blood of mice from the 13-week studies.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CARISOPRODOL

Carisoprodol was obtained in two lots. Lot 58764, used in the corn oil gavage studies, was obtained from Carter Wallace, Inc. (Cranbury, NJ). Lot 105396, used in the 0.5% methylcellulose gavage studies, was obtained from Ceres Chemical Company, Inc. (White Plains, NY). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI) (Kansas City, MO). Reports on analyses performed in support of the carisoprodol studies are on file at the National Institute of Environmental Health Sciences.

Each lot of carisoprodol was homogenized by mixing in a blender for 15 minutes before other analyses were performed. Because lot 105396 was received in two drums, samples from each drum were compared for equivalency by high-performance liquid chromatography (HPLC). The two samples were determined to be similar.

Each lot of the chemical, a white powder, was identified as carisoprodol by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy; all spectra were consistent with those expected for the structure of carisoprodol. The infrared spectra were consistent with a literature reference (*Sadtler Standard Spectra*). For lot 58764, the melting point range of 91° to 93° C was consistent with the range of 92° to 93° C reported in the literature (*Merck Index*, 1996); for lot 105396, the melting point range of 94° to 95° C was slightly high.

For lot 58764, elemental analyses for carbon, hydrogen, and nitrogen agreed with the theoretical values for carisoprodol. Because carisoprodol reacted with the reagent used in the Karl Fischer water analysis, the percent weight loss on drying, 0.03% ± 0.01%, was determined for water content. Functional group titration of alkali metal cyanates indicated a purity of 101.3% ± 1.0%. Thin-layer chromatography by two solvent systems indicated a major spot only. Because carisoprodol underwent thermal degradation at the temperatures required for volatization for packed column and capillary gas chromatography, no valid impurity profile could be developed. The cumulative data indicated an overall purity of 99% or greater. Additional analyses with HPLC were performed by the analytical chemistry laboratory to quantify an impurity detected by another laboratory using HPLC with ultraviolet (210 nm) detection and two solvent systems (Research Triangle Institute, Research Triangle Park, NC). Results ofHPLC with 210 nm ultraviolet detection were in agreement with the Research

Triangle Institute results of approximately 15% for the impurity; however, HPLC with 190 nm ultraviolet detection or with refractive index detection indicated a concentration of only 1% for the impurity.

For lot 105396, elemental analyses for carbon, hydrogen, and nitrogen agreed with the theoretical values for carisoprodol. Karl Fischer water analysis using Hydranal-Composite 2 indicated 0.029% ± 0.004% water. Functional group titration indicated a purity of 99% ± 2%. Thin-layer chromatography by two solvent systems indicated a major spot only. HPLC with ultraviolet detection (210 nm) indicated a major peak and two impurities with a cumulative area of 0.6% relative to the major peak; HPLC with refractive index detection indicated one major peak, with no impurities with areas of 0.1% or greater relative to the major peak. Concomitant analysis of lot 58764 by HPLC indicated a major peak and two impurities with a cumulative area of 8.4% relative to the major peak by ultraviolet detection and no impurities by refractive index detection; major peak comparisons of lot 105396 with lot 58764 indicated a purity of 100.5% ± 0.9% for lot 105396 relative to lot 58764. The overall purity of lot 105396 was determined to be approximately 99%.

Stability studies performed on lot 58764 by the analytical chemistry laboratory with gas chromatography indicated that carisoprodol is stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. At the study laboratories, the bulk chemical was stored at room temperature, protected from light, in plastic bags in sealed metal cans. Periodic reanalyses performed by the study laboratories using gas chromatography indicated no degradation of the bulk chemical.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Carisoprodol in Corn Oil: The dose formulations were prepared 1 week before dosing began and at least every 2 weeks thereafter by adding a weighed amount of carisoprodol to corn oil and stirring with a magnetic stirrer; additional corn oil was added and the suspension was again stirred until it appeared homogeneous. The dose formulations were stored in glass vessels at room temperature, protected from light, for up to 3 weeks. The dose formulations were stirred before and during dosing with a magnetic stirrer and stirring bar.

Homogeneity studies were performed on a 250 mg/mL formulation by the analytical chemistry laboratory and on the 7.5, 20, 120, and 320 mg/mL dose formulations by the study laboratory using gas chromatography. Homogeneity of all formulations was confirmed. The analytical chemistry laboratory also confirmed that the 250 mg/mL formulation was readily rehomogenized by stirring after 7 days of storage. Stability studies of a 5 mg/mL formulation were performed by the analytical chemistry laboratory using gas chromatography. The

stability of the 5 mg/mL formulation was confirmed for 3 weeks when stored in the dark at room temperature or for 3 hours when stored at room temperature, open to air and light.

Analyses of the dose formulations of carisoprodol were conducted at the study laboratory with gas chromatography. The dose formulations and animal room samples were analyzed at the beginning, midpoint, and end of the rat and mouse studies; the second set of dose formulations and animal room samples for the mouse study were also analyzed. All dose formulations analyzed were within 10% of the target concentrations. All animal room samples for rats and mice were within 10% of the target concentrations. The results of referee analyses performed by the analytical chemistry laboratory agreed with the results obtained by the study laboratory. Periodic analyses of the corn oil vehicle by the study laboratory demonstrated peroxide levels within the acceptable limit of 10 mEq/kg.

Carisoprodol in 0.5% Methylcellulose: The 0.5% methylcellulose vehicle was prepared by adding methylcellulose to deionized water at approximately 80° C and stirring. The dose formulations were prepared one week before dosing began and at least every 2 weeks thereafter by adding carisoprodol to 0.5% methylcellulose, stirring with a spatula, further diluting with 0.5% methylcellulose, and stirring with an overhead stirrer for approximately 30 minutes. The dose formulations were stored in amber glass bottles for up to 3 weeks at 5° C.

Homogeneity studies were performed on a 250 mg/mL formulation by the analytical chemistry laboratory and on the 20 and 160 mg/mL dose formulations by the study laboratory using gas chromatography. Homogeneity was confirmed. Additionally, the analytical chemistry laboratory confirmed that the 250 mg/mL formulation, stored for 2 weeks at 5° C or at room temperature, was readily rehomogenized by stirring. Stability studies were performed on a 5 mg/mL formulation by the analytical chemistry laboratory and on a 30 mg/mL formulation by the study laboratory using gas chromatography. The stability of the 5 mg/mL formulation was confirmed for 7 days when stored in sealed glass containers in the dark at 5° C or for 3 hours when stored at room temperature, open to air and light; no more than 6.4% carisoprodol was lost for formulations stored at 5° C or room temperature for up to 28 days. The 30 mg/mL formulation was stable for 35 days when stored sealed and protected from light at 5° C.

Analyses of the dose formulations of carisoprodol were conducted at the study laboratory with gas chromatography. The dose formulations and animal room samples were analyzed at the beginning, midpoint, and end of the studies. All dose formulations analyzed were within 10% of the target concentrations. All animal room samples were within 10% of the target concentrations. Periodic analyses of methylcellulose used

to prepare the vehicle were performed by Galbraith Laboratories, Inc. (Knoxville, TN); results indicated the methylcellulose was of acceptable purity.

13-WEEK STUDIES

Carisoprodol in Corn Oil: Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 10 days (rats) or 14 days (mice) and were approximately 5 weeks (rats) or 6 weeks (mice) old on the first day of the studies.

Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood was collected from five male and five female rats and mice at the end of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). Sera from two male rats contained antibodies to cilia-associated respiratory bacillus. All other results were negative. Additional details concerning the study design are provided in Table 1.

The doses for the 13-week studies were selected based partly on preliminary NTP studies in which rats and mice were administered carisoprodol by gavage and partly on LD_{50} values reported in the literature. Groups of 10 male and 10 female rats were administered 0, 100, 200, 400, 800, or 1,600 mg carisoprodol per kilogram body weight in corn oil by gavage for 13 weeks. Groups of 10 male and 10 female mice received 0, 75, 150, 300, 600, or 1,200 mg/kg in corn oil by gavage for 13 weeks. Rats were housed five per cage and mice were housed individually. NIH-07 open formula meal (Zeigler Brothers, Inc., Gardners, PA) and water (Bethesda municipal supply) were available *ad libitum*. Additional details on animal maintenance are provided in Table 1.

Clinical pathology studies were performed on rats designated for clinical pathology testing and on all core study rats. Blood for hematology and clinical chemistry evaluations was collected from clinical pathology study rats on days 5 and 21; blood was collected from core study rats at the end of the studies. Rats evaluated on day 5 were fasted for 16 to 18 hours before the last carisoprodol dose was administered. At all time points, the animals were anesthetized with carbon dioxide, and blood was withdrawn from the retroorbital sinus. Samples for hematology analysis were placed in collection tubes containing potassium EDTA as an anticoagulant; samples for clinical chemistry evaluations were placed in tubes with no anticoagulant. The latter samples were allowed to clot and were then centrifuged, and the serum was removed.

Hematologic determinations were made with a Serano-Baker 9000 automated cell counter (Serano-Baker Diagnostics, Allentown, PA) and reagents. The parameters that were evaluated are listed in Table 1. Differential leukocyte counts and morphologic evaluation of blood cells were determined by light microscopy from stained blood smears. Clinical chemistry variables were measured with a Geining DL analyzer. The parameters that were evaluated are listed in Table 1. Reagents were obtained from the equipment manufacturer.

Vaginal cytology and sperm morphology evaluations were performed on core study rats and mice at the end of the studies. Ten male and ten female rats from the vehicle control, 100, 400, and 1,600 mg/kg groups and 10 male and 10 female mice from the vehicle control, 75, 300, and 1,200 mg/kg groups were evaluated. The parameters that were evaluated are listed in Table 1. Methods used were those described in the NTP Statement of Work (NTP, 1984). Briefly, for the 7 days prior to the scheduled terminal sacrifice, the vaginal vaults of the female rats and mice were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Sperm motility was evaluated at necropsy in the following manner. The right testis and right epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each right cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. Four sperm morphology slides were prepared for each animal evaluated. An aliquot of killed sperm suspension was stained in a test tube, spread on a microscope slide under a coverslip and examined.

Plasma carisoprodol concentrations were measured in rats and mice designated for plasma analyses and in core study rats and mice at the end of the studies. Groups of 24 rats received a single dose of 100 or 1,600 mg/kg carisoprodol in corn oil by gavage, and groups of 24 mice received a single dose of 75 or 1,200 mg/kg; groups of nine rats and nine mice were maintained as vehicle controls. Blood was collected from the retroorbital sinus in heparinized tubes from groups of three male and three female vehicle control rats and mice at 60, 180, and 360 minutes after the corn oil vehicle was administered and from groups of three male and three female rats and mice in the dosed groups at 10, 20, 40, 60, 120, 180, 240, and 360 minutes after dosing. The special study

animals were fasted for 16 to 18 hours (rats) or 16 to 22 hours (mice) before the single dose of carisoprodol was administered. Approximately 500 μL of blood was drawn from rats, and the maximum amount available was drawn from mice. Blood was also collected from five male and five female rats per group from the 0, 100, 400, and 1,600 mg/kg groups and five male and five female mice from the 0, 75, 300, and 1,200 mg/kg groups at the end of the core studies, 1 hour after the final dose was administered. Core study animals were not fasted and received at least two consecutive daily doses of carisoprodol before being bled. Approximately 250 to 300 μL of blood was drawn from core study rats, and the maximum amount available was drawn from mice.

Blood samples were centrifuged at 4° C; plasma was collected in microcentrifuge tubes and held at −70° C until shipment to the analytical chemistry laboratory (MRI), where the samples were held at −20° C until analysis by gas chromatography. Plasma samples from each special study male rat in the 1,600 mg/kg group and from a portion of the vehicle control rats were combined with 2.7 μg/mL methaqualone (internal standard) in toluene. Plasma samples from the remaining special study and core study rats and mice were combined with buffer (pH 7.4) and 2 μg/mL methaqualone in methylene chloride. For animals with sufficiently large plasma samples, multiple extracts were prepared. The extracts were centrifuged, and the toluene and methylene chloride layers were taken for analysis; the methylene chloride layers were evaporated to dryness and then reconstituted with methanol before analysis. The samples were analyzed by gas chromatography with a nitrogen/phosphorous thermionic specific detector.

Complete necropsies were performed on all core study animals. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all vehicle control animals, all rats in the 1,600 mg/kg groups, all mice in the 1,200 mg/kg groups, and all animals that died early. Table 1 lists the organs weighed and tissues examined microscopically.

Carisoprodol in 0.5% Methylcellulose: Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 15 to 18 days and were approximately 6 or 7 weeks old on the first day of the studies.

Blood was collected from five male and five female rats and mice at the beginning of dosing, 4 weeks after the studies began, and at the end of the studies. The sera were analyzed for antibody titers to rodent viruses; all results were negative. The animals bled at 4 weeks were also evaluated for the presence of parasites and observed grossly for evidence of disease.

The doses for the 13-week studies were selected based partly on preliminary NTP studies in which rats and mice were administered carisoprodol by gavage and partly on LD_{50} values reported in the literature. Groups of 10 male and 10 female rats were administered of 0, 100, 200, 400, or 800 mg carisoprodol per kilogram body weight in 0.5% methylcellulose by gavage for 13 weeks. Groups of 10 male and 10 female mice received 0, 600, 1,200, or 1,600 mg/kg in 0.5% methylcellulose by gavage for 13 weeks. Rats were housed five per cage; mice were housed individually. NIH-07 open formula pellets (Zeigler Brothers, Inc., Gardners, PA) and water (Columbus municipal supply) were available *ad libitum*. Additional details on animal maintenance are provided in Table 1.

Plasma carisoprodol concentrations were measured in core study rats and mice at the end of the studies. Blood was collected from the retroorbital sinus of half of the surviving rats and mice from each of the dosed groups 30 minutes after the last dose was administered and from the remaining animals at 60 minutes postdosing; animals were anesthetized with a mixture of carbon dioxide and oxygen before being bled. Blood samples were centrifuged and plasma was collected and stored at or below −20° C until analysis. All samples were extracted within 28 days. The samples were initially divided into three portions. A portion of plasma from each animal was buffered to pH 4 with 0.1 M potassium phosphate. Methylene chloride was added and the tubes were rotated for approximately 10 minutes by a mechanical rotator, then centrifuged for approximately 3 minutes. The methylene chloride layer was evaporated to dryness with nitrogen, reconstituted with 20 μg/mL methaqualone in methanol, and analyzed by gas chromatography with a nitrogen phosphorus detector. Plasma samples outside the standard curve range were reextracted with another standard curve or, if the amount of plasma was insufficient for reextraction, the carisoprodol was calculated with an extrapolation of the standard curve. Additional portions of plasma were analyzed in the low standard curve range to accommodate reanalyses. Spiked plasma standards and quality control plasma samples were prepared by combining plasma with stock solutions of carisoprodol in methanol and were analyzed concomitantly with the plasma samples from dosed animals.

Complete necropsies were performed on all animals. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all animals that died early; histopathologic examinations of the liver and kidney were performed on all vehicle control rats and mice, on rats in the 800 mg/kg groups, and on mice in the 1,600 mg/kg groups. Target organs (kidneys of rats, liver of mice) were examined for animals in the lower dose groups. Table 1 lists the organs weighed and tissues examined microscopically.

Upon completion of the laboratory pathologist's histopathologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

TABLE 1
Experimental Design and Materials and Methods in the 13-Week Gavage Studies of Carisoprodol

Corn Oil Gavage Studies	0.5% Methylcellulose Gavage Studies
Study Laboratory Microbiological Associates, Inc. (Rockville, MD)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice
Animal Source Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Time Held Before Studies Rats: 10 days (core) Mice: 14 days (core)	Rats: 15 days (males) or 16 days (females) Mice: 17 days (males) or 18 days (females)
Average Age When Studies Began Rats: 5 weeks (core) Mice: 6 weeks (core)	Rats: 6 weeks Mice: 6 weeks (males), 7 weeks (females)
Date of First Dose Rats: 25 June 1987 (core, male clinical pathology) 26 June 1987 (female clinical pathology) 13 (male) or 14 (female) July 1987 (single-dose plasma) Mice: 6 July 1987 (core) 21 (male) or 22 (female) July 1987 (single-dose plasma)	Rats: 27 (male) or 28 (female) October 1992 Mice: 29 (male) or 30 (female) October 1992
Duration of Dosing 13 weeks (5 days per week)	13 weeks (5 days per week)
Date of Last Dose and Necropsy Rats: 25 September 1987 (core) Mice: 7 October 1987 (core)	Rats: 26 (male) or 27 (female) January 1993 Mice: 28 (male) or 29 (female) January 1993
Average Age at Necropsy 19 weeks	Rats: 19 weeks Mice: 19 weeks (male) or 20 weeks (female)
Size of Study Groups 10 males and 10 females (core) 10 males and 10 females (clinical pathology) 9 (control) or 24 (dosed) males and 9 (control) or 24 (dosed) females (single-dose plasma)	10 males and 10 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as corn oil gavage study
Animals per Cage Rats: 5 Mice: 1	Rats: 5 Mice: 1
Diet NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*

TABLE 1
Experimental Design and Materials and Methods in the 13-Week Gavage Studies of Carisoprodol

Corn Oil Gavage Studies	0.5% Methylcellulose Gavage Studies
Animal Room Environment	
Temperature: 21.1° to 24.4° C (rats); 20.0° to 23.3° C (mice) Relative humidity: 39% to 60% (rats); 23% to 72% (mice) Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 21.1° to 25.0° C Relative humidity: 41% to 56% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Doses	
Rats: 0, 100, 200, 400, 800, or 1,600 mg/kg in corn oil by gavage (dosing volume = 5 mL/kg body weight) Mice: 0, 75, 150, 300, 600, or 1,200 mg/kg in corn oil by gavage (dosing volume = 10 mL/kg body weight)	Rats: 0, 100, 200, 400, or 800 mg/kg in 0.5% methylcellulose by gavage (dosing volume = 5 mL/kg body weight) Mice: 0, 600, 1,200, or 1,600 mg/kg in 0.5% methylcellulose by gavage (dosing volume = 10 mL/kg body weight)
Type and Frequency of Observation	
Animals were observed twice daily; animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies.	Animals were observed twice daily; animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies.
Necropsy	
Necropsies were performed on all animals in the core studies. The following organs were weighed: brain, heart, right kidney, liver, lungs, right testis, and thymus.	Necropsies were performed on all animals. The following organs were weighed: right kidney and liver.
Histopathology	
Complete histopathologic evaluations were performed on all vehicle control rats and mice, all rats in the 1,600 mg/kg groups, all mice in the 1,200 mg/kg groups, and all animals that died early. The following tissues were examined: adrenal glands, blood smear, brain (three sections), clitoral glands (rats only), esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial glands (rats only), prostate gland, salivary glands, seminal vesicles, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurological signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). Gross lesions in rats and mice in the lower dose groups were examined. Organs examined in the lower dose groups included the kidneys and liver of male rats.	Complete histopathologic evaluations were performed on all rats and mice that died early. The tissues that were examined were the same as those listed for the corn oil gavage studies, plus the thigh muscle (if neuromuscular signs were present), the clitoral glands of female mice, and the preputial glands of male mice. Gross lesions and tissue masses in rats and mice in all dose groups were examined. Histopathologic examinations were performed on the liver (two sections) and kidneys of all vehicle control rats and mice, all rats in the 800 mg/kg groups, and all mice in the 1,600 mg/kg groups. Organs examined in the lower dose groups included the kidneys of rats and the liver of mice.

TABLE 1
Experimental Design and Materials and Methods in the 13-Week Gavage Studies of Carisoprodol

Corn Oil Gavage Studies	0.5% Methylcellulose Gavage Studies
Clinical Pathology Blood was collected from the retroorbital sinus of rats anesthetized with carbon dioxide. Rats in the clinical pathology study groups were evaluated on days 5 and 21. Core study rats were evaluated at the end of the study. ***Hematology***: hematocrit; hemoglobin concentration; erythrocyte and reticulocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and leukocyte count and differentials. ***Clinical chemistry***: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids.	None
Sperm Morphology and Vaginal Cytology Evaluations Sperm morphology and vaginal cytology evaluations were performed on core study rats in the vehicle control, 100, 400, and 1,600 mg/kg groups and mice in the vehicle control, 75, 300, and 1,200 mg/kg groups at the end of the studies. Male rats and mice were evaluated for necropsy body and reproductive tissue weights and epididymal spermatozoal data. Females were evaluated for necropsy body weight, estrous cycle length, and the percentage of cycle spent in the various estrous stages.	None
Plasma Carisoprodol Concentration Analyses Plasma carisoprodol concentrations in special study rats and mice administered a single gavage dose of 100 or 1,600 mg/kg carisoprodol were measured at 10, 20, 40, 60, 120, 180, 240, and 360 minutes after dosing; plasma carisoprodol concentrations in supplemental vehicle control rats and mice were analyzed 60, 180, and 360 minutes after the corn oil vehicle was administered. Plasma carisoprodol concentrations were measured in core study rats in the 0, 100, 400, and 1,600 mg/kg groups and mice in the 0, 75, 300, and 1,200 mg/kg groups 1 hour after the final dose was administered.	Plasma carisoprodol concentrations were measured in rats and mice at the end of the studies. Blood was collected from the retroorbital sinus of half of the surviving rats and mice from each of the dosed groups 30 minutes after the last dose was administered and from the remaining animals at 60 minutes postdosing.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

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 with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). 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 or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations.

QUALITY ASSURANCE METHODS

The 13-week studies were conducted in compliance with United States Food and Drug Administration Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance Units of Microbiological Associates, Inc., and Battelle Columbus Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1987). Carisoprodol was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX) and was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and

poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of five doses of carisoprodol. The high dose was limited by study design to 10,000 μg/plate. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, not reproducible, or 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. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

Mouse Lymphoma Mutagenicity Test Protocol

The experimental protocol is presented in detail by Myhr *et al.* (1985). Carisoprodol was supplied as a coded aliquot by Radian Corporation. The high dose of 1,000 μg/mL was determined by toxicity. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in supplemented Fischer's medium; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring cells resistant to trifluorothymidine (TFT), subcultures were exposed to medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day; to medium containing thymidine, hypoxanthine, and glycine for 1 day; and to normal medium for 3 to 5 days. For cloning, the horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, except 300 μg/mL with S9 activation, were replicated, including concurrent positive and solvent controls. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with carisoprodol continued for 4 hours, at which time the medium plus carisoprodol was removed, and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, cells were plated in medium and soft agar supplemented with TFT for selection of TFT-resistant cells, and cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. The test was initially performed without S9. Because a clearly positive response was not obtained, the test was repeated using freshly prepared S9 from the livers of Aroclor 1254-induced male F344 rats.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). All data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for carisoprodol to be considered positive, i.e., capable of inducing TFT resistance. A single significant response led to a "questionable" conclusion, and the absence of both a trend and peak response resulted in a "negative" call.

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al.* (1987). Carisoprodol was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least four doses of carisoprodol; the high dose was limited by toxicity. A single flask per dose was used.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with carisoprodol in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing carisoprodol 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 carisoprodol, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no carisoprodol. Incubation proceeded for an additional 27 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P < 0.005$) 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 carisoprodol for 10 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 carisoprodol 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. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 $\pm$ 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentages of cells with aberrations. To arrive at a statistical call for a trial, 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). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 13-week toxicity study of carisoprodol in corn oil, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were 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 2,000 polychromatic erythrocytes (PCEs) and 10,000 normochromatic erythrocytes (NCEs) in up to 10 animals per dose group.

Log transformation of the NCE data, testing for normality by the Shapiro-Wilk test, and testing for heterogeneity of variance by Cochran's test were performed before statistical analyses. The frequency of micronucleated cells among NCEs was determined by analysis of variance with the SAS GLM procedure. The NCE data for each dose group were compared with the concurrent solvent control by Student's *t*-test. The

frequency of micronucleated cells among PCEs was analyzed by the Cochran-Armitage trend test, and individual dose groups were compared to the concurrent solvent control by Kastenbaum-Bowman's binomial test.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and differing results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Report represent a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

RATS

CARISOPRODOL IN CORN OIL

One female in the 1,600 mg/kg group was accidentally killed during week 3 (Table 2). The final mean body weight gain of males administered 1,600 mg/kg was significantly less than that of the vehicle controls; final mean body weights and mean body weight gains were significantly greater in female rats in the 800 and 1,600 mg/kg groups than in the vehicle controls (Table 2 and Figure 2).

Clinical findings included lethargy, diarrhea, and rough hair coat in male and female rats in the 800 and 1,600 mg/kg groups; females in these two groups also exhibited ataxia, prostration, and urine stain in the vaginal area. Ataxia was observed in males in the 1,600 mg/kg group. One male and two females in the 800 mg/kg groups had rough hair coats.

Data for the hematology and clinical chemistry variables are listed in Table B1. At week 13, there was a minimal treatment-related increase in mean cell volumes (MCV) of dosed male rats and the 1,600 mg/kg females. Additionally, the 1,600 mg/kg male rats had a minimally increased mean cell hemoglobin (MCH) value; this is consistent with, and would reflect, the increase in MCV. For the male rats in the 1,600 mg/kg group, the increased MCV and MCH values were accompanied by a minimal decrease of erythrocyte (RBC) count; this suggests that, while there were fewer numbers of circulating RBCs, the red cells were slightly larger. At week 13, increased total protein concentrations occurred in the 1,600 mg/kg male rats. A transient decrease in alkaline phosphatase (AP) activity occurred in male rats in the 200 mg/kg and greater dose groups on day 5. There was a tendency for minimal increases in urea nitrogen concentrations in the dosed male and female rats at various time points. Creatinine concentration, another marker of renal function, was, however, unaffected suggesting that the increases in urea nitrogen concentration were nonrenal in origin. Other changes in hematology and clinical chemistry parameters were sporadic and minimal, did not demonstrate a treatment relationship, and/or were inconsistent between males and females; these were not considered toxicologically relevant.

TABLE 2
Survival and Body Weights of Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	97 ± 2	356 ± 6	259 ± 6	
100	10/10	97 ± 2	364 ± 6	267 ± 6	102
200	10/10	113 ± 2**	362 ± 5	249 ± 5	102
400	10/10	109 ± 4**	350 ± 5	241 ± 5	99
800	10/10	106 ± 3**	352 ± 11	246 ± 11	99
1,600	10/10	109 ± 3**	338 ± 7	229 ± 6**	95
Female					
0	10/10	98 ± 3	201 ± 3	103 ± 3	
100	10/10	97 ± 2	200 ± 3	103 ± 4	99
200	10/10	98 ± 3	197 ± 4	99 ± 3	98
400	10/10	95 ± 1	207 ± 3	111 ± 3	103
800	10/10	97 ± 3	213 ± 3*	117 ± 3**	106
1,600	9/10[c]	95 ± 3	222 ± 4**	125 ± 4**	110

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 3 (gavage accident)

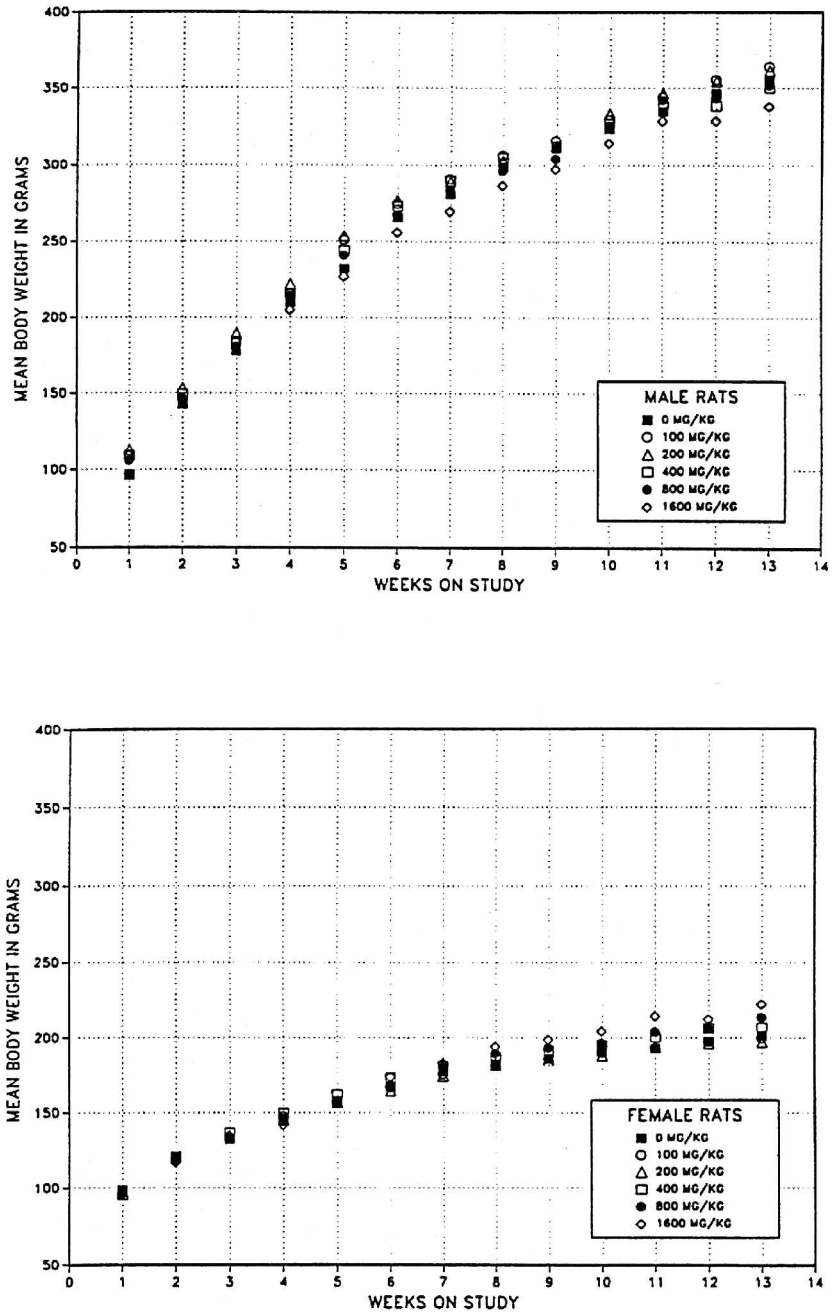


FIGURE 2
Body Weights of Rats Administered Carisoprodol in Corn Oil by Gavage for 13 Weeks

The kidney and liver weights of males and females administered 200 mg/kg or greater were generally significantly greater than those of the vehicle controls (Tables 3 and C1).

At necropsy, no gross lesions were attributed to carisoprodol administration. No microscopic lesions were observed in female rats; in males, treatment-related microscopic lesions were observed in the kidney and liver. A slight exacerbation of chronic nephropathy, evidenced by increased lesion incidences and severities, was observed in the kidney of males administered carisoprodol in corn oil at doses of 400 mg/kg or greater (Table 4). Chronic nephropathy may occur spontaneously in male F344/N rats in subchronic studies. The lesion consists of a few scattered foci of basophilic renal tubule cells surrounded by a thickened basement membrane. In four males in the 1,600 mg/kg group, minimal tubule epithelial necrosis was also present in the kidney and was evidenced by necrotic cells that were desquamated into the lumen of cortical tubules.

TABLE 3
Selected Organ Weight Data for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	362 ± 6	373 ± 6	361 ± 5	361 ± 5	364 ± 11	351 ± 6
R. Kidney						
Absolute	1.174 ± 0.030	1.242 ± 0.016	1.275 ± 0.023*	1.286 ± 0.026**	1.336 ± 0.044**	1.391 ± 0.028**
Relative	3.24 ± 0.05	3.33 ± 0.05	3.54 ± 0.03**	3.56 ± 0.05**	3.67 ± 0.04**	3.97 ± 0.07**
Liver						
Absolute	12.006 ± 0.308	12.966 ± 0.312	12.744 ± 0.404	14.246 ± 0.377**	16.661 ± 0.648**	17.750 ± 0.472**
Relative	33.15 ± 0.58	34.74 ± 0.54	35.28 ± 0.68*	39.37 ± 0.50**	45.67 ± 0.75**	50.56 ± 0.86**
Female						
n	10	10	10	10	10	9
Necropsy body wt	205 ± 3	203 ± 3	202 ± 5	212 ± 3	214 ± 4	222 ± 4**
R. Kidney						
Absolute	0.722 ± 0.014	0.739 ± 0.015	0.754 ± 0.019	0.790 ± 0.014**	0.886 ± 0.020**	0.871 ± 0.018**
Relative	3.53 ± 0.05	3.63 ± 0.06	3.74 ± 0.04*	3.73 ± 0.07*	4.14 ± 0.05**	3.92 ± 0.06**
Liver						
Absolute	6.023 ± 0.123	6.392 ± 0.115	6.728 ± 0.234*	7.951 ± 0.216**	9.163 ± 0.218**	10.829 ± 0.271**
Relative	29.45 ± 0.52	31.51 ± 0.76*	33.31 ± 0.66**	37.44 ± 0.71**	42.78 ± 0.61**	48.69 ± 0.93**

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

TABLE 4
Incidence of Kidney and Liver Lesions in Male Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Kidney[a]	10	10	10	10	10	10
Nephropathy[b]	6 (1.0)[c]	6 (1.0)	8 (1.0)	10* (1.1)	10* (1.2)	10* (1.2)
Necrosis	0	0	0	0	0	4
Liver	10	10	10	10	10	10
Hypertrophy	0	0	0	0	0	4* (1.0)

[a] Number of animals with organ microscopically examined
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

Effects in the liver were limited to centrilobular hypertrophy of hepatocytes in four males in the 1,600 mg/kg group (Table 4). This lesion was characterized by cells with slightly increased amounts of cytoplasm and increased eosinophilic staining.

There were no significant differences in sperm motility or vaginal cytology parameters between exposed and vehicle control males or females (Tables D1 and D2).

Plasma carisoprodol concentrations of rats (core groups) evaluated at the end of the study one hour after the last dose increased with increasing dose (Table E1). The plasma carisoprodol concentrations in rats (special groups) administered a single gavage dose also increased with increasing dose. In males, after a single dose of carisoprodol, plasma concentrations peaked at 20 minutes in the 100 mg/kg group and 40 minutes in the 1,600 mg/kg group; in females, concentrations peaked at 10 minutes in the 100 mg/kg group and at 120 minutes in the 1,600 mg/kg group.

CARISOPRODOL IN 0.5% METHYLCELLULOSE

Two males and one female administered 800 mg/kg and two females administered 400 mg/kg died between weeks 7 and 13 (Table 5). The mean body weights and body weight gains of dosed rats were generally similar to or slightly greater than those of the vehicle controls; the differences were significant for males in the 200 mg/kg group and females in the 100 and 800 mg/kg groups (Table 5 and Figure 3).

Clinical findings included lethargy and ataxia in males and females administered 400 mg/kg or greater and ataxia in females in the 100 and 200 mg/kg groups. Lethargy and ataxia were more severe in females than in males.

TABLE 5
Survival and Body Weights of Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	140 ± 3	366 ± 6	227 ± 4	
100	10/10	143 ± 3	376 ± 8	233 ± 6	103
200	10/10	143 ± 2	389 ± 5*	246 ± 5*	106
400	10/10	144 ± 3	387 ± 5	243 ± 4	106
800	8/10[c]	144 ± 4	364 ± 8	224 ± 5	99
Female					
0	10/10	124 ± 1	210 ± 4	86 ± 4	
100	10/10	125 ± 1	222 ± 2*	97 ± 3*	106
200	10/10	124 ± 1	215 ± 3	91 ± 2	103
400	8/10[d]	124 ± 2	221 ± 4	96 ± 3	105
800	9/10[e]	125 ± 1	223 ± 4*	98 ± 3*	106

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 10, 13
[d] Week of death: 9, 9
[e] Week of death: 7

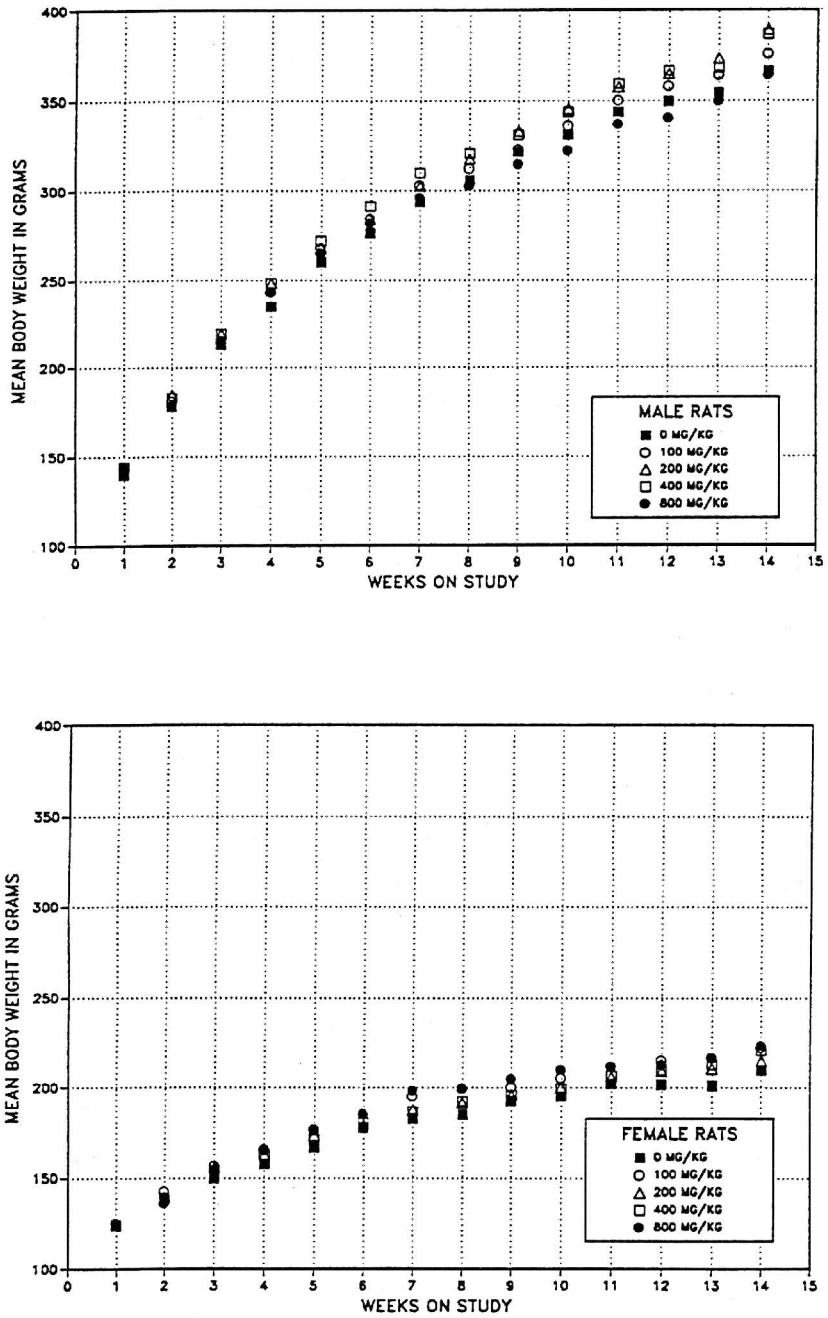


FIGURE 3
Body Weights of Rats Administered Carisoprodol in 0.5% Methylcellulose by Gavage for 13 Weeks

The absolute and relative liver weights of males administered 400 or 800 mg/kg and females administered 800 mg/kg were significantly greater than those of the vehicle controls (Tables 6 and C2). The absolute liver weights of males in the 200 mg/kg group and females in the 400 mg/kg group were also significantly greater than those of the vehicle controls. The absolute kidney weight of females in the 800 mg/kg group was significantly greater than that of the vehicle controls (Table 6).

TABLE 6
Selected Organ Weight Data for Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male					
n	10	10	10	10	8
Necropsy body wt	363 ± 6	372 ± 8	385 ± 5*	383 ± 5	359 ± 7
Liver					
Absolute	15.064 ± 0.370	15.035 ± 0.528	16.431 ± 0.323*	16.778 ± 0.434**	17.318 ± 0.424**
Relative	41.46 ± 0.65	40.33 ± 0.96	42.66 ± 0.49	43.75 ± 0.68*	48.23 ± 0.65**
Female					
n	10	10	10	8	9
Necropsy body wt	207 ± 4	220 ± 2*	215 ± 2	218 ± 4	221 ± 4*
R. Kidney					
Absolute	0.768 ± 0.019	0.770 ± 0.021	0.788 ± 0.014	0.817 ± 0.013	0.844 ± 0.020**
Relative	3.71 ± 0.08	3.50 ± 0.09	3.67 ± 0.04	3.75 ± 0.04	3.82 ± 0.04
Liver					
Absolute	7.894 ± 0.250	7.741 ± 0.130	7.949 ± 0.153	8.741 ± 0.164**	10.028 ± 0.270**
Relative	38.13 ± 1.23	35.19 ± 0.54	37.00 ± 0.57	40.13 ± 0.52	45.43 ± 0.96**

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

At necropsy, no gross lesions considered related to carisoprodol administration were observed. Microscopic examination identified the kidney as a target organ. Similar to the corn oil gavage study, administration of carisoprodol in 0.5% methylcellulose was associated with a slight exacerbation of chronic nephropathy in male

TABLE 7
Incidence of Kidney Lesions in Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male					
Number Examined Microscopically	10	10	10	10	10
Nephropathy[a]	10 (1.0)[b]	9 (1.0)	10 (1.3)	10 (1.8)	10 (1.8)
Female					
Number Examined Microscopically	10	10	10	10	9
Nephropathy	2 (1.0)	2 (1.0)	3 (1.0)	1 (1.0)	5 (1.0)

[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

and female rats (Tables 7, A1, and A2). In males administered 200 mg/kg or greater, the average severity of nephropathy was minimally increased compared to the vehicle controls. In females, the effect was manifested as a slightly increased incidence of nephropathy in the 800 mg/kg group compared to the vehicle controls.

Plasma carisoprodol concentrations of rats evaluated at the end of the study generally increased with increasing dose, but the increase was not linear or proportional with dose (Table E2). The concentration was highest at 30 minutes in rats administered 100, 200 or 800 mg/kg, and at 60 minutes in rats administered 400 mg/kg.

TOXICOKINETIC STUDIES

Results of the single-dose proportionality and bioavailability studies are shown in Tables G1 and G2. Single gavage doses of 200 to 800 mg/kg were dose proportional based on area-under-the-curve (AUC) data; absolute bioavailability values increased with increasing dose, ranging from 15% to 32%.

Results of the single-dose bioequivalence studies are shown in Tables G5 and G6. AUC values indicated that the bioavailability of carisoprodol in 0.5% methylcellulose was approximately fivefold that of carisoprodol in corn oil, and the C_{max} values of carisoprodol in 0.5% methylcellulose were approximately threefold those of carisoprodol in corn oil.

MICE

CARISOPRODOL IN CORN OIL

Two female mice each in the vehicle control and 75 mg/kg groups and one female each in the 150 and 600 mg/kg groups were accidentally killed during weeks 2 and 3, and one female in the 150 mg/kg group was found to be missing during week 2; all males survived to the end of the study (Table 8). The mean body weights and body weight gains of mice administered carisoprodol in corn oil were generally similar to those of the vehicle controls (Table 8 and Figure 4).

Clinical findings included lethargy, ataxia, and tremors in all male and female mice in the 600 and 1,200 mg/kg groups and prostration in nine males and all females in the 1,200 mg/kg groups; three females in the 600 mg/kg group also showed prostration.

TABLE 8
Survival and Body Weights of Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	21.1 ± 0.7	26.9 ± 0.7	5.8 ± 0.6	
75	10/10	21.4 ± 0.5	28.6 ± 0.7	7.2 ± 0.5	106
150	10/10	21.8 ± 0.6	29.5 ± 0.9*	7.7 ± 0.8	110
300	10/10	21.6 ± 0.6	29.2 ± 0.8	7.6 ± 0.6	109
600	10/10	21.1 ± 0.6	27.4 ± 0.5	6.2 ± 0.6	102
1,200	10/10	20.9 ± 0.7	26.9 ± 0.5	6.1 ± 0.4	100
Female					
0	8/10[c]	16.6 ± 0.4	24.2 ± 1.0	7.3 ± 0.9	
75	8/10[c]	16.5 ± 0.4	23.9 ± 0.5	7.2 ± 0.6	99
150	8/10[d]	15.6 ± 0.3	23.9 ± 0.6	8.2 ± 0.4	99
300	10/10	16.0 ± 0.4	24.8 ± 0.5	8.8 ± 0.4	102
600	9/10[e]	15.4 ± 0.4	24.2 ± 0.5	8.6 ± 0.5	100
1,200	10/10	15.8 ± 0.5	23.2 ± 0.5	7.4 ± 0.3	96

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 2, 3 (gavage accidents)
[d] Week of death: 2 (missing); 3 (gavage accident)
[e] Week of death: 3 (gavage accident)

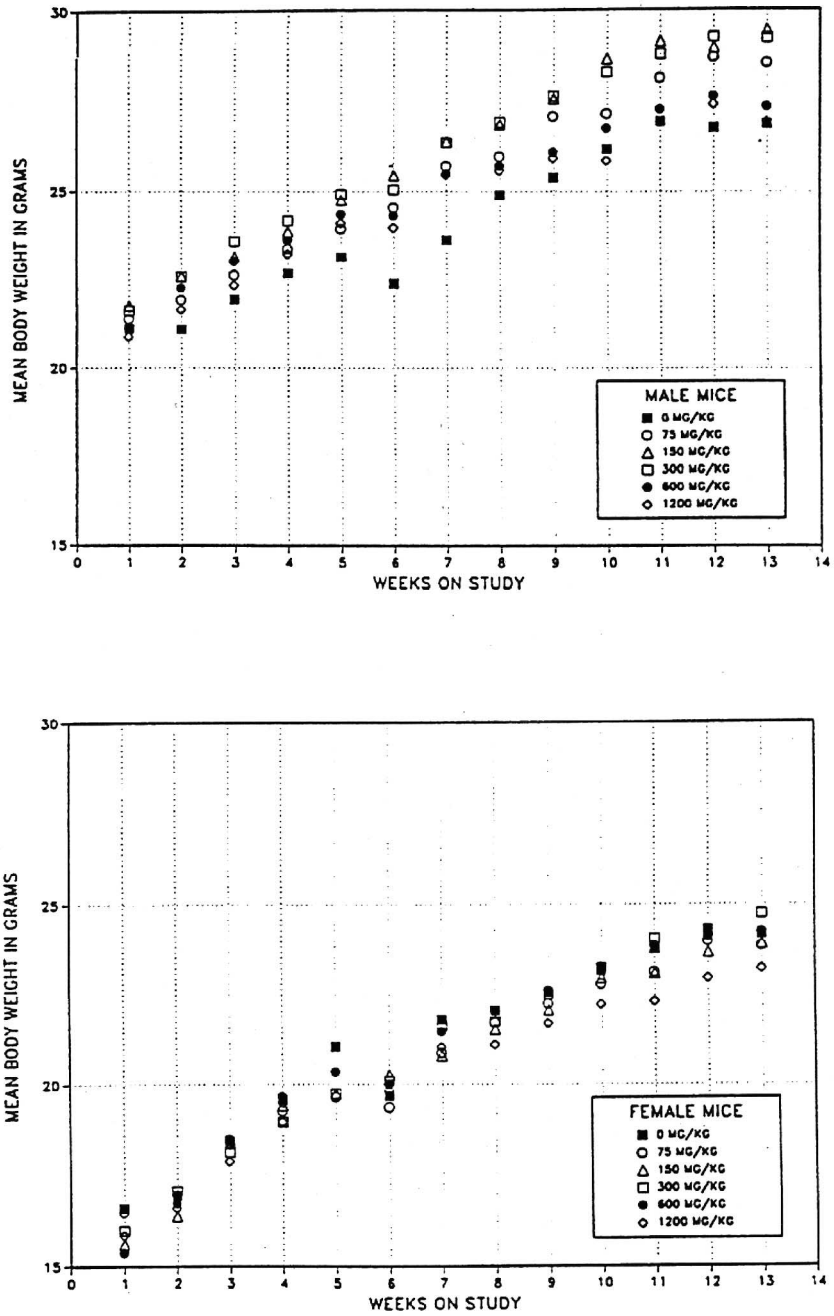


FIGURE 4
Body Weights of Mice Administered Carisoprodol in Corn Oil by Gavage for 13 Weeks

Absolute and relative liver weights were significantly greater in males administered 300 mg/kg or greater and females administered 150 mg/kg or greater than in the vehicle controls; the absolute liver weight of males in the 150 mg/kg group was also significantly greater than that of the vehicle controls (Tables 9 and C3). The absolute and relative right testis weights of males in the 1,200 mg/kg group were significantly less than those of the vehicle controls (Tables 9 and C3).

TABLE 9
Selected Organ Weight Data for Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	1,200 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	27.3 ± 0.7	29.3 ± 0.6	30.0 ± 1.1*	29.9 ± 0.7*	27.8 ± 0.4	27.6 ± 0.5
Liver						
Absolute	1.056 ± 0.035	1.133 ± 0.043	1.223 ± 0.037**	1.309 ± 0.039**	1.374 ± 0.024**	1.558 ± 0.049**
Relative	38.64 ± 0.65	38.66 ± 1.06	40.91 ± 1.08	43.75 ± 0.84**	49.46 ± 0.91**	56.49 ± 1.27**
R. Testis						
Absolute	0.121 ± 0.002	0.118 ± 0.002	0.122 ± 0.003	0.121 ± 0.002	0.115 ± 0.002	0.109 ± 0.003**
Relative	4.43 ± 0.08	4.05 ± 0.08*	4.07 ± 0.11*	4.05 ± 0.08*	4.15 ± 0.08	3.96 ± 0.11**
Female						
n	8	8	8	10	8	10
Necropsy body wt	24.6 ± 0.8	24.0 ± 0.5	24.7 ± 0.5	24.8 ± 0.5	24.7 ± 0.5	23.2 ± 0.6
Liver						
Absolute	0.996 ± 0.035	1.025 ± 0.017	1.143 ± 0.028**	1.177 ± 0.038**	1.333 ± 0.032**	1.270 ± 0.036**
Relative	40.56 ± 0.71	42.79 ± 0.71	46.33 ± 0.66**	47.50 ± 1.01**	54.03 ± 0.97**	54.72 ± 0.72**

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

No dose-related lesions were observed grossly at necropsy. Histopathologic examination revealed perivascular inflammation in the lungs of one male and five females in the vehicle control groups and eight females in the 1,200 mg/kg group; this finding was considered to be a Type III hypersensitivity reaction unrelated to carisoprodol treatment. No microscopic lesions were considered related to carisoprodol administration.

Sperm motility of males in this group was also significantly less than that of the vehicle controls (Table D3) but was within the normal range (83.3 ± 12.6). There were no significant differences in vaginal cytology parameters between dosed and vehicle control females (Table D4).

Carisoprodol was detected in the plasma of only one male mouse each in the 300 and 1,200 mg/kg groups and in four females in the 1,200 mg/kg group at the end of 13 weeks (Table E3). In mice administered a single gavage dose of carisoprodol, plasma concentrations increased with increasing dose. In males, plasma carisoprodol concentrations peaked at 20 minutes in the 75 mg/kg group and at 40 to 120 minutes in the 1,200 mg/kg group; in females, concentrations peaked at 60 minutes in the 75 mg/kg group and at 120 minutes in the 1,200 mg/kg group.

CARISOPRODOL IN 0.5% METHYLCELLULOSE

One male and one female administered 1,600 mg/kg died of undetermined causes during week 1; one male each in the vehicle control and 600 mg/kg groups, two males in the 1,600 mg/kg group, one vehicle control female, and two females in the 1,200 mg/kg group were accidentally killed during weeks 1 and 2 (Table 10). The final mean body weights and body weight gains of all groups of males and females administered carisoprodol in 0.5% methylcellulose were significantly less than those of the vehicle controls (Table 10 and Figure 5).

Ataxia, lethargy, convulsions, and prostration were observed in all dosed groups of males and females during the first week of the study; lethargy and ataxia continued to be observed throughout the study, although the mice developed some tolerance to dosing.

TABLE 10
Survival and Body Weights of Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	9/10[c]	24.1 ± 0.3	36.5 ± 0.7	12.4 ± 0.6	
600	9/10[d]	23.6 ± 0.3	32.9 ± 0.5**	9.2 ± 0.5**	90
1,200	10/10	23.6 ± 0.3	31.1 ± 0.5**	7.6 ± 0.5**	85
1,600	7/10[e]	24.1 ± 0.3	31.2 ± 0.4**	7.1 ± 0.4**	85
Female					
0	9/10[d]	19.1 ± 0.2	29.8 ± 0.8	10.6 ± 0.7	
600	10/10	19.3 ± 0.3	26.0 ± 0.6**	6.8 ± 0.4**	87
1,200	8/10[f]	19.0 ± 0.2	25.8 ± 0.7**	6.9 ± 0.6**	87
1,600	9/10[g]	19.3 ± 0.2	26.7 ± 0.5**	7.4 ± 0.4**	90

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' or Dunnett's test
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 2 (gavage accident)
[d] Week of death: 1 (gavage accident)
[e] Week of death: 1, 2, 2 (week 2 deaths were gavage accidents)
[f] Week of death: 1, 2 (gavage accidents)
[g] Week of death: 1

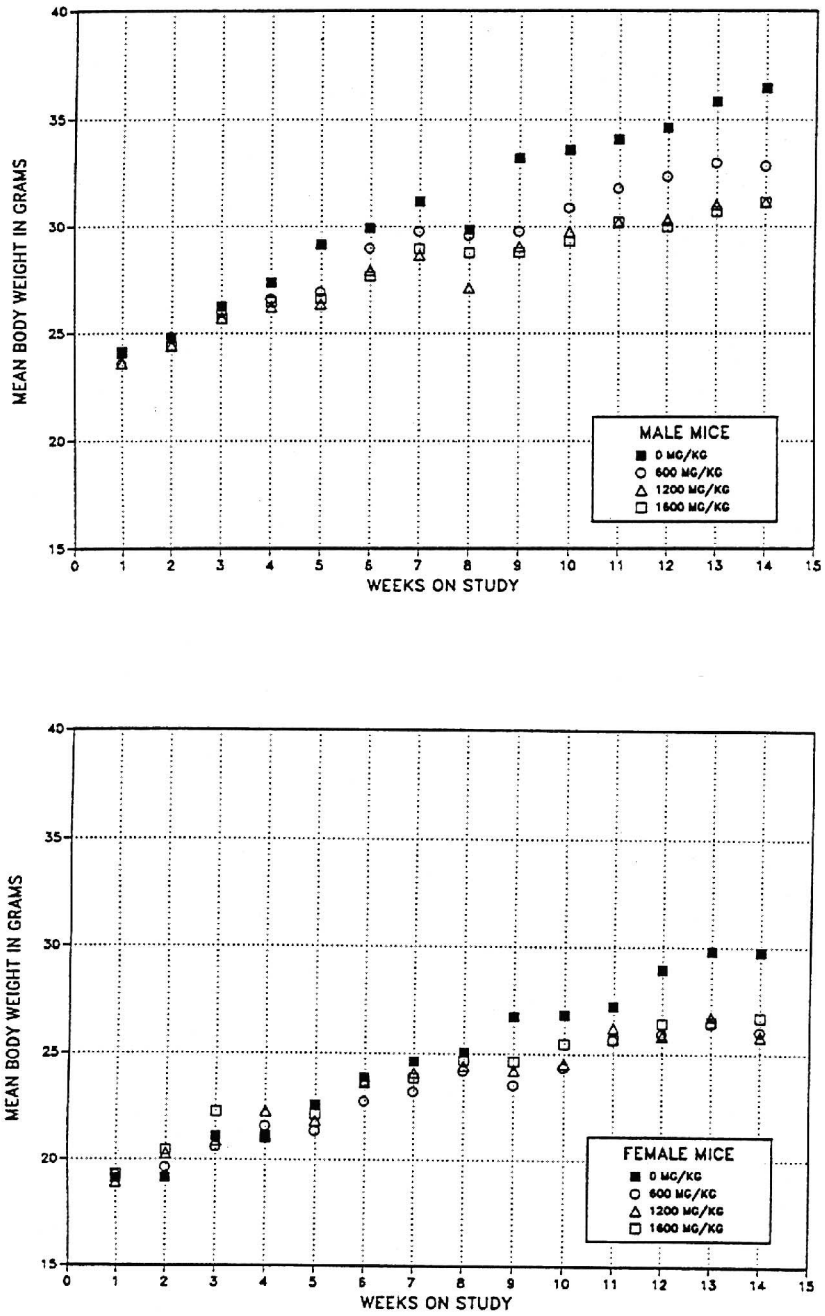


FIGURE 5
Body Weights of Mice Administered Carisoprodol in 0.5% Methylcellulose by Gavage for 13 Weeks

The absolute kidney weights of all dosed groups of males and females were significantly less than those of the vehicle controls (Table C4). Relative liver weights of all dosed groups of males and of females in the 1,600 mg/kg group were significantly greater than those of the vehicle controls. The absolute organ weight differences generally reflected body weight differences.

At necropsy, no gross lesions considered related to carisoprodol administration were observed. The incidence of centrilobular hypertrophy of the liver was significantly increased in male mice in all dosed groups and in females in the 1,200 and 1,600 mg/kg groups (Tables 11, A3, and A4). The severity of centrilobular hypertrophy was minimal in males in the 600 mg/kg group and in both groups of females; the severity in the 1,200 and 1,600 mg/kg groups of males was mild.

Plasma carisoprodol concentrations of female mice evaluated at the end of the study generally increased with increasing dose, but the increase was not linear or proportional with dose (Table E4). In males, plasma carisoprodol concentrations appeared to have no pattern. Carisoprodol concentrations were higher at 30 minutes postdosing in all groups of males and females compared to those at 60 minutes. Carisoprodol concentrations were generally greater in males than in females receiving the same dose.

TABLE 11
Incidence of Liver Lesions in Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose

	Vehicle Control	600 mg/kg	1,200 mg/kg	1,600 mg/kg
Male				
Number Examined Microscopically	10	10	10	10
Centrilobular Hypertrophy[a]	0	9** (1.0)[b]	10** (2.0)	7** (2.0)
Female				
Number Examined Microscopically	10	10	10	10
Centrilobular Hypertrophy	0	0	8** (1.0)	9** (1.0)

** Significantly different (P≤0.01) from the vehicle control group by the Fisher exact test
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

TOXICOKINETIC STUDIES

Results of the single-dose proportionality and bioavailability studies are shown in Tables G3 and G4. Single gavage doses of 300 to 1,200 mg/kg were dose proportional based on AUC data; absolute bioavailability values increased with increasing dose, ranging from 18% to 38%.

Results of the single-dose bioequivalence studies are shown in Tables G7 and G8. AUC values indicated that the bioavailability of carisoprodol in 0.5% methylcellulose was slightly greater than that of carisoprodol in corn oil, and the C_{max} values of carisoprodol in 0.5% methylcellulose were 1.5 to 1.75 times those of carisoprodol in corn oil.

GENETIC TOXICOLOGY

Carisoprodol (10 to 10,000 μg/plate) was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without induced rat or hamster liver S9 (Table F1; Zeiger *et al.*, 1987). In the L5178Y mouse lymphoma cell assay, significant increases in the number of mutant colonies were observed in three of four trials conducted in the absence of exogenous metabolic activation (S9); with S9, no mutagenic activity was observed (Table F2). Relative total growth, although depressed at the mutagenically active concentrations, remained at acceptable levels.

In cytogenetic tests with cultured Chinese hamster ovary cells, carisoprodol induced a small and inconsistent increase in sister chromatid exchanges that was concluded to represent an equivocal response, with and without S9 (Table F3). In the absence of S9, results of a single trial showed a significant induction of sister chromatid exchanges at the two lowest concentrations of carisoprodol tested (5 and 16 μg/mL), and the frequency of sister chromatid exchanges decreased with increasing dose. The first trial with S9 tested viable concentrations up to 500 μg/mL carisoprodol; results were negative. A second trial performed with S9 gave weakly positive results, with significant increases in sister chromatid exchanges noted at the lowest concentration (500 μg/mL) and the highest viable concentration (1,250 μg/mL). Carisoprodol induced chromosomal aberrations in cultured Chinese hamster ovary cells at the highest concentration tested in each of two trials conducted in the absence of S9 (Table F4). The initial trial with S9, which used a maximum viable concentration of 500 μg/mL, gave negative results. However, two subsequent trials, each using higher maximum concentrations of carisoprodol (1,000 or 1,250 μg/mL), gave positive results.

In contrast to the positive results obtained in the *in vitro* chromosomal aberrations assay, carisoprodol was negative in the *in vivo* mouse peripheral blood micronucleus test (Table F5). No increases in the frequencies of micronucleated erythrocytes were seen in either male or female mice administered carisoprodol for 13 weeks by gavage.

DISCUSSION

Carisoprodol administered in corn oil by gavage for 13 weeks at doses up to 1,600 mg/kg had no effect on survival. Mean body weights of males in the 1,600 mg/kg group decreased and those of females in the 800 and 1,600 mg/kg groups increased compared to the controls. However, two male rats and one female rat in the 800 mg/kg groups and two females in the 400 mg/kg group in the carisoprodol in 0.5% methylcellulose study died between weeks 7 and 13. Carisoprodol in corn oil induced ataxia, lethargy, and prostration in the 800 and 1,600 mg/kg groups; similar clinical findings were observed at lower doses of carisoprodol administered in 0.5% methylcellulose. These effects indicated that carisoprodol was more potent when administered in 0.5% methylcellulose than in corn oil. This was supported by toxicokinetic data which showed that carisoprodol administered in 0.5% methylcellulose is fivefold more available and has a threefold greater C_{max} than carisoprodol administered in corn oil. Thus, the differences in effects between the two carisoprodol studies in rats were probably related to the fact that carisoprodol administered in 0.5% methylcellulose was more readily available to the rats than carisoprodol administered in corn oil.

Carisoprodol administered in corn oil also induced generally dose-related increases in kidney weights of rats. However, carisoprodol in 0.5% methylcellulose did not have any effects on kidney weights other than an increase in the absolute right kidney weight of females administered 800 mg/kg. The causes of the differences in kidney weight effects between carisoprodol administered in corn oil and in 0.5% methylcellulose were not clear. Carisoprodol in corn oil induced a slight exacerbation of chronic nephropathy in male rats administered 400 mg/kg or greater but not in females. In the carisoprodol in 0.5% methylcellulose study, exacerbation of chronic nephropathy was also observed in males administered 200 mg/kg or greater. The male rat kidney appeared to be more sensitive to the effects of carisoprodol than the female rat kidney.

There were no consistent effects on clinical chemistry or reproductive toxicity in rats administered carisoprodol. Hematology parameters in rats fluctuated during the 13 weeks of the study. These differences were probably secondary to dehydration, which resulted from ataxia, lethargy, and prostration induced by carisoprodol.

Carisoprodol administered in corn oil induced dose-related increases in the liver weights of rats. At a dose of 1,600 mg/kg, carisoprodol induced centrilobular hypertrophy of hepatocytes in male rats. Carisoprodol in 0.5% methylcellulose also induced dose-related increases in liver weights, but no liver lesions were observed

at 800 mg/kg, the highest dose administered. The increases in liver weights were probably due to induction of cytochrome P450 enzymes (Kato, 1966; Kato and Takanaka, 1968; Topham *et al.*, 1972).

Doses of up to 1,200 mg/kg carisoprodol in corn oil had no effect on survival (after adjustment for accidental deaths) or mean body weights of mice. Carisoprodol administered in 0.5% methylcellulose also had no effect on survival at doses of up to 1,600 mg/kg but did induce decreases in mean body weights and body weight gains at doses as low as 600 mg/kg. These differences in body weight effects were probably related to the greater availability of carisoprodol administered in 0.5% methylcellulose compared to administration in corn oil. Toxicokinetic data indicated that carisoprodol administered in 0.5% methylcellulose had a greater bioavailability and a 1.5- to 1.75-fold greater C_{max} than carisoprodol administered in corn oil. Carisoprodol administered in corn oil or in 0.5% methylcellulose induced lethargy, ataxia, prostration, and tremors in male and female mice.

Carisoprodol in corn oil induced generally dose-related increases in liver weights of male and female mice. No accompanying histopathologic changes were observed. Carisoprodol in 0.5% methylcellulose induced increases in relative liver weights of male and female mice but also induced increases in the incidences of centrilobular hypertrophy in all dosed groups of males and in females administered 1,200 or 1,600 mg/kg. The differences in histopathology findings between mice treated with carisoprodol in corn oil and in 0.5% methylcellulose were probably due to the greater availability of carisoprodol administered in 0.5% methylcellulose.

Carisoprodol administered in corn oil induced decreases in testis weights of mice. A dose of 1,200 mg/kg carisoprodol in corn oil also induced a decrease in epididymal spermatozoal motility but had no effect on vaginal cytology parameters. No effects on testis weights or sperm motility parameters were observed in dosed rats. Results of reproduction and fertility analyses in continuous breeding studies in Swiss (CD-1®) mice indicated no reproductive toxicity in first- or second-generation mice, but third-generation mice in the 1,200 mg/kg group had 22% fewer live pups than did the controls (Grizzle *et al.*, 1995). Thus, carisoprodol was considered to be a mild reproductive toxicant in mice.

In genetic toxicity studies, carisoprodol was not mutagenic in *Salmonella typhimurium* with or without S9 and did not induce increases in the frequency of micronuclei in mouse peripheral blood erythrocytes. However, carisoprodol did induce mutations in L5178Y mouse lymphoma cells without, but not with, S9. Results of a sister chromatid exchange test with carisoprodol in cultured Chinese hamster ovary cells were considered equivocal with and without S9. Chromosomal aberrations in cultured Chinese hamster ovary cells were clearly

increased by carisoprodol treatment, particularly in the presence of S9. These results were in agreement with results reported in the literature.

In conclusion, the no-observed-adverse-effect level (NOAEL) of carisoprodol administered in corn oil or in 0.5% methylcellulose was determined to be 100 mg/kg, compared to the clinical dose of 20 mg/kg per day for adult humans and 5 to 7.5 mg/kg per day for children. Carisoprodol induced an increase in liver weight in rats and mice and enhanced nephropathy in male rats. Carisoprodol was more readily available biologically in 5% methylcellulose than in corn oil. An oral dose of 100 mg/kg in rats and mice was estimated to be comparable to an adult dose of 350 mg four times per day in humans; both give an approximate blood level of 2 μg/mL at 60 minutes after administration.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose A-2
TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose A-4
TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose A-6
TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose A-8

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Disposition Summary					
Animals initially in study	10	10	10	10	10
Early deaths					
Natural deaths					2
Survivors					
Terminal sacrifice	10	10	10	10	8
Animals examined microscopically	10	10	10	10	10
Alimentary System					
Liver	(10)	(1)			(10)
Congestion					2 (20%)
Hepatodiaphragmatic nodule	3 (30%)				
Inflammation, chronic, focal		1 (100%)			
Necrosis					1 (10%)
Cardiovascular System					
None					
Endocrine System					
None					
General Body System					
None					
Genital System					
None					
Hematopoietic System					
Thymus					(2)
Hemorrhage					2 (100%)
Integumentary System					
None					
Musculoskeletal System					
None					
Nervous System					
None					

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Respiratory System					
Lung					(2)
Congestion					1 (50%)
Special Senses System					
None					
Urinary System					
Kidney	(10)	(10)	(10)	(10)	(10)
Nephropathy	10 (100%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)

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

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Disposition Summary					
Animals initially in study	10	10	10	10	10
Early deaths					
Natural deaths				2	1
Survivors					
Terminal sacrifice	10	10	10	8	9
Animals examined microscopically	10	10	10	10	10
Alimentary System					
Liver	(10)		(1)	(2)	(9)
Hepatodiaphragmatic nodule					2 (22%)
Inflammation, chronic, focal			1 (100%)		
Cardiovascular System					
None					
Endocrine System					
None					
General Body System					
None					
Genital System					
None					
Hematopoietic System					
Thymus				(2)	(1)
Hemorrhage				2 (100%)	
Integumentary System					
None					
Musculoskeletal System					
None					
Nervous System					
None					

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Respiratory System					
Lung				(2)	(1)
Congestion					1 (100%)
Nose				(2)	(1)
Respiratory epithelium, hemorrhage					1 (100%)
Special Senses System					
None					
Urinary System					
Kidney	(10)	(10)	(10)	(10)	(9)
Mineralization	9 (90%)	10 (100%)	10 (100%)	10 (100%)	8 (89%)
Nephropathy	2 (20%)	2 (20%)	3 (30%)	1 (10%)	5 (56%)

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

	Vehicle Control	600 mg/kg	1,200 mg/kg	1,600 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Dosing accidents	1	1		2
Natural death				1
Survivors				
Terminal sacrifice	9	9	10	7
Animals examined microscopically	10	10	10	10
Alimentary System				
Esophagus	(1)	(1)		(3)
Muscularis, inflammation, chronic active		1 (100%)		
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)			
Inflammation, granulomatous	1 (10%)			
Centrilobular, hypertrophy		9 (90%)	10 (100%)	7 (70%)
Salivary glands	(1)	(1)		(3)
Inflammation, chronic active	1 (100%)			
Cardiovascular System				
Heart	(1)	(1)		(3)
Epicardium, foreign body	1 (100%)			
Epicardium, inflammation, suppurative	1 (100%)			
Endocrine System				
None				
General Body System				
None				
Genital System				
None				
Hematopoietic System				
Spleen	(1)	(1)		(3)
Hematopoietic cell proliferation	1 (100%)			
Thymus	(1)	(1)		(3)
Inflammation, granulomatous	1 (100%)			
Thymocyte, autolysis				3 (100%)
Integumentary System				
None				

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose

	Vehicle Control	600 mg/kg	1,200 mg/kg	1,600 mg/kg
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(1)	(1)		(3)
Inflammation, chronic active	1 (100%)			
Inflammation, suppurative				1 (33%)
Mediastinum, foreign body	1 (100%)			1 (33%)
Mediastinum, inflammation, suppurative				2 (67%)
Special Senses System				
None				
Urinary System				
Kidney	(10)	(1)		(10)
Inflammation, chronic active	1 (10%)			
Renal tubule, regeneration	2 (20%)			2 (20%)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

	Vehicle Control	600 mg/kg	1,200 mg/kg	1,600 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Dosing accidents	1		2	
Natural death				1
Survivors				
Terminal sacrifice	9	10	8	9
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Inflammation, granulomatous	2 (20%)	3 (30%)	3 (30%)	
Centrilobular, hypertrophy			8 (80%)	9 (90%)
Hepatocyte, necrosis	1 (10%)			
Cardiovascular System				
Heart	(1)		(2)	(1)
Epicardium, foreign body	1 (100%)			
Epicardium, inflammation, suppurative	1 (100%)		1 (50%)	
Endocrine System				
None				
General Body System				
None				
Genital System				
None				
Hematopoietic System				
Thymus	(1)		(2)	(1)
Foreign body	1 (100%)			
Inflammation, suppurative	1 (100%)		1 (50%)	
Thymocyte, autolysis			1 (50%)	
Thymocyte, necrosis			1 (50%)	
Integumentary System				
None				
Musculoskeletal System				
None				

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose

	Vehicle Control	600 mg/kg	1,200 mg/kg	1,600 mg/kg
Nervous System				
None				
Respiratory System				
Lung	(1)		(2)	(1)
Congestion, acute			1 (50%)	
Mediastinum, foreign body	1 (100%)		1 (50%)	
Mediastinum, inflammation, suppurative	1 (100%)		2 (100%)	
Special Senses System				
None				
Urinary System				
None				

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

APPENDIX B
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE B1 **Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil** **B-2**

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Male						
Hematology						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	9	10	10	9
Hematocrit (%)						
Day 5	42.0 ± 0.4	41.5 ± 0.6	41.0 ± 0.6	41.8 ± 0.4	42.0 ± 0.4	41.8 ± 0.5
Day 21	43.1 ± 0.5	42.6 ± 0.6	42.4 ± 0.4	40.8 ± 1.8	43.9 ± 0.4	42.8 ± 0.5
Week 13	48.6 ± 0.5	49.0 ± 2.0	45.5 ± 3.3	47.4 ± 0.7	48.0 ± 0.4	48.0 ± 0.5
Hemoglobin (g/dL)						
Day 5	15.1 ± 0.1	14.8 ± 0.2	14.7 ± 0.2	14.8 ± 0.1	14.8 ± 0.1	14.9 ± 0.1
Day 21	15.5 ± 0.1	15.5 ± 0.2	15.5 ± 0.2	15.5 ± 0.1	15.7 ± 0.1	15.1 ± 0.1
Week 13	16.2 ± 0.3	16.1 ± 0.4	16.0 ± 0.1	15.7 ± 0.2	15.8 ± 0.1	15.6 ± 0.1
Erythrocytes ($10^6/\mu L$)						
Day 5	7.07 ± 0.06	6.81 ± 0.14	6.81 ± 0.14	6.44 ± 0.18**	6.79 ± 0.13	6.86 ± 0.14
Day 21	7.68 ± 0.09	7.42 ± 0.11	7.42 ± 0.10	7.13 ± 0.30	7.70 ± 0.11	7.44 ± 0.08
Week 13	9.43 ± 0.10	9.13 ± 0.20	9.34 ± 0.10	9.07 ± 0.16	9.10 ± 0.06*	8.96 ± 0.08**
Reticulocytes ($10^6/\mu L$)						
Day 5	0.18 ± 0.02	0.18 ± 0.02	0.25 ± 0.05	0.31 ± 0.05*	0.34 ± 0.05*	0.24 ± 0.04*
Day 21	0.19 ± 0.01[b]	0.19 ± 0.02[b]	0.16 ± 0.02[c]	0.20 ± 0.02[b]	0.17 ± 0.01[d]	0.20 ± 0.02[e]
Week 13	0.12 ± 0.01	0.16 ± 0.02	0.10 ± 0.00	0.13 ± 0.01	0.09 ± 0.01	0.13 ± 0.01
Mean cell volume (fL)						
Day 5	59.5 ± 0.3	61.0 ± 0.6	60.2 ± 0.6	65.3 ± 1.7*	62.0 ± 1.4	61.0 ± 1.1
Day 21	56.1 ± 0.3	57.4 ± 0.3	57.3 ± 0.4	57.2 ± 0.6	57.2 ± 0.6	57.6 ± 0.5
Week 13	50.5 ± 1.2	53.7 ± 1.5*	52.3 ± 0.1*	52.3 ± 0.2*	52.8 ± 0.2**	53.6 ± 0.3**
Mean cell hemoglobin (pg)						
Day 5	21.4 ± 0.2	21.8 ± 0.2	21.6 ± 0.3	23.0 ± 0.5*	21.9 ± 0.4	21.7 ± 0.3
Day 21	20.3 ± 0.2	20.9 ± 0.2	20.9 ± 0.2	22.2 ± 1.3	20.4 ± 0.2	20.3 ± 0.1
Week 13	17.2 ± 0.2	17.6 ± 0.2*	17.1 ± 0.1	17.3 ± 0.1	17.4 ± 0.1	17.6 ± 0.1*
Mean cell hemoglobin concentration (g/dL)						
Day 5	36.0 ± 0.3	35.7 ± 0.3	35.9 ± 0.2	35.3 ± 0.4	35.2 ± 0.3	35.6 ± 0.4
Day 21	36.2 ± 0.4	36.5 ± 0.4	36.5 ± 0.4	39.0 ± 2.7	35.8 ± 0.3	35.4 ± 0.3
Week 13	33.3 ± 0.5	33.0 ± 0.6	32.8 ± 0.2	33.1 ± 0.2	32.9 ± 0.1	32.8 ± 0.2
Platelets ($10^3/\mu L$)						
Day 5	1,075.7 ± 31.2	1,053.7 ± 32.4	1,095.2 ± 37.0	1,179.1 ± 47.2	1,122.9 ± 37.7	1,067.9 ± 24.5
Day 21	859.3 ± 8.1	866.7 ± 19.2	890.1 ± 9.9	881.5 ± 6.0	909.9 ± 21.7	899.3 ± 21.2
Week 13	649.5 ± 11.7	673.8 ± 18.3	682.0 ± 10.4	727.3 ± 15.0**	697.1 ± 10.0	671.2 ± 17.6
Leukocytes ($10^3/\mu L$)						
Day 5	6.20 ± 0.24	7.85 ± 0.90	6.24 ± 0.50	13.26 ± 3.40*	9.50 ± 2.03	7.25 ± 0.75
Day 21	5.20 ± 0.29	5.94 ± 0.32	5.29 ± 0.42	5.52 ± 0.40	5.38 ± 0.31[f]	5.89 ± 0.49
Week 13	6.16 ± 0.23	6.59 ± 0.37	7.69 ± 0.36	6.77 ± 0.40[f]	6.96 ± 0.26	4.79 ± 0.22
Segmented neutrophils ($10^3/\mu L$)						
Day 5	0.66 ± 0.05	0.74 ± 0.11	0.71 ± 0.04	1.04 ± 0.22	0.76 ± 0.13	0.83 ± 0.16
Day 21	0.77 ± 0.11	0.75 ± 0.07	0.83 ± 0.10	0.79 ± 0.10	0.79 ± 0.09[f]	0.86 ± 0.13
Week 13	1.01 ± 0.08	1.16 ± 0.15	0.98 ± 0.09	1.13 ± 0.10[f]	1.18 ± 0.09	1.22 ± 0.10
Lymphocytes ($10^3/\mu L$)						
Day 5	5.49 ± 0.26	7.07 ± 0.88	5.43 ± 0.46	12.05 ± 3.24*	8.60 ± 1.91	6.31 ± 0.60
Day 21	4.36 ± 0.29	5.12 ± 0.31	4.43 ± 0.37	4.66 ± 0.30	4.50 ± 0.26[f]	4.94 ± 0.43
Week 13	4.93 ± 0.19	5.19 ± 0.27	6.31 ± 0.31*	5.42 ± 0.40[f]	5.47 ± 0.23	3.42 ± 0.27

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	9	10	10	9
Monocytes ($10^3/\mu L$)						
Day 5	0.05 ± 0.02	0.04 ± 0.03	0.07 ± 0.02	0.08 ± 0.03	0.10 ± 0.04	0.07 ± 0.03
Day 21	0.05 ± 0.02	0.06 ± 0.02	0.03 ± 0.02	0.08 ± 0.04	0.08 ± 0.02[f]	0.07 ± 0.03
Week 13	0.17 ± 0.03	0.16 ± 0.04	0.37 ± 0.06	0.14 ± 0.03[f]	0.30 ± 0.06	0.11 ± 0.03
Eosinophils ($10^3/\mu L$)						
Day 5	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.07 ± 0.04	0.05 ± 0.02	0.02 ± 0.01
Day 21	0.03 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.02[f]	0.02 ± 0.01
Week 13	0.06 ± 0.02	0.08 ± 0.03	0.02 ± 0.02	0.06 ± 0.02[f]	0.04 ± 0.02	0.04 ± 0.02
Clinical Chemistry						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	9	10
Urea nitrogen (mg/dL)						
Day 5	16.8 ± 0.4	16.9 ± 0.5	16.5 ± 0.4	16.5 ± 0.4	18.6 ± 0.5*	19.2 ± 0.7*
Day 21	14.6 ± 0.8	15.5 ± 0.8	15.6 ± 0.5	15.9 ± 1.1	15.2 ± 0.2	16.5 ± 1.1
Week 13	17.3 ± 1.8	20.9 ± 1.0	21.2 ± 0.5	20.4 ± 1.4	23.5 ± 0.3**	22.3 ± 0.7**
Creatinine (mg/dL)						
Day 5	0.63 ± 0.02	0.64 ± 0.01	0.68 ± 0.01	0.64 ± 0.02	0.66 ± 0.01	0.67 ± 0.01
Day 21	0.74 ± 0.08	0.99 ± 0.17	0.79 ± 0.09	0.84 ± 0.11	0.72 ± 0.10	0.62 ± 0.07
Week 13	0.56 ± 0.07	0.68 ± 0.06	0.69 ± 0.02	0.63 ± 0.07	0.73 ± 0.05	0.75 ± 0.03
Total protein (g/dL)						
Day 5	5.9 ± 0.1	5.7 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1
Day 21	6.6 ± 0.1	6.7 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.6 ± 0.1
Week 13	7.7 ± 0.5[f]	8.1 ± 0.2[f]	8.2 ± 0.2	8.2 ± 0.2	8.4 ± 0.1	8.7 ± 0.1**
Albumin (g/dL)						
Day 5	4.0 ± 0.1	3.9 ± 0.0	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.0
Day 21	4.1 ± 0.0	4.2 ± 0.1	4.1 ± 0.1	4.1 ± 0.0	4.1 ± 0.1	4.0 ± 0.0
Week 13	4.9 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.6 ± 0.2	4.9 ± 0.1	4.9 ± 0.1
Alanine aminotransferase (IU/L)						
Day 5	41 ± 2	37 ± 2	36 ± 1	36 ± 1	35 ± 1	46 ± 3
Day 21	43 ± 3	49 ± 3	49 ± 3	47 ± 3	47 ± 3	40 ± 2
Week 13	56 ± 7	46 ± 4	42 ± 4	42 ± 2	45 ± 2	83 ± 33
Alkaline phosphatase (IU/L)						
Day 5	458 ± 10	436 ± 12	427 ± 6*	400 ± 11**	389 ± 15**	383 ± 16**
Day 21	477 ± 11	518 ± 15	509 ± 13	494 ± 15	520 ± 14	490 ± 22
Week 13	249 ± 21	260 ± 15	218 ± 16	244 ± 12	246 ± 6	243 ± 19
Creatine kinase (IU/L)						
Day 5	286 ± 26	276 ± 18	350 ± 52	314 ± 38	291 ± 21	249 ± 21
Day 21	236 ± 23	210 ± 18[f]	244 ± 24	289 ± 24	247 ± 27	238 ± 34
Week 13	172 ± 34	150 ± 22	169 ± 18	111 ± 13	191 ± 53	193 ± 39

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	9	10
Sorbitol dehydrogenase (IU/L)						
Day 5	7 ± 0	7 ± 0	7 ± 0	8 ± 0	8 ± 0	8 ± 1
Day 21	9 ± 0[d]	11 ± 1	9 ± 1[b]	10 ± 1[d]	9 ± 1[b]	8 ± 1[f]
Week 13	16 ± 5[f]	10 ± 1	13 ± 2[f]	11 ± 1[f]	13 ± 1	14 ± 2[f]
Bile acids (μmol/L)						
Day 5	15.2 ± 6.5	8.9 ± 1.5	10.2 ± 1.9	13.0 ± 2.3	21.2 ± 2.5**	16.8 ± 2.3*
Day 21	48.2 ± 14.8	35.8 ± 10.5	49.8 ± 13.7[f]	50.2 ± 12.0	24.8 ± 8.2	25.7 ± 9.2
Week 13	25.3 ± 6.4[d]	30.3 ± 10.0[f]	23.4 ± 3.9[d]	22.8 ± 3.5	17.8 ± 4.0	24.0 ± 5.9[f]
Female						
Hematology						
n						
Day 5	10	10	10	10	10	10
Day 21	9	10	10	10	10	10
Week 13	9	7	10	10	10	8
Hematocrit (%)						
Day 5	40.8 ± 0.8	40.2 ± 0.5	42.1 ± 0.5	42.3 ± 0.4	40.9 ± 0.5	40.9 ± 0.5
Day 21	42.5 ± 0.4	42.5 ± 0.6	42.0 ± 0.4	42.8 ± 0.3	42.3 ± 0.5[f]	42.1 ± 0.4
Week 13	49.4 ± 1.4	47.8 ± 0.8	48.4 ± 0.6	48.0 ± 0.4	47.7 ± 0.6	47.1 ± 1.2
Hemoglobin (g/dL)						
Day 5	15.6 ± 0.3	15.1 ± 0.2	15.5 ± 0.1	15.7 ± 0.2	15.3 ± 0.2	15.0 ± 0.2
Day 21	15.6 ± 0.1	15.6 ± 0.2	15.4 ± 0.1	15.7 ± 0.1	15.5 ± 0.1	15.2 ± 0.1
Week 13	16.3 ± 0.4	15.7 ± 0.2	16.0 ± 0.1	15.8 ± 0.1	15.7 ± 0.1	15.4 ± 0.3
Erythrocytes (10^6/μL)						
Day 5	7.10 ± 0.14	6.93 ± 0.10	7.19 ± 0.10	7.52 ± 0.27	7.07 ± 0.11	7.07 ± 0.13
Day 21	7.66 ± 0.08	7.60 ± 0.12	7.50 ± 0.08	7.66 ± 0.09	7.54 ± 0.10	7.48 ± 0.07
Week 13	8.62 ± 0.23	8.27 ± 0.13	8.44 ± 0.09	8.38 ± 0.05	8.27 ± 0.10	8.11 ± 0.21
Reticulocytes (10^6/μL)						
Day 5	0.20 ± 0.03[g]	0.19 ± 0.02[e]	0.23 ± 0.02[e]	0.23 ± 0.03[g]	0.25 ± 0.04[g]	0.23 ± 0.02[e]
Day 21	0.12 ± 0.01[c]	0.12 ± 0.01[h]	0.14 ± 0.02[c]	0.12 ± 0.00[b]	0.12 ± 0.01[b]	0.12 ± 0.01[c]
Week 13	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.00	0.10 ± 0.01	0.10 ± 0.01
Mean cell volume (fL)						
Day 5	57.3 ± 1.0	58.0 ± 0.5	58.5 ± 0.6	58.7 ± 1.0	57.9 ± 0.5	57.9 ± 0.6
Day 21	55.6 ± 0.5	56.0 ± 0.3	56.0 ± 0.3	56.0 ± 0.5	55.7 ± 0.3	56.3 ± 0.5
Week 13	57.3 ± 0.1	57.8 ± 0.1	57.4 ± 0.2	57.3 ± 0.2	57.7 ± 0.1	58.1 ± 0.1**
Mean cell hemoglobin (pg)						
Day 5	22.0 ± 0.3	21.8 ± 0.1	21.6 ± 0.2	21.8 ± 0.3	21.6 ± 0.2	21.2 ± 0.2
Day 21	20.3 ± 0.2	20.5 ± 0.1	20.6 ± 0.2	20.5 ± 0.2	20.5 ± 0.1	20.3 ± 0.2
Week 13	18.9 ± 0.1	19.1 ± 0.1	19.0 ± 0.1	18.9 ± 0.1	19.0 ± 0.1	19.1 ± 0.2

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 5	10	10	10	10	10	10
Day 21	9	10	10	10	10	10
Week 13	9	7	10	10	10	8
Mean cell hemoglobin concentration (g/dL)						
Day 5	38.3 ± 0.5	37.6 ± 0.3	36.9 ± 0.3*	37.2 ± 0.4	37.4 ± 0.3	36.7 ± 0.2**
Day 21	36.6 ± 0.2	36.8 ± 0.2	36.8 ± 0.2	36.7 ± 0.2	36.8 ± 0.2	36.1 ± 0.2
Week 13	32.9 ± 0.2	32.9 ± 0.2	33.1 ± 0.2	33.0 ± 0.3	32.9 ± 0.2	32.8 ± 0.3
Platelets ($10^3/\mu L$)						
Day 5	1,056.9 ± 35.1	1,102.1 ± 35.2	1,044.6 ± 30.3	1,109.3 ± 26.7	1,002.3 ± 20.1[f]	990.4 ± 14.7
Day 21	769.2 ± 17.2	826.2 ± 15.1	806.4 ± 17.5	776.3 ± 18.1	756.8 ± 21.8[f]	757.9 ± 21.1
Week 13	693.6 ± 18.6	695.4 ± 13.7	682.7 ± 9.6	651.1 ± 28.1	685.2 ± 14.0	674.3 ± 36.7
Leukocytes ($10^3/\mu L$)						
Day 5	6.41 ± 0.31[f]	6.12 ± 0.25	6.84 ± 0.32	6.22 ± 0.28	5.50 ± 0.17*	4.90 ± 0.31*
Day 21	6.63 ± 0.34	6.41 ± 0.27	6.62 ± 0.28	7.11 ± 0.43	6.78 ± 0.39	6.95 ± 0.34
Week 13	5.27 ± 0.36	5.27 ± 0.55	6.74 ± 0.33*	5.72 ± 0.37[f]	6.02 ± 0.29	4.60 ± 0.35
Segmented neutrophils ($10^3/\mu L$)						
Day 5	0.88 ± 0.11[f]	0.75 ± 0.08	0.82 ± 0.08	0.78 ± 0.07	0.65 ± 0.07	0.73 ± 0.10
Day 21	0.94 ± 0.10	0.87 ± 0.08	0.93 ± 0.14	1.25 ± 0.15	0.95 ± 0.14	1.13 ± 0.10
Week 13	0.79 ± 0.08	0.80 ± 0.10	0.98 ± 0.10	0.99 ± 0.11[f]	1.33 ± 0.10**	1.04 ± 0.12*
Lymphocytes ($10^3/\mu L$)						
Day 5	5.43 ± 0.25[f]	5.31 ± 0.30	5.93 ± 0.33	5.33 ± 0.27	4.79 ± 0.21	4.07 ± 0.25*
Day 21	5.58 ± 0.31	5.46 ± 0.27	5.59 ± 0.28	5.73 ± 0.35	5.73 ± 0.32	5.68 ± 0.31
Week 13	4.30 ± 0.32	4.26 ± 0.48	5.55 ± 0.30	4.53 ± 0.31[f]	4.50 ± 0.26	3.41 ± 0.29
Monocytes ($10^3/\mu L$)						
Day 5	0.04 ± 0.02[f]	0.07 ± 0.02	0.07 ± 0.04	0.08 ± 0.01	0.08 ± 0.02	0.06 ± 0.02
Day 21	0.11 ± 0.04	0.07 ± 0.02	0.10 ± 0.02	0.10 ± 0.03	0.08 ± 0.03	0.14 ± 0.03
Week 13	0.16 ± 0.03	0.19 ± 0.03	0.20 ± 0.03	0.18 ± 0.05[f]	0.17 ± 0.04	0.13 ± 0.03
Eosinophils ($10^3/\mu L$)						
Day 5	0.04 ± 0.02[f]	0.02 ± 0.01	0.03 ± 0.02	0.07 ± 0.02	0.01 ± 0.01	0.00 ± 0.00
Day 21	0.04 ± 0.02	0.06 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.07 ± 0.02	0.02 ± 0.01
Week 13	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02[f]	0.04 ± 0.02	0.01 ± 0.01
Clinical Chemistry						
n						
Day 5	10	10	10	10	10	10
Day 21	9	10	10	10	10	10
Week 13	10	10	10	10	10	8
Urea nitrogen (mg/dL)						
Day 5	13.9 ± 0.6	17.0 ± 0.5*	15.0 ± 0.5	14.6 ± 0.7	14.3 ± 0.7	17.1 ± 0.7*
Day 21	15.9 ± 0.7	16.5 ± 0.8	14.9 ± 0.7	16.4 ± 0.8	15.1 ± 0.7	17.2 ± 1.3
Week 13	17.6 ± 0.7	17.3 ± 1.1	17.6 ± 0.9	18.9 ± 1.3	18.6 ± 0.9	20.0 ± 1.2
Creatinine (mg/dL)						
Day 5	0.71 ± 0.07	0.62 ± 0.04	0.62 ± 0.02	0.65 ± 0.02	0.62 ± 0.01	0.68 ± 0.02
Day 21	0.62 ± 0.04	0.61 ± 0.03	0.58 ± 0.04	0.57 ± 0.03	0.59 ± 0.02	0.60 ± 0.02
Week 13	0.68 ± 0.05	0.76 ± 0.02	0.62 ± 0.04	0.66 ± 0.04	0.65 ± 0.04	0.59 ± 0.06[f]

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 5	10	10	10	10	10	10
Day 21	9	10	10	10	10	10
Week 13	10	10	10	10	10	8
Total protein (g/dL)						
Day 5	6.5 ± 0.2	6.4 ± 0.2	6.5 ± 0.2	6.7 ± 0.1	6.5 ± 0.1	6.5 ± 0.1[f]
Day 21	6.4 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.0 ± 0.2
Week 13	7.3 ± 0.2	7.4 ± 0.2	7.2 ± 0.3	7.5 ± 0.1	7.4 ± 0.2	7.7 ± 0.1
Albumin (g/dL)						
Day 5	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
Day 21	4.3 ± 0.1	4.3 ± 0.1	4.1 ± 0.0*	4.2 ± 0.0	4.2 ± 0.1	4.0 ± 0.1*
Week 13	4.8 ± 0.1	4.9 ± 0.1	4.8 ± 0.0	4.9 ± 0.0	4.8 ± 0.1	4.9 ± 0.1
Alanine aminotransferase (IU/L)						
Day 5	38 ± 2	38 ± 4[f]	40 ± 3	47 ± 2	43 ± 3	41 ± 2[f]
Day 21	32 ± 2	36 ± 1	35 ± 1	36 ± 2	38 ± 1*	43 ± 2*
Week 13	40 ± 4	40 ± 4	35 ± 2	33 ± 2	34 ± 2	39 ± 3
Alkaline phosphatase (IU/L)						
Day 5	384 ± 14	395 ± 20	387 ± 11	390 ± 16	386 ± 12	336 ± 8
Day 21	410 ± 14	399 ± 12	413 ± 7	387 ± 8	458 ± 23	462 ± 22
Week 13	221 ± 20	219 ± 15	205 ± 13	213 ± 14	227 ± 17	262 ± 13
Creatine kinase (IU/L)						
Day 5	344 ± 64	359 ± 57	271 ± 22	342 ± 49	254 ± 22	312 ± 56
Day 21	160 ± 38	203 ± 29	166 ± 36	143 ± 20	207 ± 44	215 ± 29
Week 13	145 ± 26	153 ± 30	100 ± 15	171 ± 55	117 ± 31	149 ± 32
Sorbitol dehydrogenase (IU/L)						
Day 5	6 ± 1	8 ± 0	5 ± 0	6 ± 1	6 ± 0	6 ± 0
Day 21	8 ± 1	9 ± 1	9 ± 2[f]	10 ± 2	13 ± 2	9 ± 1
Week 13	8 ± 1	9 ± 2	8 ± 1	9 ± 1	7 ± 1	9 ± 1
Bile acids (μmol/L)						
Day 5	16.5 ± 5.4	22.1 ± 8.5	14.7 ± 1.5	17.1 ± 3.0[f]	29.0 ± 4.5*	25.1 ± 7.5
Day 21	26.2 ± 6.0	20.7 ± 8.1	18.9 ± 2.1	31.9 ± 10.3	25.2 ± 6.6	28.2 ± 7.5
Week 13	37.6 ± 11.5	31.0 ± 5.5	23.6 ± 5.7	18.4 ± 3.9	29.4 ± 3.9	21.0 ± 3.7[f]

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=7
[c] n=3
[d] n=8
[e] n=6
[f] n=9
[g] n=5
[h] n=4

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

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil C-2

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose C-4

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil C-5

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose C-7

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	362 ± 6	373 ± 6	361 ± 5	361 ± 5	364 ± 11	351 ± 6
Brain						
Absolute	1.941 ± 0.013	1.999 ± 0.021	2.013 ± 0.011	1.877 ± 0.110	1.985 ± 0.018	1.960 ± 0.014
Relative	5.37 ± 0.08	5.37 ± 0.09	5.59 ± 0.07	5.20 ± 0.30	5.49 ± 0.16	5.60 ± 0.10
Heart						
Absolute	1.117 ± 0.033	1.109 ± 0.020	1.084 ± 0.013	1.113 ± 0.021	1.103 ± 0.027	1.122 ± 0.020
Relative	3.08 ± 0.07	2.98 ± 0.06	3.01 ± 0.05	3.08 ± 0.03	3.04 ± 0.04	3.20 ± 0.06
R. Kidney						
Absolute	1.174 ± 0.030	1.242 ± 0.016	1.275 ± 0.023*	1.286 ± 0.026**	1.336 ± 0.044**	1.391 ± 0.028**
Relative	3.24 ± 0.05	3.33 ± 0.05	3.54 ± 0.03**	3.56 ± 0.05**	3.67 ± 0.04**	3.97 ± 0.07**
Liver						
Absolute	12.006 ± 0.308	12.966 ± 0.312	12.744 ± 0.404	14.246 ± 0.377**	16.661 ± 0.648**	17.750 ± 0.472**
Relative	33.15 ± 0.58	34.74 ± 0.54	35.28 ± 0.68*	39.37 ± 0.50**	45.67 ± 0.75**	50.56 ± 0.86**
Lung						
Absolute	1.837 ± 0.085	1.799 ± 0.068	2.045 ± 0.161	2.219 ± 0.124	2.059 ± 0.144	1.730 ± 0.113
Relative	5.08 ± 0.24	4.84 ± 0.23	5.68 ± 0.47	6.14 ± 0.32	5.69 ± 0.41	4.93 ± 0.31
R. Testis						
Absolute	1.479 ± 0.026	1.498 ± 0.020	1.528 ± 0.018	1.497 ± 0.022	1.554 ± 0.032	1.520 ± 0.023
Relative	4.09 ± 0.06	4.02 ± 0.03	4.24 ± 0.03	4.15 ± 0.07	4.28 ± 0.08*	4.34 ± 0.04**
Thymus						
Absolute	0.339 ± 0.018	0.366 ± 0.013	0.341 ± 0.017	0.341 ± 0.011	0.332 ± 0.018	0.306 ± 0.014
Relative	0.94 ± 0.06	0.98 ± 0.03	0.94 ± 0.04	0.94 ± 0.03	0.91 ± 0.04	0.87 ± 0.04

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1,600 mg/kg
Female						
n	10	10	10	10	10	9
Necropsy body wt	205 ± 3	203 ± 3	202 ± 5	212 ± 3	214 ± 4	222 ± 4**
Brain						
Absolute	1.857 ± 0.013	1.871 ± 0.008	1.842 ± 0.017	1.883 ± 0.021	1.886 ± 0.013	1.844 ± 0.012
Relative	9.09 ± 0.12	9.22 ± 0.10	9.16 ± 0.18	8.88 ± 0.07	8.82 ± 0.11	8.30 ± 0.10**
Heart						
Absolute	0.711 ± 0.015	0.728 ± 0.016	0.715 ± 0.019	0.790 ± 0.018*	0.771 ± 0.023*	0.827 ± 0.024**
Relative	3.48 ± 0.06	3.58 ± 0.08	3.55 ± 0.10	3.73 ± 0.09	3.60 ± 0.10	3.72 ± 0.07
R. Kidney						
Absolute	0.722 ± 0.014	0.739 ± 0.015	0.754 ± 0.019	0.790 ± 0.014**	0.886 ± 0.020**	0.871 ± 0.018**
Relative	3.53 ± 0.05	3.63 ± 0.06	3.74 ± 0.04*	3.73 ± 0.07*	4.14 ± 0.05**	3.92 ± 0.06**
Liver						
Absolute	6.023 ± 0.123	6.392 ± 0.115	6.728 ± 0.234*	7.951 ± 0.216**	9.163 ± 0.218**	10.829 ± 0.271**
Relative	29.45 ± 0.52	31.51 ± 0.76*	33.31 ± 0.66**	37.44 ± 0.71**	42.78 ± 0.61**	48.69 ± 0.93**
Lung						
Absolute	1.140 ± 0.044	1.181 ± 0.049	1.177 ± 0.031	1.194 ± 0.034	1.251 ± 0.036	1.164 ± 0.028
Relative	5.58 ± 0.23	5.80 ± 0.21	5.84 ± 0.16	5.63 ± 0.16	5.85 ± 0.17	5.25 ± 0.16
Thymus						
Absolute	0.254 ± 0.012	0.273 ± 0.014	0.270 ± 0.012	0.307 ± 0.010*	0.288 ± 0.012	0.291 ± 0.012
Relative	1.25 ± 0.07	1.34 ± 0.06	1.34 ± 0.04	1.45 ± 0.05	1.35 ± 0.06	1.31 ± 0.05

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

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male					
n	10	10	10	10	8
Necropsy body wt	363 ± 6	372 ± 8	385 ± 5*	383 ± 5	359 ± 7
R. Kidney					
Absolute	1.298 ± 0.020	1.343 ± 0.037	1.383 ± 0.016	1.362 ± 0.025	1.383 ± 0.036
Relative	3.58 ± 0.05	3.61 ± 0.06	3.59 ± 0.04	3.56 ± 0.06	3.85 ± 0.08**
Liver					
Absolute	15.064 ± 0.370	15.035 ± 0.528	16.431 ± 0.323*	16.778 ± 0.434**	17.318 ± 0.424**
Relative	41.46 ± 0.65	40.33 ± 0.96	42.66 ± 0.49	43.75 ± 0.68*	48.23 ± 0.65**
Female					
n	10	10	10	8	9
Necropsy body wt	207 ± 4	220 ± 2*	215 ± 2	218 ± 4	221 ± 4*
R. Kidney					
Absolute	0.768 ± 0.019	0.770 ± 0.021	0.788 ± 0.014	0.817 ± 0.013	0.844 ± 0.020**
Relative	3.71 ± 0.08	3.50 ± 0.09	3.67 ± 0.04	3.75 ± 0.04	3.82 ± 0.04
Liver					
Absolute	7.894 ± 0.250	7.741 ± 0.130	7.949 ± 0.153	8.741 ± 0.164**	10.028 ± 0.270**
Relative	38.13 ± 1.23	35.19 ± 0.54	37.00 ± 0.57	40.13 ± 0.52	45.43 ± 0.96**

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

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	1,200 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	27.3 ± 0.7	29.3 ± 0.6	30.0 ± 1.1*	29.9 ± 0.7*	27.8 ± 0.4	27.6 ± 0.5
Brain						
Absolute	0.448 ± 0.003	0.456 ± 0.004	0.454 ± 0.004	0.462 ± 0.005	0.448 ± 0.006	0.447 ± 0.004
Relative	16.50 ± 0.46	15.61 ± 0.29	15.29 ± 0.53	15.51 ± 0.39	16.14 ± 0.24	16.27 ± 0.31
Heart						
Absolute	0.132 ± 0.004	0.133 ± 0.004	0.131 ± 0.003	0.135 ± 0.004	0.126 ± 0.002	0.128 ± 0.004
Relative	4.84 ± 0.08	4.55 ± 0.10	4.40 ± 0.11**	4.52 ± 0.10	4.52 ± 0.09	4.64 ± 0.09
R. Kidney						
Absolute	0.249 ± 0.007	0.249 ± 0.006	0.264 ± 0.010	0.265 ± 0.005	0.256 ± 0.005	0.245 ± 0.005
Relative	9.15 ± 0.21	8.50 ± 0.11*	8.77 ± 0.12	8.87 ± 0.14	9.21 ± 0.15	8.91 ± 0.19
Liver						
Absolute	1.056 ± 0.035	1.133 ± 0.043	1.223 ± 0.037**	1.309 ± 0.039**	1.374 ± 0.024**	1.558 ± 0.049**
Relative	38.64 ± 0.65	38.66 ± 1.06	40.91 ± 1.08	43.75 ± 0.84**	49.46 ± 0.91**	56.49 ± 1.27**
Lung						
Absolute	0.191 ± 0.008	0.183 ± 0.007	0.223 ± 0.013	0.198 ± 0.010	0.176 ± 0.004	0.206 ± 0.017
Relative	7.00 ± 0.21	6.26 ± 0.19	7.51 ± 0.52	6.58 ± 0.22	6.32 ± 0.11	7.53 ± 0.69
R. Testis						
Absolute	0.121 ± 0.002	0.118 ± 0.002	0.122 ± 0.003	0.121 ± 0.002	0.115 ± 0.002	0.109 ± 0.003**
Relative	4.43 ± 0.08	4.05 ± 0.08*	4.07 ± 0.11*	4.05 ± 0.08*	4.15 ± 0.08	3.96 ± 0.11**
Thymus						
Absolute	0.033 ± 0.002	0.035 ± 0.002	0.032 ± 0.003	0.043 ± 0.003*	0.040 ± 0.003	0.039 ± 0.002
Relative	1.19 ± 0.09	1.18 ± 0.05	1.06 ± 0.09	1.42 ± 0.07	1.43 ± 0.11	1.42 ± 0.06

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	1,200 mg/kg
Female						
n	8	8	8	10	8	10
Necropsy body wt	24.6 ± 0.8	24.0 ± 0.5	24.7 ± 0.5	24.8 ± 0.5	24.7 ± 0.5	23.2 ± 0.6
Brain						
Absolute	0.469 ± 0.007	0.461 ± 0.003	0.470 ± 0.006	0.467 ± 0.005	0.469 ± 0.006	0.460 ± 0.004
Relative	19.19 ± 0.52	19.25 ± 0.41	19.11 ± 0.43	18.91 ± 0.30	19.01 ± 0.30	19.89 ± 0.40
Heart						
Absolute	0.121 ± 0.005	0.116 ± 0.004	0.122 ± 0.005	0.119 ± 0.003	0.120 ± 0.004	0.117 ± 0.005
Relative	4.92 ± 0.14	4.82 ± 0.15	4.93 ± 0.17	4.82 ± 0.14	4.87 ± 0.11	5.04 ± 0.19
R. Kidney						
Absolute	0.186 ± 0.008	0.178 ± 0.004	0.193 ± 0.005	0.191 ± 0.006	0.187 ± 0.010	0.183 ± 0.007
Relative	7.56 ± 0.20	7.45 ± 0.20	7.83 ± 0.13	7.70 ± 0.22	7.60 ± 0.42	7.88 ± 0.18
Liver						
Absolute	0.996 ± 0.035	1.025 ± 0.017	1.143 ± 0.028**	1.177 ± 0.038**	1.333 ± 0.032**	1.270 ± 0.036**
Relative	40.56 ± 0.71	42.79 ± 0.71	46.33 ± 0.66**	47.50 ± 1.01**	54.03 ± 0.97**	54.72 ± 0.72**
Lung						
Absolute	0.192 ± 0.016	0.190 ± 0.012	0.232 ± 0.022	0.213 ± 0.015	0.190 ± 0.007	0.197 ± 0.018
Relative	7.84 ± 0.59	7.93 ± 0.52	9.37 ± 0.81	8.70 ± 0.75	7.71 ± 0.27	8.48 ± 0.72
Thymus						
Absolute	0.049 ± 0.004	0.050 ± 0.003	0.049 ± 0.004	0.052 ± 0.003	0.048 ± 0.004	0.044 ± 0.003
Relative	1.97 ± 0.13	2.09 ± 0.11	2.01 ± 0.16	2.11 ± 0.08	1.95 ± 0.15	1.87 ± 0.11

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

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

	Vehicle Control	600 mg/kg	1,200 mg/kg	1,600 mg/kg
Male				
n	9	9	10	7
Necropsy body wt	36.1 ± 0.7	32.7 ± 0.5**	31.1 ± 0.5**	31.0 ± 0.4**
R. Kidney				
Absolute	0.307 ± 0.007	0.283 ± 0.008*	0.257 ± 0.005**	0.259 ± 0.004**
Relative	8.54 ± 0.23	8.66 ± 0.20	8.28 ± 0.12	8.34 ± 0.13
Liver				
Absolute	1.811 ± 0.027	1.723 ± 0.038	1.770 ± 0.033	1.887 ± 0.045
Relative	50.26 ± 0.64	52.64 ± 0.76*	57.06 ± 0.92**	60.77 ± 0.90**
Female				
n	9	10	8	9
Necropsy body wt	29.2 ± 0.8	25.8 ± 0.6**	25.8 ± 0.7**	26.5 ± 0.5*
R. Kidney				
Absolute	0.188 ± 0.005	0.163 ± 0.004**	0.173 ± 0.003*	0.173 ± 0.002*
Relative	6.46 ± 0.18	6.33 ± 0.16	6.72 ± 0.11	6.55 ± 0.10
Liver				
Absolute	1.362 ± 0.035	1.205 ± 0.036**	1.251 ± 0.040	1.461 ± 0.031
Relative	46.68 ± 0.93	46.62 ± 0.78	48.46 ± 0.94	55.14 ± 0.77**

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

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1 Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil D-2

TABLE D2 Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil D-2

TABLE D3 Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil D-3

TABLE D4 Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil D-3

TABLE D1
Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	100 mg/kg	400 mg/kg	1,600 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	362 ± 6	373 ± 6	361 ± 5	351 ± 6
R. Cauda epididymis	0.2000 ± 0.0083	0.2385 ± 0.0216	0.2058 ± 0.0065	0.2131 ± 0.0067
R. Epididymis	0.4528 ± 0.0140	0.4627 ± 0.0095	0.4588 ± 0.0093	0.4616 ± 0.0085
R. Testis	1.479 ± 0.026	1.498 ± 0.020	1.497 ± 0.022	1.520 ± 0.023
Epididymal spermatozoal measurements				
Motility (%)	68.95 ± 0.97	69.58 ± 1.45	70.22 ± 1.06	68.43 ± 0.91
Abnormal (%)	0.70 ± 0.08	0.72 ± 0.11	0.70 ± 0.12	0.92 ± 0.13
Concentration (10^6/g cauda epididymal tissue)	476 ± 24	394 ± 28	435 ± 28	421 ± 33

[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (necropsy body and tissue weights) or Dunn's test (epididymal spermatozoal measurements).

TABLE D2
Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	100 mg/kg	400 mg/kg	1,600 mg/kg
n	10	10	10	9
Necropsy body wt (g)	205 ± 3	203 ± 3	212 ± 3	222 ± 4**
Estrous cycle length (days)	4.78 ± 0.22[b]	5.00 ± 0.15	4.67 ± 0.29[b]	5.00 ± 0.22[c]
Estrous stages (% of cycle)				
Diestrus	41.4	34.3	34.3	33.3
Proestrus	18.6	15.7	20.0	12.7
Estrus	21.4	27.1	25.7	23.8
Metestrus	18.6	22.9	20.0	25.4
Uncertain diagnosis	0.0	0.0	0.0	4.8

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, dosed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in one of ten animals.
[c] Estrous cycle was longer than 12 days or unclear in two of nine animals.

TABLE D3
Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	75 mg/kg	300 mg/kg	1,200 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	27.3 ± 0.7	29.3 ± 0.6	29.9 ± 0.7*	27.6 ± 0.5
R. Cauda epididymis	0.0206 ± 0.0010	0.0242 ± 0.0014	0.0243 ± 0.0014	0.0210 ± 0.0005
R. Epididymis	0.0491 ± 0.0016	0.0533 ± 0.0024	0.0517 ± 0.0022	0.0461 ± 0.0009[b]
R. Testis	0.121 ± 0.002	0.118 ± 0.002	0.121 ± 0.002	0.109 ± 0.003**
Epididymal spermatozoal measurements				
Motility (%)	76.29 ± 1.49[b]	67.86 ± 3.31	75.03 ± 1.06	68.07 ± 2.42*
Abnormal (%)	1.70 ± 0.13	1.48 ± 0.17	1.38 ± 0.17	1.92 ± 0.20
Concentration (10^6/g cauda epididymal tissue)	707 ± 56	650 ± 55	610 ± 33	779 ± 50

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test (necropsy body weight) or Dunn's test (epididymal spermatozoal motility)
** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (right caudal and epididymal weights) or Dunn's test (abnormal sperm and epididymal spermatozoal concentration).
[b] n=9

TABLE D4
Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

	Vehicle Control	75 mg/kg	300 mg/kg	1,200 mg/kg
n	8	8	10	10
Necropsy body wt (g)	24.6 ± 0.8	24.0 ± 0.5	24.8 ± 0.5	23.2 ± 0.6
Estrous cycle length (days)	4.38 ± 0.18	4.25 ± 0.16	4.60 ± 0.22	4.60 ± 0.22
Estrous stages (% of cycle)				
Diestrus	23.2	21.4	24.3	25.7
Proestrus	25.0	17.9	25.7	27.1
Estrus	30.4	33.9	31.4	28.6
Metestrus	21.4	25.0	18.6	18.6
Uncertain diagnosis	0.0	1.8	0.0	0.0

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (necropsy body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.

APPENDIX E
PLASMA CARISOPRODOL CONCENTRATIONS

TABLE E1 Plasma Carisoprodol Concentrations for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil E-2
TABLE E2 Plasma Carisoprodol Concentrations for Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose E-3
TABLE E3 Plasma Carisoprodol Concentrations for Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil E-4
TABLE E4 Plasma Carisoprodol Concentrations for Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose E-5

TABLE E1
Plasma Carisoprodol Concentrations for Rats in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

Time	Vehicle Control	100 mg/kg	400 mg/kg	1,600 mg/kg
Special Study Groups				
n	3	3		3
Male				
10 minutes		1.32 ± 0.48		0.60 ± 0.23
20 minutes		2.70 ± 0.30		3.37 ± 0.59
40 minutes		1.10 ± 0.12		9.10 ± 1.07
60 minutes	0.32 ± 0.06	0.90 ± 0.38		4.90 ± 1.33*
120 minutes		0.33 ± 0.07		6.30 ± 1.45
180 minutes	0.30 ± 0.58	0.30 ± 0.05		5.57 ± 2.45
240 minutes		0.28 ± 0.06		7.93 ± 3.79
360 minutes	0.35 ± 0.03	0.25 ± 0.03		2.15 ± 1.30
Female				
10 minutes		6.58 ± 1.15		13.07 ± 3.66
20 minutes		2.32 ± 1.19		16.97 ± 2.13
40 minutes		5.80 ± 2.41		23.33 ± 2.88
60 minutes	0.67 ± 0.10	0.78 ± 0.07		30.73 ± 2.10*
120 minutes		0.43 ± 0.12		49.13 ± 1.51
180 minutes	0.60 ± 0.13	0.70 ± 0.00		37.47 ± 6.04
240 minutes		0.50 ± 0.19		15.22 ± 6.75
360 minutes	0.57 ± 0.19	0.57 ± 0.06		24.90 ± 11.20
Core Study Groups				
n	5	5	5	5
Male				
13 weeks	—[b]	—	2.24 ± 1.14[c]	5.23 ± 1.90
Female				
13 weeks	—	1.10[d]	19.40 ± 7.38	29.44 ± 6.02

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
[a] Data are given as mean ± standard error (μg/mL). Plasma was collected after a single dose for special study groups and 1 hour after the final dose for core study groups.
[b] All values were below the limit of detection (0.6 μg/mL).
[c] Mean for three rats; values for two rats were below the limit of detection.
[d] Value for one rat; values for four rats were below the limit of detection.

TABLE E2
Plasma Carisoprodol Concentrations for Rats in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

Time	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male				
n	5	5	5	4
30 minutes	1.90 ± 0.48	9.51 ± 2.27	10.87 ± 1.47	16.17 ± 3.02
60 minutes	1.12 ± 0.26	4.84 ± 1.04	12.15 ± 1.89	10.01 ± 2.07
Female				
n	5	5	4	5
30 minutes	9.02 ± 2.02	21.21 ± 3.03	10.72 ± 1.05	23.25 ± 3.66
60 minutes	4.00 ± 0.47	10.65 ± 1.83	17.78 ± 8.93	8.53 ± 3.62[b]

[a] Data are given as mean ± standard error (μg/mL).
[b] n=4

TABLE E3
Plasma Carisoprodol Concentrations for Mice in the 13-Week Gavage Study of Carisoprodol in Corn Oil[a]

Time	Vehicle Control	75 mg/kg	300 mg/kg	1,200 mg/kg
Special Study Groups				
n	3	3		3
Male				
10 minutes		4.50 ± 0.55		23.37 ± 0.68
20 minutes		6.20 ± 0.78		13.13 ± 3.93
40 minutes		1.85 ± 0.65[b]		54.87 ± 6.00
60 minutes	1.67 ± 0.41	2.90 ± 0.21*		43.95 ± 22.85*[b]
120 minutes		1.20 ± 0.00[b]		52.87 ± 15.79
180 minutes	2.55 ± 0.05[b]	2.17 ± 0.19		30.53 ± 18.17
240 minutes		1.13 ± 0.12		33.50 ± 11.99
360 minutes	1.93 ± 0.19	8.73 ± 7.43		17.37 ± 8.23
Female				
10 minutes		1.83 ± 0.78		36.60 ± 4.86
20 minutes		1.37 ± 0.26		49.03 ± 10.16
40 minutes		1.57 ± 0.18		74.20 ± 9.90
60 minutes	0.97 ± 0.03	2.03 ± 0.07*		49.43 ± 2.90**
120 minutes		1.90 ± 0.06		93.03 ± 18.55
180 minutes	0.73 ± 0.12	1.80 ± 0.00*		46.93 ± 15.31**
240 minutes		1.90 ± 0.00		70.87 ± 24.65
360 minutes	1.77 ± 0.47	1.83 ± 0.03		32.73 ± 3.09
Core Study Groups				
n	5	5	5	5
Male				
13 weeks	—[c]	—	1.50[d]	15.70[d]
Female				
13 weeks	—	—	—	8.10 ± 5.06[e]

* Significantly different (P≤0.05) from the control group by Shirley's test
** P≤0.01
[a] Data are given as mean ± standard error (μg/mL). Plasma was collected after a single dose for special study groups and 1 hour after the final dose for core study groups.
[b] n=2; one animal had an insufficient volume of plasma for analysis.
[c] All values were below the limit of detection (1.2 μg/mL).
[d] Value for one mouse; values for four mice were below the limit of detection.
[e] Mean for four mice; value for one mouse was below the limit of detection.

TABLE E4
Plasma Carisoprodol Concentrations for Mice in the 13-Week Gavage Study of Carisoprodol in 0.5% Methylcellulose[a]

Time	600 mg/kg	1,200 mg/kg	1,600 mg/kg
Male			
n	5	5	4
30 minutes	27.10 ± 5.80	36.58 ± 9.46	14.86 ± 3.49
60 minutes	18.51 ± 2.21[b]	18.26 ± 2.73	13.01 ± 4.16[c]
Female			
n	5	4	5
30 minutes	8.50 ± 2.77	19.11 ± 2.85	21.84 ± 5.75
60 minutes	1.02 ± 0.28	6.02 ± 1.38[c]	9.51 ± 2.21[b]

[a] Data are given as mean ± standard error (μg/mL).
[b] n=4
[c] n=3

APPENDIX F
GENETIC TOXICOLOGY

TABLE F1 Mutagenicity of Carisoprodol in *Salmonella typhimurium* F-2
TABLE F2 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Carisoprodol F-4
TABLE F3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Carisoprodol F-10
TABLE F4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Carisoprodol F-12
TABLE F5 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Carisoprodol by Gavage for 13 Weeks F-14

TABLE F1
Mutagenicity of Carisoprodol in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	105 ± 2.6	104 ± 6.4	143 ± 5.8	163 ± 7.7	107 ± 13.9	171 ± 4.2
	100	99 ± 4.5	99 ± 2.4	140 ± 5.0	167 ± 7.0	89 ± 11.8	161 ± 9.1
	333	141 ± 3.4	103 ± 6.2	156 ± 2.6	195 ± 4.3	196 ± 10.4	168 ± 7.8
	1,000	96 ± 5.5	109 ± 14.2	125 ± 5.0	187 ± 4.0	137 ± 11.3	180 ± 4.9
	3,333	20 ± 6.0	77 ± 12.8	145 ± 2.3	167 ± 14.7	117 ± 10.8	136 ± 10.6
	10,000	91 ± 5.8[c]	92 ± 6.6[c]	145 ± 10.4[c]	174 ± 6.0[c]	125 ± 13.9[c]	156 ± 3.8[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		1,529 ± 53.2	808 ± 92.5	1,316 ± 62.6	2,434 ± 94.1	676 ± 23.9	983 ± 19.5
TA1535	0	16 ± 2.7	9 ± 2.4	17 ± 2.0	8 ± 1.5	12 ± 1.2	10 ± 2.5
	100	11 ± 0.9	5 ± 2.3	14 ± 0.9	9 ± 1.2	17 ± 2.1	9 ± 1.9
	333	16 ± 1.5	6 ± 1.7	12 ± 1.2	9 ± 1.2	15 ± 1.5	11 ± 1.8
	1,000	12 ± 0.9	8 ± 1.5	18 ± 2.2	9 ± 3.6	14 ± 1.2	12 ± 2.3
	3,333	2 ± 1.2	8 ± 1.0	14 ± 2.3	12 ± 1.2	17 ± 0.6	10 ± 2.6
	10,000	Toxic	8 ± 0.9[c]	17 ± 1.2[c]	7 ± 1.5[c]	13 ± 1.5[c]	7 ± 1.2[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		897 ± 38.7	242 ± 36.5	250 ± 14.1	105 ± 3.8	136 ± 26.8	145 ± 29.2

Strain	Dose (μg/plate)	-S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2
TA1537	0	11 ± 1.3	12 ± 2.0	9 ± 1.3	6 ± 0.0	9 ± 2.3
	10			7 ± 1.9		
	33		8 ± 1.5	8 ± 0.7		
	100	9 ± 2.3	10 ± 1.2	8 ± 1.2	10 ± 0.9	11 ± 0.6
	333	9 ± 2.7	8 ± 0.9	8 ± 1.5	7 ± 0.6	10 ± 1.9
	1,000	Toxic	8 ± 1.2	9 ± 0.6	8 ± 0.3	15 ± 1.8
	3,333	Toxic	Toxic		9 ± 1.2	11 ± 0.6
	10,000	Toxic			6 ± 0.9[c]	6 ± 0.9[c]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		308 ± 69.8	105 ± 19.1	570 ± 7.6	190 ± 7.9	33 ± 3.3

Strain	Dose (μg/plate)	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA1537	0	18 ± 2.3	10 ± 1.7
	100	21 ± 1.3	12 ± 2.3
	333	24 ± 3.5	10 ± 0.0
	1,000	21 ± 2.0	9 ± 1.0
	3,333	16 ± 2.9	8 ± 1.9
	10,000	12 ± 0.9[c]	5 ± 0.9[c]
Trial summary		Negative	Negative
Positive control		96 ± 5.4	62 ± 8.3

TABLE F1
Mutagenicity of Carisoprodol in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0	28 ± 1.5	12 ± 3.7	38 ± 5.4	13 ± 1.0	42 ± 1.2	18 ± 2.7
	100	26 ± 0.9	20 ± 2.6	41 ± 3.4	12 ± 1.0	40 ± 3.8	16 ± 2.4
	333	29 ± 3.2	18 ± 1.2	35 ± 4.2	14 ± 3.2	46 ± 8.2	19 ± 2.0
	1,000	30 ± 1.8	16 ± 4.3	44 ± 2.3	12 ± 0.7	46 ± 2.1	20 ± 1.2
	3,333	5 ± 2.9	13 ± 1.5	39 ± 1.7	16 ± 0.3	40 ± 1.2	17 ± 0.6
	10,000	11 ± 2.3[c]	15 ± 1.5[c]	19 ± 5.1[c]	12 ± 1.5[c]	32 ± 3.0[c]	18 ± 2.7[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		198 ± 14.2	164 ± 15.6	874 ± 65.4	1,185 ± 19.5	248 ± 17.4	318 ± 20.4

[a] Studies were performed at Case Western Reserve University. The detailed protocol and these data are presented by Zeiger *et al*. (1987). 0 μg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Precipitate on plate
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE F2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Carisoprodol[a]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
-S9						
Trial 1						
Ethanol[c]						
		84	77	92	36	
		100	105	102	34	
		89	113	99	37	
		112	105	89	26	34
Methyl methanesulfonate[d]						
	5	77	70	496	216	
		94	69	506	179	198*
Carisoprodol						
	250	107	77	105	33	
		90	70	73	27	30
	375	87	64	85	33	
		90	92	86	32	
		84	91	74	29	31
	500	80	76	64	27	
		80	48	83	34	
		99	74	80	27	29
	750	105	54	131	41	
		80	42	117	49	
		104	53	108	35	42
	1,000	Lethal				
		Lethal				
		Lethal				

TABLE F2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Carisoprodol

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 2						
Ethanol						
		88	104	107	41	
		74	96	94	43	
		97	100	125	43	42
Methyl methanesulfonate						
	5	42	33	646	519	
		28	25	589	697	608*
Carisoprodol						
	300	78	87	95	40	
		76	64	120	53	
		62	79	83	45	46
	400	84	54	163	65	
		72	37	158	73	
		64	67	108	57	65*
	500	62	17	222	119	
		70	12	211	100	
		67	50	151	75	98*
	600	Lethal				
		Lethal				

TABLE F2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Carisoprodol

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 3						
Ethanol						
		117	113	101	29	
		82	92	80	33	
		90	95	84	31	31
Methyl methanesulfonate						
	5	71	69	511	240	
		82	49	573	234	
		71	49	617	290	255*
Carisoprodol						
	300	85	83	87	34	
		97	79	66	23	
		91	91	58	21	26
	400	96	55	81	28	
		68	75	59	29	
		72	74	136	63	40
	500	93	44	140	50	
		100	52	164	54	
		92	70	128	47	50*
	600	84	17	225	89	
		75	34	113	50	
		105	39	152	48	63*
	700	99	78	108	36	
		82	67	101	41	
		86	81	98	38	38
	800	Lethal				
		Lethal				

TABLE F2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Carisoprodol

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 4						
Ethanol						
		107	80	98	31	
		104	109	96	31	
		115	111	81	23	28
Methyl methanesulfonate						
	5	73	51	725	333	
		83	45	608	243	
		92	39	751	271	282*
Carisoprodol						
	500	67	21	123	62	
		109	31	154	47	
		77	17	96	42	50*
	550	105	21	142	45	
		103	18	145	47	
		86	32	91	35	42
	600	80	15	156	65	
		70	15	143	68	
		88	27	116	44	59*
	650	75	11	158	70	
		Lethal				
	700	86	13	164	63	
		91	24	167	61	62*
		Lethal				
	750	63	4	224	119	
		Lethal				
		Lethal				
	800	Lethal				
		Lethal				
		Lethal				

TABLE F2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Carisoprodol

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 1						
Ethanol						
		114	115	143	42	
		72	75	204	95	
		109	117	189	58	
		102	93	168	55	62
Methyl cholanthrene[d]						
	2.5	70	21	628	301	
		56	21	637	383	
		67	23	705	352	345*
Carisoprodol						
	200	89	92	99	37	
		89	68	143	54	
		106	82	159	50	47
	400	98	76	148	50	
		112	96	123	37	
		83	84	76	30	39
	500	100	77	107	36	
		106	66	94	29	
		111	86	127	38	34
	600	119	109	131	37	
		96	54	150	52	
		97	79	136	47	45
	700	105	31	102	32	
		83	14	154	62	
		88	45	139	53	49
	800	86	7	184	72	
		116	19	170	49	60
		Lethal				
	1,000	Lethal				
		Lethal				
		Lethal				

TABLE F2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Carisoprodol

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Ethanol						
		69	89	116	56	
		112	115	150	45	
		93	96	129	46	49
Methyl cholanthrene						
	2.5	51	24	683	448	
		45	16	576	427	437*
Carisoprodol						
	300	68	80	101	49	
	400	67	70	145	72	
		57	60	121	71	72
	500	85	71	114	45	
		94	83	175	62	53
	600	105	47	75	24	
		82	72	82	33	29
	700	78	32	96	41	
		69	7	127	62	51
	800	Lethal				
		Lethal				

* Positive response ($P \leq 0.05$) versus the solvent control
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Myhr *et al*. (1985).
[b] Mutant fraction (MF) (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at $MF/10^6$ cells treated).
[c] Solvent control
[d] Positive control

TABLE F3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Carisoprodol[a]

Compound	Concentration (μg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Summary: Equivocal								
Dimethylsulfoxide[c]		50	1,040	310	0.29	6.2	26.0	
Mitomycin-C[d]	0.003	50	1,049	854	0.81	17.1	26.0	173.12
	0.005	50	1,050	1,131	1.07	22.6	26.0	261.37
Carisoprodol	5	50	1,048	415	0.39	8.3	26.0	32.85*
	16	50	1,046	404	0.38	8.1	26.0	29.58*
	50	50	1,050	335	0.31	6.7	26.0	7.04
	160	50	1,047	281	0.26	5.6	26.0	-9.96
	500	0						
					P=0.992[e]			
+S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,052	459	0.43	9.2	27.0	
Cyclophosphamide[d]	1.5	50	1,048	1,159	1.10	23.2	27.0	153.47
	1.5	50	1,050	1,148	1.09	23.0	27.0	150.59
Carisoprodol	5	50	1,038	397	0.38	7.9	27.0	-12.34
	16	50	1,047	361	0.34	7.2	27.0	-20.98
	50	50	1,045	393	0.37	7.9	27.0	-13.81
	160	50	1,041	365	0.35	7.3	27.0	-19.64
	500	50	1,046	393	0.37	7.9	27.0	-13.89
	1,600	0						
					P=0.990			

TABLE F3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Carisoprodol

Compound	Concentration (μg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome (%)
+S9 (continued)								
Trial 2								
Summary: Weakly positive								
Dimethylsulfoxide		50	1,038	324	0.31	6.5	27.0	
Cyclophosphamide	1.5	50	1,044	1,445	1.38	28.9	27.0	343.43
Carisoprodol	500	50	1,039	397	0.38	7.9	27.0	22.41*
	750	50	1,043	352	0.33	7.0	27.0	8.12
	1,000	50	1,038	378	0.36	7.6	27.0	16.67
	1,250	50	1,042	422	0.40	8.4	27.0	29.75*
	1,500	0						
					P=0.004			

* Positive response (≥20% increase over solvent control)
[a] Study was performed at Environmental Health Research and Testing, Inc. The protocol is presented by Galloway *et al.* (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine.
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Solvent control
[d] Positive control
[e] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose

TABLE F4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Carisoprodol[a]

Compound	Concentration (μg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
-S9					
Trial 1					
Harvest time: 12 hours					
Summary: Equivocal					
Dimethylsulfoxide[b]		100	0	0.00	0.0
Mitomycin-C[c]	0.5	100	50	0.50	37.0
Carisoprodol	5	100	4	0.04	4.0
	16	100	1	0.01	1.0
	50	100	1	0.01	1.0
	160	100	0	0.00	0.0
	500	100	7	0.07	6.0*
	1,600	0			
					P=0.063[d]
Trial 2					
Harvest time: 12 hours					
Summary: Weakly positive					
Dimethylsulfoxide		100	0	0.00	0.0
Mitomycin-C	0.5	100	113	1.13	58.0
Carisoprodol	250	100	1	0.01	1.0
	500	100	0	0.00	0.0
	750	100	2	0.02	2.0
	1,000	100	3	0.03	2.0
	1,250	100	6	0.06	5.0*
	1,500	0			
					P=0.006
+S9					
Trial 1					
Harvest time: 12 hours					
Summary: Negative					
Dimethylsulfoxide		100	1	0.01	1.0
Cyclophosphamide[c]	50	100	117	1.17	63.0
Carisoprodol	16	100	8	0.08	3.0
	50	100	2	0.02	2.0
	160	100	3	0.03	3.0
	500	100	4	0.04	3.0
	1,600	0			
					P=0.204

TABLE F4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Carisoprodol

Compound	Concentration (μg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
+S9 (continued)					
Trial 2[e]					
Harvest time: 12 hours					
Summary: Positive					
Dimethylsulfoxide		100	3	0.03	3.0
Carisoprodol	500	100	5	0.05	4.0
	750	100	7	0.07	6.0
	1,000	100	20	0.20	17.0*
	1,250	100	115	1.15	64.0*
					P<0.001
Trial 3					
Harvest time: 12 hours					
Summary: Weakly positive					
Dimethylsulfoxide		100	4	0.04	1.0
Cyclophosphamide	50	100	85	0.85	61.0
Carisoprodol	50	100	0	0.00	0.0
	100	100	2	0.02	2.0
	250	100	1	0.01	1.0
	500	100	0	0.00	0.0
	750	100	1	0.01	1.0
	1,000	100	50	0.50	40.0*
					P<0.001

* Positive response (P≤0.05) versus the solvent control
[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Galloway *et al*. (1987).
[b] Solvent control
[c] Positive control
[d] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[e] Positive control was lost due to technical error.

TABLE F5
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Carisoprodol by Gavage for 13 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocyes Scored	Micronucleated Cells/1,000 Cells[b]	
			PCEs	NCEs
Male				
Corn oil[c]		10	1.74 ± 0.33	1.39 ± 0.16
Carisoprodol	300	10	1.33 ± 0.20	1.28 ± 0.07
	600	10	1.66 ± 0.39	1.41 ± 0.13
	1,200	10	1.20 ± 0.41	1.55 ± 0.13
			P=0.833[d]	P=0.383[e]
Female				
Corn oil		8	1.21 ± 0.31	1.12 ± 0.08
Carisoprodol	300	10	1.87 ± 0.34	0.87 ± 0.12
	600	8	1.81 ± 0.39	0.93 ± 0.12
	1,200	10	1.87 ± 0.37	1.27 ± 0.25
			P=0.186	P=0.670

[a] Study was performed by the U.S. Department of Agriculture (Western Regional Research Center). The detailed protocol is presented by MacGregor *et al*. (1990). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Solvent control
[d] Significance of micronucleated PCEs/1,000 PCEs tested by Cochran-Armitage trend test
[e] Significance of micronucleated NCEs/1,000 NCEs tested by analysis of variance

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TOXICOKINETIC STUDIES

INTRODUCTION

Studies were performed to compare the proportionality and bioavailability of a single dose of carisoprodol administered by two routes, intravenous and gavage dosing, in F344/N rats and $B6C3F_1$ mice. Additional studies were performed to compare the bioequivalence of a single gavage dose of carisoprodol administered in two vehicles, corn oil and 0.5% methylcellulose.

MATERIALS AND METHODS

Carisoprodol (lot 105396) was obtained from Ceres Chemical Company, Inc. (White Plains, NY). The same lot (lot 105396) was used for the 13-week core studies of carisoprodol in 0.5% methylcellulose; bulk and dose analysis methods are described in the Materials and Methods section of this Toxicity Study Report. Dose formulations were prepared by mixing carisoprodol with dimethylacetamide:water (50:50), 0.5% methylcellulose, or corn oil. Homogeneity analyses were performed on 30 and 160 mg/mL formulations of carisoprodol in 0.5% methylcellulose and in corn oil by gas chromatography with flame ionization detection. Homogeneity was confirmed. The stability of a 6 mg/mL formulation of carisoprodol in aqueous dimethylacetamide was analyzed by gas chromatography with flame ionization detection; stability was confirmed for 14 days when the dose formulation was stored at room temperature or at 5° C, sealed and protected from ultraviolet light.

Initially, pilot studies were conducted to estimate the plasma concentration ranges for groups of 10 male rats and 10 male mice administered a single intravenous injection of carisoprodol in aqueous dimethylacetamide (rats: 40 mg/kg; mice: 60 mg/kg) or a single gavage dose of carisoprodol in 0.5% methylcellulose (rats: 200 or 800 mg/kg; mice: 300 or 1,200 mg/kg). A single animal per group was anesthetized with a carbon dioxide:oxygen mixture and bled by cardiac puncture at each of eight or ten time points: 15, 30, 60, 90, 120, 240, 360, and 480 minutes (gavage) or 2, 7, 12, 20, 30, 40, 60, 90, 150, and 240 minutes (injection).

For the single-dose proportionality and bioavailability studies, male rats and male mice were obtained from Charles River Laboratories (rats: Kingston, NY; mice: Portage, MI). Rats and mice were approximately 11 weeks old at receipt. Animals were quarantined for 20 days (rats) or 18 days (mice) and were approximately 14 weeks old on the day of treatment. Blood was collected from 10 rats and 10 mice before the day of treatment. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative. Rats and mice were housed individually. Feed and water were available *ad libitum*.

Fifteen male rats were weighed and then administered a single intravenous dose of 16 mg carisoprodol per kilogram body weight in aqueous dimethylacetamide at a dosing volume of 2 mL/kg. Groups of three rats were then bled at each of two time points: 2 and 210 minutes, 10 and 120 minutes, 20 and 90 minutes, 40 and 180 minutes, or 60 and 150 minutes. Thirty male mice were weighed and then administered a single intravenous dose of 32 mg/kg carisoprodol in aqueous dimethylacetamide at a dosing volume of 4 mL/kg. Groups of three mice were bled at one time point: 2, 10, 20, 40, 60, 90, 120, 150, 180, or 210 minutes.

Twelve male rats were weighed and then administered a single gavage dose of 200, 400, or 800 mg/kg carisoprodol in 0.5% methylcellulose at a dosing volume of 5 mL/kg; groups of three rats per dose were then bled at each of two time points: 5 and 240 minutes, 10 and 60 minutes, 20 and 120 minutes, or 40 and 180 minutes (200 mg/kg group); 5 and 300 minutes, 15 and 180 minutes, 30 and 240 minutes, or 60 and 120 minutes (400 mg/kg group); or 5 and 480 minutes, 15 and 120 minutes, 30 and 360 minutes, or 60 and

240 minutes (800 mg/kg group). Seventy-two male mice were weighed and then received a single gavage dose of 300, 600, or 1,200 mg/kg carisoprodol in 0.5% methylcellulose at a dosing volume of 10 mL/kg. Groups of three mice were bled at one time point: 5, 15, 30, 60, 120, 180, 240, or 300 minutes (300 mg/kg group); 5, 15, 30, 60, 120, 240, 360, or 420 minutes (600 mg/kg group); or 5, 15, 45, 120, 240, 360, 480, or 600 minutes (1,200 mg/kg group).

Animals were anesthetized with a carbon dioxide:oxygen mixture; blood was withdrawn by retroorbital puncture (rats) or cardiac puncture (mice) and collected in tubes containing heparin. Plasma was separated by centrifugation and stored at −20° C until analyses were performed. Plasma samples were diluted with blank plasma, if necessary, to obtain 200 μL samples; phosphate buffer (400 μL, pH 4) and methylene chloride (1 mL) were added. A 500 μL aliquot of each sample was evaporated to dryness with nitrogen at approximately 35° C and then reconstituted in methanol (100 μL) containing an internal standard. Plasma was analyzed by gas chromatography with a nitrogen-phosphorus detector. Spiked plasma standard curves at high and low concentrations were prepared and extracted with samples that were expected to be in the high or low ranges. Each standard curve consisted of three rodent plasma standards at the lowest and highest concentrations, one standard at each of the intermediate concentrations, and one blank. The concentrations of samples that were not in the expected ranges were calculated with standard curves from the appropriate ranges. Quality control samples (at least one for every 10 plasma samples) were concomitantly extracted with the plasma samples and the standard curves.

For the single-dose bioequivalence studies, male rats and male mice were obtained from Simonsen Laboratories (Gilroy, CA). Rats were approximately 12 weeks and mice were approximately 11 or 12 weeks old at receipt. Animals were quarantined for 14 days and were approximately 14 weeks old (rats) or 13 or 14 weeks old (mice) on the day of treatment. Blood was collected from five rats and five mice before the day of treatment. The sera were analyzed for antibody titers to rodent viruses. All results were negative. Rats and mice were housed individually. Feed and water were available *ad libitum*.

Groups of 20 male rats and 40 male mice, approximately 14 weeks of age, were weighed and then administered a single gavage dose of 800 mg/kg (rats) or 1,200 mg/kg (mice) carisoprodol in 0.5% aqueous methylcellulose or corn oil. The dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice. Groups of five rats receiving each vehicle were bled at each of two time points: 5 and 120 minutes, 15 and 60 minutes, 30 and 360 minutes, or 240 and 480 minutes. Groups of five mice receiving each vehicle were bled at a single time point: 5, 15, 30, 60, 120, 180, 300, or 420 minutes. Blood was collected and plasma analyses were performed as described for the single-dose proportionality and bioavailability studies. Dried extracts were stored at approximately −20° C until they were reconstituted for analysis.

Area-under-the-curve (AUC) values were calculated by a software program (SigmaPlot®, Jandel Corporation, San Rafael, CA) using the trapezoidal method; the software program was also used to calculate linear regression values. Semi-logarithmic plots of plasma concentration versus time and AUC versus dose were prepared. Absolute bioavailability was determined with the following equation:

$$\text{absolute bioavailability} = AUC_{oral}/AUC_{intravenous} \times dose_{intravenous}/dose_{oral}$$

RESULTS

Pilot Studies

Measurable amounts of carisoprodol were present in plasma samples taken up to 240 minutes after intravenous injection in rats and mice, up to 360 minutes after gavage dosing in rats, and up to 480 minutes after gavage dosing in mice (data not shown).

Single-Dose Proportionality and Bioavailability Studies

Rats: Plasma carisoprodol concentration data are provided in Table G1 and Figures G1 through G4. Single gavage doses of 200 to 800 mg/kg were dose proportional based on AUC data; absolute bioavailability values increased with increasing dose, ranging from 15% to 32% (Table G2 and Figure G5).

Mice: Plasma carisoprodol concentration data for mice are provided in Table G3 and Figures G6 through G9. Single gavage doses of 300 to 1,200 mg/kg were dose proportional based on AUC data; absolute bioavailability values increased with increasing dose, ranging from 18% to 38% (Table G4 and Figure G5).

Single-Dose Bioequivalence Studies

Rats: Plasma carisoprodol concentration data are provided in Table G5 and Figure G10. AUC values indicated that the bioavailability of carisoprodol in 0.5% methylcellulose was approximately fivefold that of carisoprodol in corn oil. The C_{max} values of the dose in 0.5% methylcellulose were approximately threefold those of the dose in corn oil (Table G6).

Mice: Plasma carisoprodol concentration data are provided in Table G7 and Figure G11. AUC values indicated no significant difference in the bioavailability of carisoprodol between the vehicles; however, the C_{max} values of the dose in 0.5% methylcellulose were 1.5 to 1.75 times those of the dose in corn oil (Table G8).

TABLE G1
Plasma Concentrations of Carisoprodol in Male Rats After a Single Intravenous Dose of 16 mg/kg Carisoprodol in Aqueous Dimethylacetamide or a Single Gavage Dose of 200, 400, or 800 mg/kg Carisoprodol in 0.5% Methylcellulose[a]

Time (minutes)	Intravenous	Gavage		
	16 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
n	3	3	3	3
2	19.8 ± 2.6			
5		3.2 ± 2.9	5.5 ± 4.3	2.2 ± 0.5
10	10.2 ± 0.5	7.1 ± 1.7		
15			14.1 ± 2.5	11.4 ± 1.8
20	5.6 ± 0.3	11.2 ± 8.7		
30			16.8 ± 5.1	18.4 ± 8.8
40	1.5 ± 0.2	5.3 ± 4.6		
60	0.57 ± 0.14	3.7 ± 1.5	10.6 ± 7.0	25.9 ± 5.8
90	0.26[b]			
120	—[c]	1.0[b]	7.0 ± 2.6	17.9 ± 2.8
150	—			
180	—	0.27[b]	3.5 ± 0.6	
210	—			
240		0.47 ± 0.23	4.1 ± 4.7	8.9 ± 6.5
300			2.5 ± 2.3	
360				4.6 ± 3.4
480				2.9 ± 1.3

[a] Data are given as mean ± standard deviation (μg/mL).
[b] Mean of two samples; third sample was below the limit of detection. No standard deviation was calculated.
[c] All values were below the limit of detection.

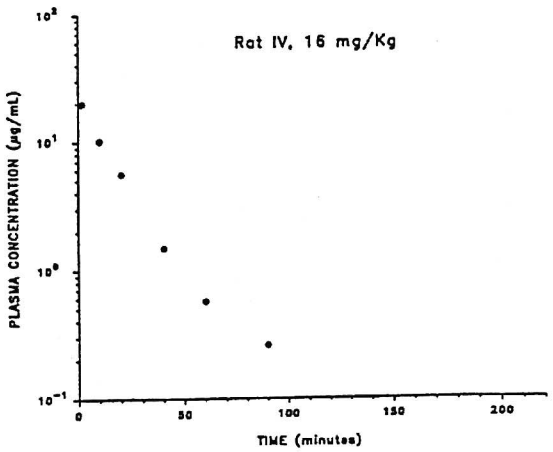

FIGURE G1
Plasma Concentrations of Carisoprodol in Male Rats After a Single Intravenous Injection of 16 mg/kg in Aqueous Dimethylacetamide

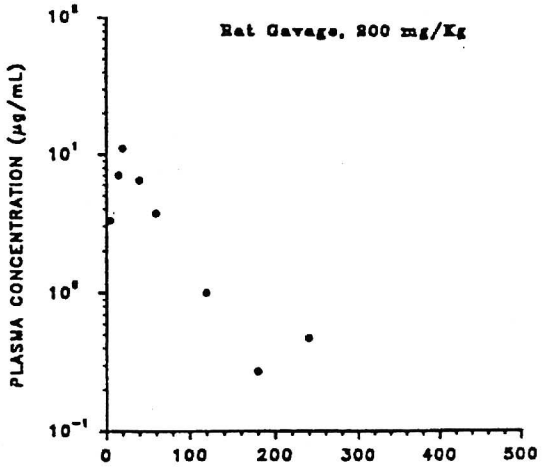

FIGURE G2
Plasma Concentrations of Carisoprodol in Male Rats After a Single Gavage Dose of 200 mg/kg in 0.5% Methylcellulose

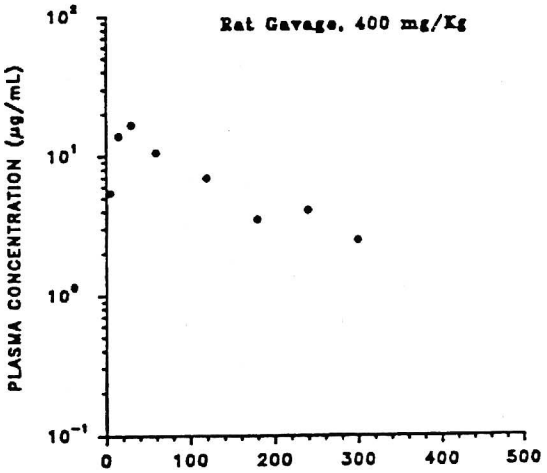

FIGURE G3
Plasma Concentrations of Carisoprodol in Male Rats After a Single Gavage Dose of 400 mg/kg in 0.5% Methylcellulose

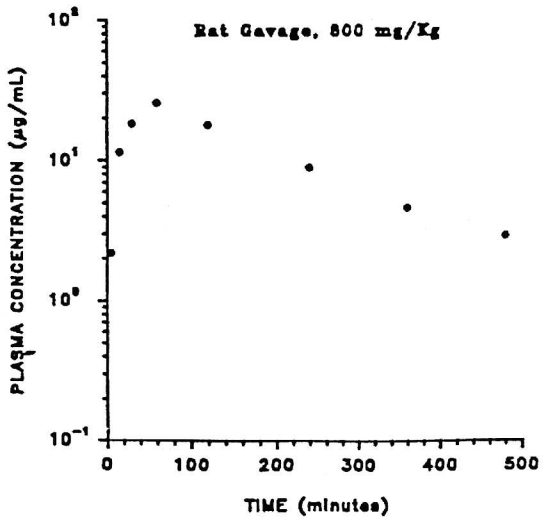

FIGURE G4
Plasma Concentrations of Carisoprodol in Male Rats After a Single Gavage Dose of 800 mg/kg in 0.5% Methylcellulose

TABLE G2
Toxicokinetic Data for Male Rats After a Single Gavage Dose of Carisoprodol in 0.5% Methylcellulose[a]

Dose (mg/kg)	C_{max} (μg/mL)	t_{max} (minutes)	$t_{1/2}$ (minutes)	Absolute Bioavailability (%)	AUC (μg/mL/minute)
200	11.2	20	60	14.6	578.1
400	16.8	30	100	25.3	2,008.5
800	25.8	60	120	32.3	5,134.8

[a] The data were calculated from plasma concentration-time curves, where each point represents the mean for three rats. C_{max} = maximum mean plasma concentration; t_{max} = time of maximum mean plasma concentration; $t_{1/2}$ = elimination half-life; AUC = area under the curve

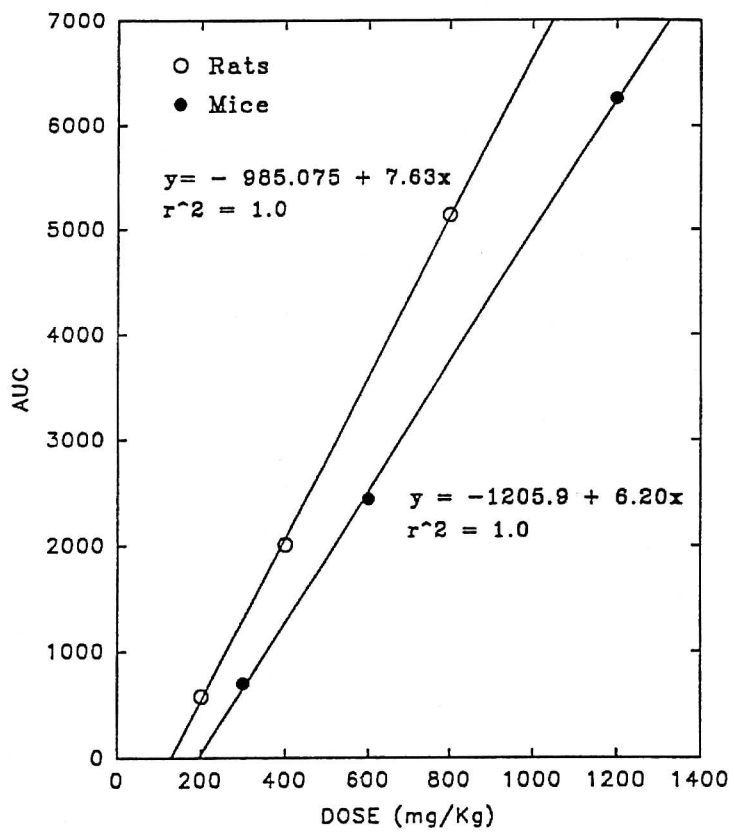

FIGURE G5
Area Under the Curve Versus Dose for Male Rats and Male Mice Administered a Single Gavage Dose of Carisoprodol in 0.5% Methylcellulose

TABLE G3
Plasma Concentrations of Carisoprodol in Male Mice After a Single Intravenous Dose of 32 mg/kg Carisoprodol in Aqueous Dimethylacetamide or a Single Gavage Dose of 300, 600, or 1,200 mg/kg Carisoprodol in 0.5% Methylcellulose[a]

Time (minutes)	Intravenous (32 mg/kg)	Gavage 300 mg/kg	Gavage 600 mg/kg	Gavage 1,200 mg/kg
n	3	3	3	3
2	38.5 ± 8.3			
5		13.7 ± 5.3	15.0 ± 6.1	22.2 ± 6.9
10	13.5 ± 4.7			
15		15.7 ± 4.7	40.0 ± 12.7	47.4 ± 8.7
20	6.5 ± 2.4			
30		10.3 ± 3.0	25.2 ± 7.7	
40	1.2 ± 0.6			
45				54.9 ± 15.8
60	0.14[b]	4.5 ± 1.2	18.9 ± 6.7	
90	0.77[c]			
120	0.9[c]	—[d]	5.0 ± 0.9	11.0 ± 3.9
150	—			
180	—	—		
210	—			
240		—	—	8.8 ± 4.3
300		—		
360			—	0.16[b]
420			—	
480				1.6[b]
600				2.4[b]

[a] Data are given as mean ± standard deviation (μg/mL).
[b] Result of one sample; two samples were below the limit of detection. No standard deviation was calculated.
[c] Mean of two samples; third sample was below the limit of detection. No standard deviation was calculated.
[d] All values were below the limit of detection.

TABLE G4
Toxicokinetic Data for Male Mice After a Single Gavage Dose of Carisoprodol in 0.5% Methylcellulose[a]

Dose (mg/kg)	C_{max} (μg/mL)	t_{max} (minutes)	$t_{½}$ (minutes)	Absolute Bioavailability (%)	AUC (μg/mL/minute)
300	15.7	15	25	17.6	699.0
600	40.0	15	30	29.7	2,442.5
1,200	54.9	45	90	38.3	6,252.2

[a] The data were calculated from plasma concentration-time curves, where each point represents the mean for three mice. C_{max} = maximum mean plasma concentration; t_{max} = time of maximum mean plasma concentration; $t_{½}$ = elimination half-life; AUC = area under the curve

TABLE G5
Plasma Concentrations of Carisoprodol in Male Rats After a Single Gavage Dose of 800 mg/kg Carisoprodol in Corn Oil or 0.5% Methylcellulose[a]

Time (minutes)	Corn Oil	0.5% Methylcellulose
n	5	5
5	1.2 ± 1.7[b]	3.3 ± 2.1[b]
15	4.5 ± 1.7	10.8 ± 4.0
30	6.8 ± 4.3	22.0 ± 6.6
60	2.7 ± 1.1	15.4 ± 3.9
120	6.7 ± 4.3	17.3 ± 5.8
240	1.4 ± 0.9	17.5 ± 9.8
360	1.6 ± 1.7	14.7 ± 7.5
480	1.3 ± 0.7	8.0 ± 4.5

[a] Data are given as mean ± standard deviation (μg/mL).
[b] Mean of four samples; one sample was below the limit of detection.

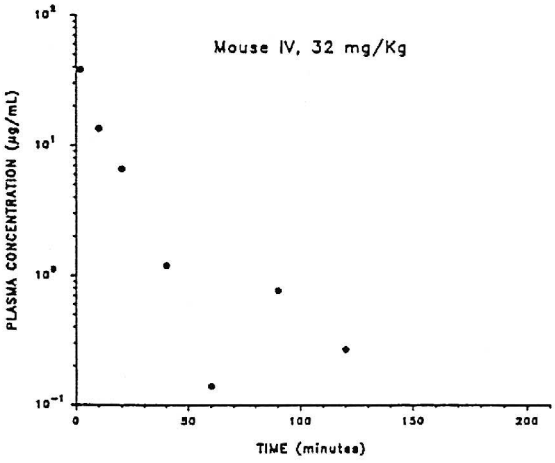

FIGURE G6
Plasma Concentrations of Carisoprodol in Male Mice After a Single Intravenous Injection of 32 mg/kg in Aqueous Dimethylacetamide

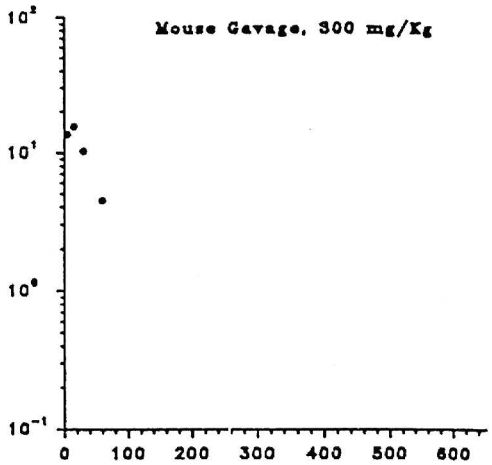

FIGURE G7
Plasma Concentrations of Carisoprodol in Male Mice After a Single Gavage Dose of 300 mg/kg in 0.5% Methylcellulose

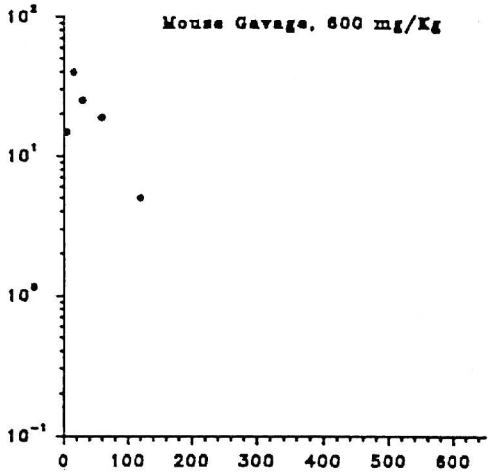

FIGURE G8
Plasma Concentrations of Carisoprodol in Male Mice After a Single Gavage Dose of 600 mg/kg in 0.5% Methylcellulose

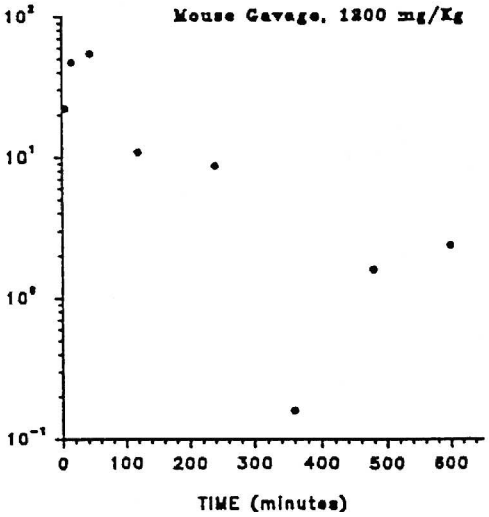

FIGURE G9
Plasma Concentrations of Carisoprodol in Male Mice After a Single Gavage Dose of 1,200 mg/kg in 0.5% Methylcellulose

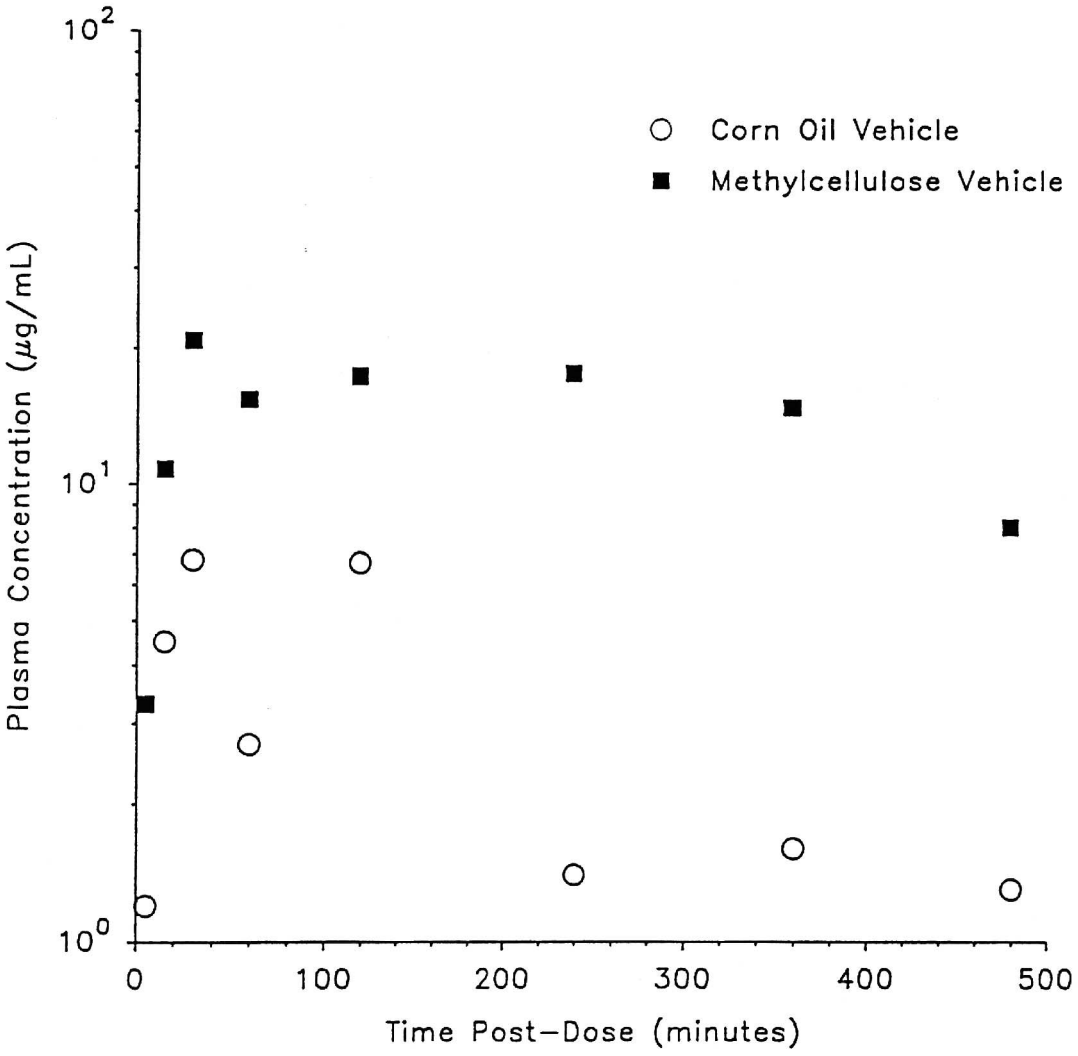

FIGURE G10
Plasma Concentrations of Carisoprodol in Male Rats After a Single Gavage Dose of 800 mg/kg in Corn Oil of 0.5% Methylcellulose

TABLE G6
Toxicokinetic Data for Male Rats After a Single Gavage Dose of 800 mg/kg Carisoprodol in Corn Oil or 0.5% Methylcellulose[a]

Route	C_{max} (μg/mL)	t_{max} (minutes)	$t_{1/2}$ (minutes)	AUC (μg/mL/minute)
Corn oil	6.8	30	130	1,378
0.5% Methylcellulose	20.8	30	140	7,214

[a] The data were calculated from plasma concentration-time curves, where each point represents the mean for three rats. C_{max} = maximum mean concentration; t_{max} = time of maximum mean concentration; $t_{1/2}$ = elimination half-life; AUC = area under the curve

TABLE G7
Plasma Concentrations of Carisoprodol in Male Mice After a Single Gavage Dose of 1,200 mg/kg Carisoprodol in Corn Oil or 0.5% Methylcellulose[a]

Time (minutes)	Corn Oil	0.5% Methylcellulose
n	5	5
5	7.4 ± 3.9	25.9 ± 12.1
15	36.8 ± 7.5	54.5 ± 12.3
30	35.8 ± 8.8	82.2 ± 33.4
60	55.7 ± 10.5	78.2 ± 38.7
120	43.7 ± 21.9	56.7 ± 22.1
180	22.5 ± 9.4	46.2 ± 12.7
300	14.5 ± 12.2	29.1 ± 12.1
420	6.8 ± 8.9	5.9 ± 8.9[b]

[a] Data are given as mean ± standard deviation (μg/mL).
[b] Mean of three samples; two samples were below the limit of detection.

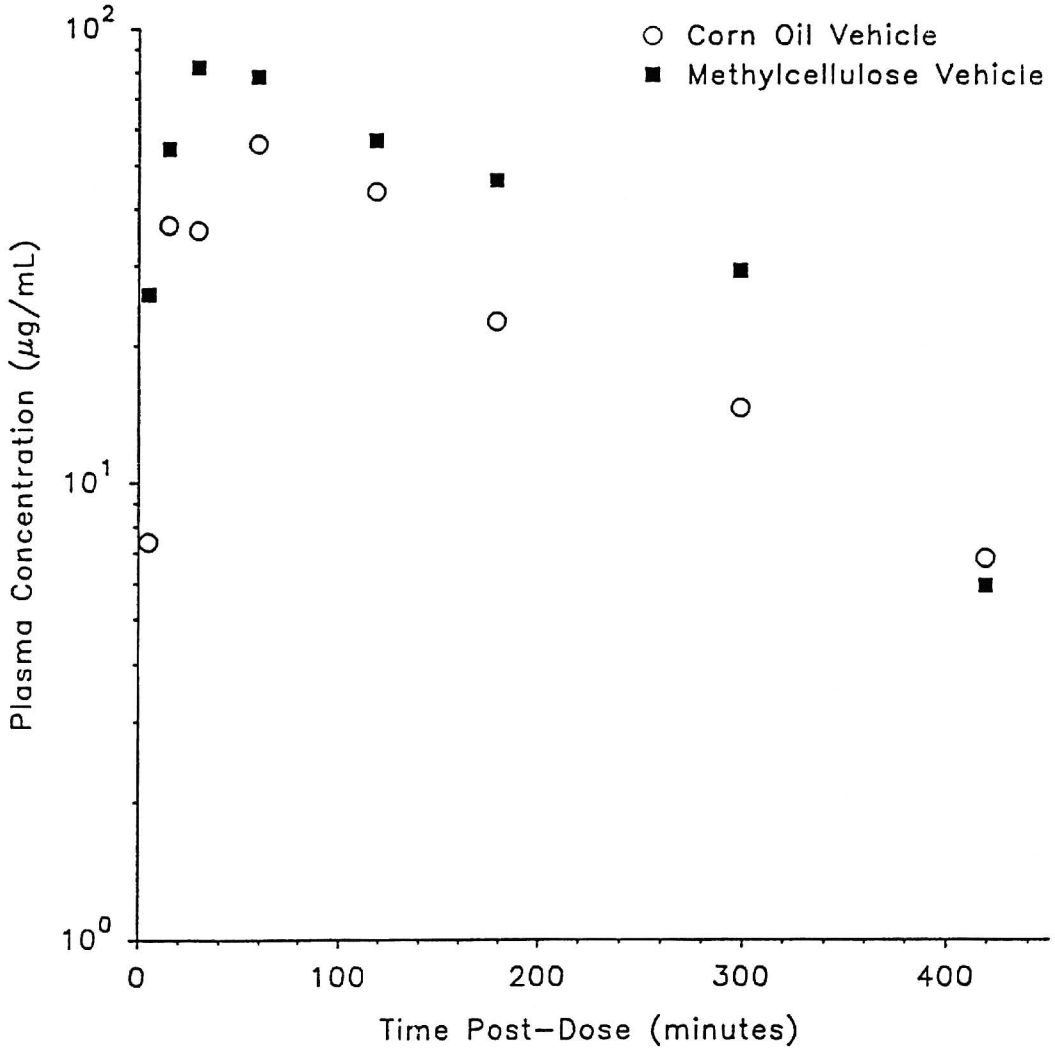

FIGURE G11
Plasma Concentrations of Carisoprodol in Male Mice After a Single Gavage Dose of 1,200 mg/kg in Corn Oil or 0.5% Methylcellulose

TABLE G8
Toxicokinetic Data for Male Mice After a Single Gavage Dose of 1,200 mg/kg Carisoprodol in Corn Oil or 0.5% Methylcellulose[a]

Route	C_{max} (μg/mL)	t_{max} (minutes)	$t_{1/2}$ (minutes)	AUC (μg/mL/minute)
Corn oil	55.7	60	105	10,604
0.5% Methylcellulose	82.2	30	120	17,584

[a] The data were calculated from plasma concentration-time curves, where each point represents the mean for three mice. C_{max} = maximum mean concentration; t_{max} = time of maximum mean concentration; $t_{1/2}$ = elimination half-life; AUC = area under the curve